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Yongsheng Li *Editor*

Lipid Metabolism in Tumor Immunity

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Introduction

Immunotherapy is the third revolution of cancer treatment. It has been widely applied in a variety of tumors and even in metastatic and advanced cancers by enhancing the immune system. Different from traditional chemotherapy or targeted therapy, patients who respond to immunotherapy have a great chance of high-quality long-term survival. On the whole, the side effects of immunotherapy are significantly lower than traditional chemotherapy and the multi-target drugs. Therefore, cancer immunotherapy was rated No. 1 of the top ten scientific and technological breakthroughs by *Science* in 2013, and was awarded the Nobel Prize in 2018. It is currently the most promising method for anti-tumor treatment. The current main approaches of tumor immunotherapy to enhance anti-tumor immunity are immune checkpoint blockade, chimeric antigen T cells (CAR-T), or DC vaccines. These advancements have achieved world-renowned effects, making tumor immunotherapy enter the 2.0 era. However, immunosuppression, immune tolerance, and cytokine storm remain unsurpassed problems.

Like immunity, metabolism is also the basis of the body's life activities. A large number of studies have shown that metabolic reprogramming is an important mechanism leading to tumor immune escape, and correcting the metabolic pattern of tumor cells and tumor-associated immune cells can significantly play a role in anti-tumor therapy. Therefore, combined metabolic therapy is expected to bring about a revolution in tumor immunotherapy. However, we also need to consider the complexity and heterogeneity of the tumor microenvironment when we combine metabolic therapy. Clarifying the metabolic characteristics and interaction mechanism of the tumor and the immune system is an important basis for combined therapy, which is expected to provide new and effective strategies for tumor immunotherapy.

It is now clear that lipid metabolism reprogramming plays an important role in the proliferation and migration of tumor cells, and its lipid metabolites will also modify the tumor microenvironment and affect the recruitment and function of tumor-related immune cells. Moreover, the lipid metabolism pattern of tumor-related immune cells also determines its functional phenotype, mediating tumor immunoediting, *i.e.*, clearance, balance, and escape. Compared with glucose metabolites and amino acid metabolites, lipids are more complex, including eight types, which also determine that the extraction and analysis procedures are more complex and diverse. Therefore, creating a standard lipid metabolism detection method and revealing the molecular mechanism of lipid metabolism regulating tumor immunity has important

clinical and scientific value for understanding tumor immune escape and developing effective tumor immunotherapy strategies.

Under this clinical and academic background, as the head of the Immunometabolism Group of the Tumor Metabolism Committee of Chinese Anti-Cancer Association and the chairman of the Metabolic and Immunity Committee of Chongqing Immunological Society, I was invited by Springer Nature to write *Lipid Metabolism in Tumor Immunity*. With the collective efforts of the Immunometabolism Group of the Tumor Metabolism Committee of the Chinese Anti-Cancer Association and the Metabolism and Immunity Committee of the Chongqing Society of Immunology, the book will be divided into 12 chapters to elaborate on this topic in depth.

In detail, Prof. Xianlin Han, the proposer of "lipidomics", and his colleagues from the University of Texas wrote Chap. 1, Lipid metabolism and lipidomics applications in cancer research; Prof. Yuping Wang from Lanzhou University wrote Chap. 2, Applications of lipidomics in tumor diagnosis and therapy; Prof. Lianjun Zhang and his colleague from Suzhou Institute of Systems Medicine wrote Chap. 3, Overview: Lipid metabolism in the tumor microenvironment; Prof. Rui Zhang and his colleagues from Naval Medical University wrote Chap. 4, Lipid metabolism in cancer cells; Prof. Meihua Sui and her colleague from Zhejiang University wrote Chap. 5, Lipid metabolism in tumor-associated nature killer cells; Prof. Hongming Miao and his colleague from Army Medical University wrote Chap. 6, Lipid metabolism in tumor-associated macrophages; Prof. Yan Li and his colleagues from Nanjing University wrote Chap. 7, Lipid metabolism in tumor-associated myeloid derived suppressor cells; Prof. Jingyuan Wan and his colleague from Chongqing Medical University wrote Chap. 8, Lipid metabolism in tumor-associated fibroblasts; Prof. Fang Wan and her colleague from Inner Mongolia Agricultural University wrote Chap. 9, Lipid metabolism in tumor-associated B cells; Prof. Wei Yang and his colleagues from Southern Medical University wrote Chap. 10, Lipid metabolism in tumor-infiltrating T cells; Prof. Yaxi Chen and her colleague from Chongqing Medical University wrote Chap. 11, Lipid metabolism and tumor antigen presentation; and Prof. Qianjin Liao and his colleagues from Central South University wrote Chap. 12, Lipid metabolism and immune checkpoints.

The whole book was compiled and finalized by Prof. Yongsheng Li from Chongqing University Cancer Hospital. The authors who participated in writing this book are all junior and senior scientists who are hard-working in the front-line research of lipid metabolism and tumor immunity. They have rich experience in lipid metabolism research and also pay attention to the development of tumor immunity and immunotherapy research. Therefore, the content of this book reflects the latest progress in lipid metabolism and tumor immunity. This book aims to analyze research examples and demonstrate the role of lipid metabolism in different areas of tumor immunity, so that readers can better apply this book to solve practical problems faced by life sciences.

This book can be used as a reference book for researchers, clinicians, graduate students studying in the field of life sciences, and undergraduates in lipid metabolism and tumor immunity research. It is also a reference book for staff engaged in data analysis in related fields and interested in finding disease

biomarkers. The publication of this book is supposed to greatly promote the popularization and application of lipid metabolomics technology, and will also provide the necessary reference for relevant departments to rationally plan the development of lipid metabolomics.

Chongqing, China Yongsheng Li

Contents

Lipid Metabolism and Lipidomics Applications in Cancer Research

1

Meixia Pan, Chao Qin, and Xianlin Han

Abstract

Lipids are the critical components of cellular and plasma membrane, which constitute an impermeable barrier of cellular compartments, and play important roles on numerous cellular processes including cell growth, proliferation, differentiation, and signaling. Alterations in lipid metabolism have been implicated in the development and progression of cancers. However, unlike other biomolecules, the diversity in the structures and characteristics of lipid species results in the limited understanding of their metabolic alterations in cancers. Lipidomics is an emerging discipline that studies lipids in a large scale based on analytical chemistry principles and technological tools. Multidimensional mass spectrometry-based shotgun lipidomics (MDMS-SL) uses direct infusion to avoid difficulties from alterations in concentration, chromatographic anomalies, and ion-pairing alterations to improve resolution and achieve rapid and accurate qualitative and quantitative analysis. In this chapter, lipids and lipid metabolism relevant to cancer research are introduced, followed by a brief description of MDMS-SL and other shotgun lipidomics techniques and some applications for cancer research.

Keywords

Lipids · Lipid metabolism · Cancer · Immunity
Lipidomics · Mass spectrometry

Abbreviations

Cer	Ceramide
dPE	Phosphatidylethanolamine
ESI	Electrospray ionization
GPL	Glycerophospholipids
IP3	Inositol triphosphate
LC	Liquid chromatography
LysoPA	Lysophosphatidic acid
LysoPC	Choline lysoglycerophospholipid
LysoPE	Ethanolamine lysoglycerophospholipid
LysoPI	Lyso-phosphatidylinositol
Lyso-pPE	Lysoplasmenylethanolamine
LysoPS	Lyso-phosphatidylserine

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MALDI	Matrix-assisted laser desorption/ionization
MDMS-SL	Multidimensional mass spectrometry-based shotgun lipidomics
MS	Mass spectrometry
NEFA	Nonesterified fatty acid
PA	Phosphatidic acid
PC	Choline glycerophospholipid
PE	Ethanolamine glycerophospholipid
PG	Phosphatidylglycerol
PI	Phosphatidylinositol
PIP2	Phosphatidylinositol diphosphate
pPC	Plasmenylcholine
pPE	Plasmenylethanolamine
PS	Serine glycerophospholipid
Q	Quadrupole
QqQ	Triple quadrupole
SLE	Systemic lupus erythematosus
TAG	Triacylglycerol
TOF	Time of flight

1.1 Lipid Classes and Their Functions

Lipids are the crucial component of cellular membrane, which constitutes an impermeable barrier of cellular compartments and provides appropriate motifs for membrane protein function [1]. In addition to the role of energy storage, many lipids play distinct and critical roles in a variety of cellular functions such as signal transduction, in which lipids serve as active second messengers and hormones [2]. Lipids involved in metabolism are highly complex in terms of type and concentration and vary constantly under physiological, pathological, and environmental conditions [3].

According to the definition of the LIPID MAPS consortium, lipids are small hydrophobic or amphipathic molecules, which might originate in whole or in part by carbanion-based condensations of ketoacyl thioesters and/or by carbocation-based condensations of isoprene units [4]. The lipids have been classified into eight categories [4]. (1) *Fatty acyls*: The fatty acyl groups include various molecules synthesized by chain elongation of acetyl coenzyme A (CoA) primers with malonyl-CoA groups. The fatty acyls may contain cyclic functional groups and/or be substituted by heteroatoms. Fatty acyls, characterized in repeating a series of methylene groups, are the simplest lipids in structure. Therefore, fatty acyls are the basic components of other complex lipids [5]. (2) *Glycerolipids*: The glycerolipid groups include monoacylglycerol, diacylglycerol, triacylglycerol (TAG), and glycolipids. Glycerolipids are characterized in containing a glycerol backbone with fatty acyl chains connected to the hydroxyl groups of glycerol. Glycerolipids can be hydrolyzed into glycerol, nonesterified fatty acid (NEFA), and/or alkyl variants. Glycerolipids contribute to energy storage, energy metabolism, and signal transduction [5]. (3) *Glycerophospholipids*: Glycerophospholipids (GPLs) are the lipids having at least one glycerol hydroxyl group esterified with one phosphate or phosphonate group, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidic acid (PA), cardiolipin, bis(monoacylglycerol)phosphate and lysophospholipids, etc. GPLs are the critical and predominant components of cellular and plasma membranes. GPLs contribute to second messenger generation and are involved in cellular metabolism and signal transduction [5]. (4) *Sphingolipids*: Sphingolipid groups are characteristic in containing a long chain sphingoid as the core structure, such as ceramide (Cer), sphingomyelin, cerebroside, sulfatide, and gangliosides. Sphingolipids are involved in plasma membranes and cellular signaling [5]. (5) *Sterol lipids*: Sterol lipids are compounds with core characteristic in four fused rings. Sterol lipids are divided into subcategories of cholesterol and its derivatives, steroids, bile acids and the derivatives, etc. [4]. Cholesterol and its derivatives are critical component of cellular membranes [6]. The steroids, which act as hormones and signaling molecules, are involved in various biological and metabolic processes [7]. (6) *Prenol lipids*: The prenol lipids are synthe-

sized from the five-carbon precursors isopentenyl diphosphate and dimethylallyl diphosphate mainly produced through the mevalonate pathway [8]. (7) *Saccharolipids*: The saccharolipids are a group of lipids containing a sugar backbone straightly connected with fatty acids. Saccharolipids exist in the form of glycans or phosphorylated derivatives [5]. (8) *Polyketides*: The polyketides are a group of metabolites having the characteristics alike lipids. They are derived from plant and microbial [4].

The lipids act as cellular membrane components, energy storage depots, and signaling molecules to exert their biological functions (Table 1.1) [5].

Briefly, the polar lipids are largely involved in construction of cellular and plasma membranes, including the majority of glycerophospholipids and sphingolipids. Some of their metabolism intermediates (e.g., lysolipids, diacylglycerol, Cer, and NEFA) contribute to membrane structures with very low abundance, which could be crucial in regulation of membrane structure and cell functions. Any changes in membrane lipid compositions and lipid species could alter membrane permeability, fluidity, and stability, leading to alterations in cell functions. The effects of lipid composition alterations on cellular functions include the following: (1) alter the matrix that interacts with membrane proteins (e.g., ion channels), which in turn affects the protein configurations and functions; (2) affect the roles of microenvironments in cell communication and cytosolic ion distribution—the anionic lipids play important roles on the stability of microenvironments; and (3) influence the processes of membrane fusion, vesicle transport, contact point formation, etc., particularly the changes in the lipids with different special shapes, such as plasmalogen, cardiolipin, and lysolipids. For example, cholesterol constitutes the functional domain of lipid rafts on membrane.

Table 1.1 The major biological functions of lipid classes [5]

Cellular functions	Lipid classes
Membrane structure construction	Glycerophospholipids (e.g., PC, PE, PI, PS, PG, PA, etc.), sphingolipids (e.g., sphingomyelin, cardiolipin, cerebroside, sulfatide, gangliosides, etc.), glycolipids, sterol lipids (e.g., cholesterol, etc.)
Energy storage and metabolism	Glycerolipids (e.g., NEFA, TAG, diacylglycerol, monoacylglycerol, acyl CoA, acylcarnitine, etc.)
Signal transduction	Glycerolipids (e.g. diacylglycerol, monoacylglycerol, acyl CoA, acylcarnitine, NEFA, oxidized fatty acid), sphingolipids (e.g., Cer, sphingosine, sphingosine-1-phosphate), psychosine, *N*-acylethanolamine, lysolipids, etc.
Other functions	Plasmalogen, acylcarnitine, cardiolipin, PS, etc.

Some glycerolipids are involved in energy metabolism and act as energy storage depots, such as NEFA, acylcarnitine, acyl CoA, diacylglycerol, and TAG. The changes in these lipids suggest alterations in energy metabolism and energy homeostasis. Excessive energy deposited in the storage depots could result in increased diacylglycerol and TAG levels. Elevated acylcarnitine indicates either mitochondrial dysfunction or excess fatty acid oxidation. Elevated NEFA and acyl CoA species are usually associated with either increased lipid turnover including lipolysis or de novo biosynthesis. Therefore, accumulation of the lipids involved in energy metabolism could lead to lipotoxicity [9–12], which is related to obesity and insulin resistance [5]. The fatty acyl profiles in lipid species also provide a wealth of information about fatty acid metabolism from lipid uptake, biosynthesis to lipolysis, and lipid oxidation in mammalian systems. High levels of fatty acyls containing $n-3$ and/or $n-6$ fatty acid in lipid classes indicate either excess uptake of extracellular fatty acids or reduced utility of these fatty acids through oxidation. Accumulation of fatty acyls 16:0, 16:1, 18:0, and 18:1 strongly suggests increased de novo synthesis. Fatty acid de novo synthesis and their food sources are also related to the location and number of double bond(s) in fatty acids. For instance, the 18:1 ($n-9$) fatty acyl isomer is the most abundant monoenoic fatty acyl in plant and animal tissues. The 18:1 ($n-7$) fatty acyl isomer is a common

monoenoic fatty acyl group in bacterial lipids and usually exists as minor component in most plants and animal tissue. The 18:1 ($n-12$) fatty acyl isomer accounts for 50% or more of lipids in seed oils of the Umbelliferae family including carrot, parsley, and coriander [13].

Recently, some lipid species are found to act as second messengers involved in cellular signal transduction, such as sphingolipids and glycerophospholipids. These lipid species could straightly bind to and subsequently activate a target protein such as a receptor, kinase, and phosphatase, leading to a specific cellular functional regulation. Lipids contributing to the signaling regulation have been summarized by Han et al. (Table 1.2) [5].

Additionally, some of lipid classes exert specific functions in various cellular processes. For instance, acylcarnitine is involved in fatty acyl transportation in and out of the mitochondria. Cardiolipin is involved in electron transport chain to generate ATP in mitochondria. LysoPC species contribute to inflammatory disorders [27–30]. Plasmalogen species, the component of biological membrane, play plenty of roles in cellular functions such as antioxidant. PS is precursor of mitochondrial PE in mammalian cells [5].

1.2 Lipid Metabolism and Cancer

Alterations in lipid metabolism affect numerous cellular processes including cell growth, proliferation, differentiation, and motility [2]. The cross talk among lipids and cell functions usually occurs during metabolic processes including non-oncogenic and oncogenic metabolic states [5]. There are at least three metabolic networks that are common and associated with lipids. (1) *Sphingolipid metabolic pathway and network*: Ceramide is the center of this pathway, which could interpret the alterations in sphingolipid classes and the subclasses containing different sphingoid backbones, e.g., dihydrosphingolipids. (2) *Glycerophospholipid biosynthesis pathway* and network: This network is different from species of animals/plants, yeast, and/or bacteria. The fatty acyl chain remodeling is observed in most newly synthesized glycerophospholipid species, which is associated with the activities of transacylase/acyltransferase and fatty acyl hydrolysis. Alterations in individual lipid species indicate hydrolysis and remodeling activities. The alterations in lipid classes suggest possible combination outcomes of hydrolysis activity, which can be supported by the changes of the corresponding lyso glycerophspholipid levels. (3) *Glycerolipid metabolism pathway and*

Table 1.2 Summary of lipid species involved in cellular signaling

Lipid species	Signaling pathways
Sphingolipids	1. Cell stress response, programmed cell death [14, 15], and cell aging [16] (*Cer*) 2. Activate phospholipase A_2 to release arachidonic acid through formation of ionophore [17] (*Ceramide-1-phospate*) 3. Vesicular trafficking, cell division, survival, and phagocytosis [18–20] (*Ceramide-1-phospate*) 4. Endocytosis, cell cycle, and apoptosis through interaction with protein kinases [21] (*Sphingosine*) 5. Cell survival and migration, inflammation [22] (*Sphingosine-1-phsphate*)
Glycerophospholipids	1. Agonize inward rectifying potassium channels [23] (PIP_2) 2. Generate second messengers IP_3 and diacylglycerol (PIP_2) 3. Release intracellular calcium ions (IP_3) 4. Active the members of protein kinase C family [24, 25] 5. A protein kinase B to increase binding to extracellular proteins and ultimately enhance cell survival [26] (PIP_3)
G-protein-coupled receptor activators	Activate G-protein-coupled receptors such as lysoglycerophospholipids (e.g., *Lyso-PA*), sphingosine-1-phsphate, platelet-activating factor, endocannabinoids, eicosanoids, fatty acid-hydroxy fatty acid, and retinol derivatives, etc.
Nuclear receptor and transcription factor activators	Bind to nuclear receptors and activate transcription factors, e.g., steroid hormones, retinoic acid, eicosanoids, NEFA, etc.

network: Monoacylglycerol, diacylglycerol, and TAG are included in this network, in which diacylglycerol could be composed in various cellular compartments and therefore can be composed in TAG species. There is homeostasis among monoacylglycerol, diacylglycerol, and TAG synthesis. The activity of sphingomyelin synthase is closely linked to the above network. Acyl CoA is a critical metabolite that participates in the synthesis, degradation, and remodeling of many lipids and thus changes the lipid profiles [5].

1.2.1 Lipid Metabolic Alterations in Cancer

The "metabolic switch" in cancer cells has been observed [31]. The carcinogenic phenotypes are produced by a series of mutation events that combine to alter a variety of signal transduction pathways, which converge to alter core cellular metabolism to meet the needs for rapid dividing cells such as rapid ATP generation to maintain high rate of metabolism, increased molecular biosynthesis, strengthened maintenance of proper cellular redox status, and dissemination of cancer cells to form distant metastases [5]. Since lipids are one of the main energy sources and basic component of living cells, it is no doubt that development of cancer (i.e., uncontrolled cell proliferation and growth) is closely associated with lipid metabolism. Cancer cells exhibit specific alterations in different aspects of lipid metabolism (Fig. 1.1) such as a high rate of de novo lipid synthesis, cholesterol synthesis through mevalonate pathway, increased dependence on lipid oxidation, etc. [2].

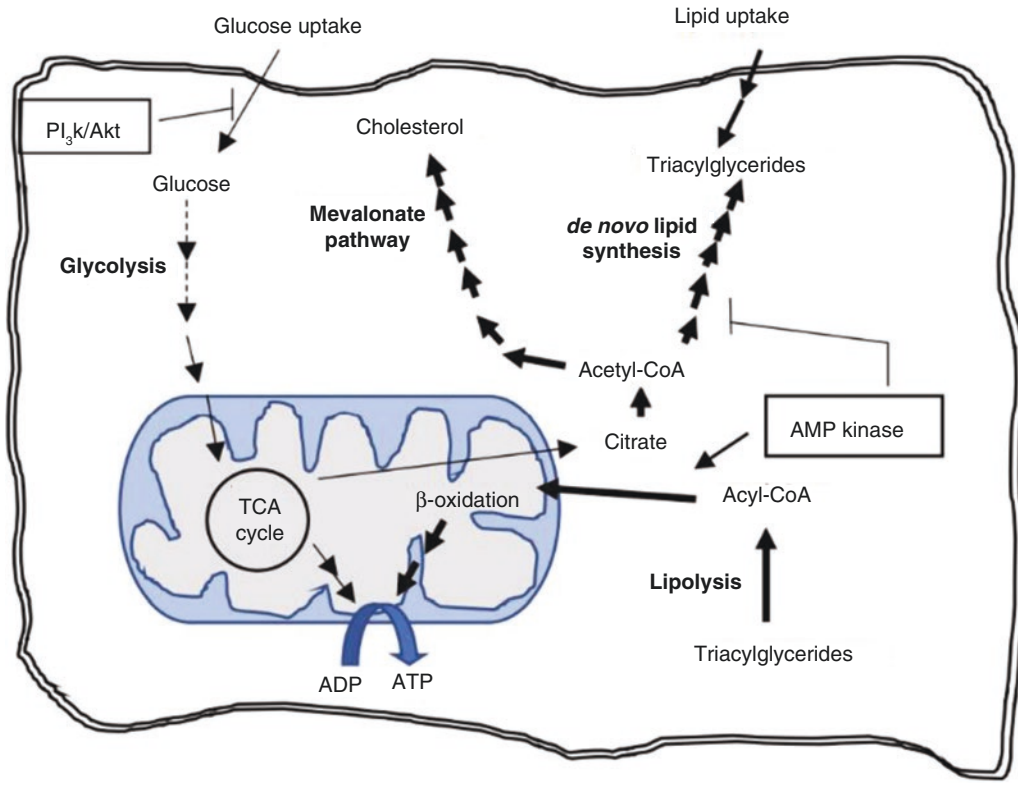

Fig. 1.1 Regulation of lipid metabolism by oncogenic signaling transduction pathways. Briefly, glucose utilization for ATP production decreases, although PI_3K/Akt pathway activation promotes glucose uptake. High rate of de novo lipid synthesis exhibits. The fatty acids mobilized from lipid stores are degraded in the mitochondria through β-oxidation to provide energy. AMP kinase is activated to prevent lipid synthesis and stimulate β-oxidation. Sterol regulatory element-binding protein also contributes to the regulation of fatty acid and cholesterol biosynthesis pathways

1.2.1.1 Alterations in Lipid Synthesis

It was found that fatty acid synthase expression was enhanced in the earliest stages of cancer development in lung cancer, prostate cancer, and breast cancer [32–34] and was more obvious as cancer progresses [35–37], indicating that the cancer cells exhibit high rates of de novo lipid synthesis starting at a relatively early-stage tumor development [33, 38]. It has been observed that cancer cells endogenously synthesize 95% of fatty acids, despite having adequate extracellular fatty acids [39]. Cholesterol is one of the key components of biological membranes contributing to modulate the fluidity of the lipid bilayer and forms lipid rafts that coordinate the activation of some signal transduction pathway [40]. Accumulation of cholesterol has been observed in prostate cancer cells. The abnormal cholesterol metabolism could influence signal transduction events at the membrane by promoting tumor cell growth, inhibiting apoptotic signals, and potentially stimulating other malignant cellular behaviors [41]. The enzyme activities of fatty acid and cholesterol biosynthesis in cancer cells are regulated by sterol regulatory element-binding proteins [42, 43] and phosphoinositide 3-kinase (PI_3K)/Akt/PKB (protein kinase B) [44]. Mutant p53 increases the expression of genes involved in the cholesterol biosynthesis pathway [45].

1.2.1.2 Lipid Remodeling

The rapid growth and proliferation in cancer cells require a large amount of lipids for lipogenesis, biological membrane construction, and functioning maintenance. Cellular and plasma membrane are largely composed of cholesterol, glycerophospholipids, and sphingolipids [40, 46]. Activation of de novo lipogenesis results in a dramatic difference on membrane lipid composition from that of normal cells, i.e., a lipid remodeling in plasma membrane. For example, phospholipid composition toward more saturated and monounsaturated acyl species modulates membrane biophysics and functions [47]. One example of relationship between genetic changes in cancer and membrane lipid remodeling that drives tumor growth has recently been reported. The lysophosphatidylcholine acyltransferase, a key membrane lipid remodeling enzyme [48], could shape the lipid composition of plasma membrane in growth factor receptor-driven cancers by increasing saturated phosphatidylcholine content which is involved in oncogenic signal transduction [49]. The structures of hormones and growth factors, such as prostaglandins, leukotrienes, lysophosphatidic acid, or steroid hormones, are also composed of lipids [50]. Therefore, the levels of fatty acyls are associated with lipid hormone synthesis and affect tumor-promoting signaling processes [51].

1.2.1.3 Lipid Oxidation

It has been recognized that reprogramming of energy metabolism is one of the emerging hallmarks in cancer progress [52]. Although increased glucose uptake is reprogrammed in most cancer cells, the glucose in cancer cells tends to be used for anabolic processes, such as ribose production, protein glycosylation, and serine synthesis, rather than oxidized for ATP production [31]. Fatty acid is an extreme substitute energy source. Fatty acid oxidation is highly relevant to ATP production and NADPH homeostasis, which might be crucial for cancer cell growth and survival [31]. AMP kinase activity is essential for the cancer cells to respond to the stressful environments and activate a catabolic switch to increase ATP and NADPH reserves [31]. Increased dependence on lipid oxidation as main energy source in cancer cells has been observed. One such example is prostate cancer, which generally displays a low rate of glucose utilization [53], and overexpression of some β-oxidation enzymes [54], indicating that fatty acid oxidation might be a dominant bioenergetic pathway [38].

1.2.1.4 Signaling Lipids

Bioactive lipids, acting as second messengers and hormones, play important role in signaling regulation. For example, fatty acids, fatty acid and sterol derivatives, and eicosanoids modulate the gene expression through binding and activation of the nuclear hormone receptors, e.g., peroxisome proliferator-activated receptors. These transcription factors control genes that regulate lipid homeostasis that promotes the progression

of many diseases including cancer [55]. LysoGPL species are involved in cell proliferation, survival, and migration through the regulation of G-protein-coupled receptors [56]. Increased LysoPA level exhibits in malignant effusions, and its receptors are aberrantly expressed in several human cancers [57]. The intimate and causal relationship between metabolic alterations and cancers makes the metabolic alterations to be a kind of potential target for cancer treatment. For example, the use of inhibitory drugs directed against LysoPA receptors could be effective in suppressing tumor metastasis [57]. Hydrolysis products of PI and its phosphorylated derivatives such as PIP, PIP_2, and PIP_3 or themselves are second messengers and cellular regulators to activate the PI_3K/AKT signaling pathway, which is significant in chemotherapy and radiotherapy for human cancer [58].

In addition to the alternations aforementioned, cancer cells exhibit another dimension of complexity that contains a repertoire of recruited, ostensibly normal cells to create the "tumor microenvironment" of developing tumor [52]. Adipocytes are important producers of a variety of cytokines that contribute to inflammation and angiogenesis which are associated with cancer development [59]. Adipocytes have been recognized recently as important components of tumor microenvironment [59], indicating that the lipid metabolism is involved in cancer development.

1.2.2 Lipid Metabolism and Tumor Immunity

Cancer is characteristic in uncontrolled cell growth and proliferation which required fatty acids for biosynthesis of membrane structure and signaling molecules [60]. The metabolic pathways or networks disrupted in various cancer cells might be different from their genetic and tissue background [61], tissue origin and functional phenotype [62, 63], and tumor microenvironment [64]. Metabolite-mediated communication and metabolic changes between tumor cells and tumor-infiltrating immune cells have been observed [65]. Tumor-derived cytokines and the subsequent signal transduction induce the expression of lipid transport receptors, leading to increased lipid uptake. In the tumor microenvironment, the availability and use of fatty acids in T cells are affected by competition with tumor cells. The lipid accumulation in the tumor microenvironment increases the oxidative metabolism and activates the immunosuppressive mechanisms [66]. The lipid accumulation in dendritic cells could reduce their ability to process and present antigens thereby stimulating T cells. Reduction in lipid content due to the inhibition of fatty acid synthesis could restore the functions of dendritic cells [67], indicating that lipid accumulation in tumor microenvironment can affect the functions of immune cells.

Accumulation of cholesterol, sphingomyelin, and saturated phosphatidylcholine species has been found in immunoisolated T-cell receptor activation domains, suggesting that the lipid composition in immune cell membrane is involved in its function and signaling [68]. It has been reported that various fatty acids drive the differentiation and proliferation of T cells in the gut. The medium- and long-chain fatty acids support the differentiation of pro-inflammatory Th1 and Th17 cells, whereas the short-chain fatty acid, propionate, promotes the development of T_{reg} cells [69]. Moreover, a high rate of cholesterol esterification in the tumor could impair T-cell responses. Elevated cholesterol content in plasma membranes of $CD8^+$ T cells might increase their proliferation and improve their effector function [70]. The studies indicate that lipid metabolism in tumor and immune cells in the tumor microenvironment plays a crucial role on immunosuppression regulation [65].

Macrophages might be affected by abnormal lipid metabolism in cancer. Macrophages are important components of innate immunity that assist the host defense against infections but also maintain the tissue homeostasis [71]. One of the important characteristics of macrophages is their plasticity and ability to adopt various activation states in response to their microenvironment and fit their functional requirements. They are crucial partners for tumor cell migration, invasion, and metastasis [71]. Macrophages are elite produc-

ers of eicosanoids, which are hydrolyzed from phospholipid membranes by cytosolic phospholipase A2 and other related lipid mediators during inflammation [72]. The metabolic phenotype among macrophages is different in the energy metabolism. Fatty acid synthesis predominates in M1 macrophages (normal macrophage), which is characterized by aerobic glycolysis, fatty acid synthesis, and a truncated tricarboxylic acid cycle, leading to accumulation of succinate and citrate, contributing in a specific manner of their pro-inflammatory phenotype [71]. M2 macrophages are a phenotype of tumor-associated microphages. They are dependent on fatty acid oxidation to fuel their bioenergetic demands. The metabolic signature of M2 macrophages is characterized by fatty acid oxidation and an oxidative tricarboxylic acid cycle, functioning as anti-inflammatory component and mediator to tissue homeostasis [71, 73]. Thus, studies on macrophages lipidomes might reveal their metabolic reprogramming, which in turn sheds light on the therapeutic approaches to the diseases with a high macrophage involvement, such as cancer.

1.3 Introduction to Lipidomics in Cancer Research

1.3.1 Introduction to Lipidomics

There are hundreds of thousands of individual lipid molecular species in cells. Regarding the lipid distribution in normal mammalian cell, phospholipids (a phosphodiester linked to the $sn-3$ position of glycerides) are one of the predominant lipids in mammalian cells accounting for about 60 mol% of total lipids. Glycolipids and sphingolipids account for about 10 mol% of total lipids. The distribution of nonpolar lipids, such as TAG and cholesterol, are different from cell types and compartments, ranging from 0.1 to 40 mol%. Metabolites, such as NEFAs, lysolipids, diacylglycerol, ceramides, acylcarnitines, and acyl CoA, are usually less than 5 mol% of total lipids, but they can be accumulated and contribute to deleterious pathophysiologic sequelae during different pathologic conditions [74]. Each lipid aforementioned interacts in different compartments and within each bilayer compartment. Many lateral separational domains regulate specific interactions and cell functions (e.g., caveolae [75]). Moreover, the neighbor interactions (annular lipids) significantly regulate membrane dynamics, which in turn affects transmembrane protein kinetics and functions, leading to cellular physiologic function regulation and adaptation [74]. Accordingly, the lipid profiling of cells might be different under different pathologic conditions, closely related to cellular functions. Given the modern lipidomics approaches, a snapshot of the entire spectrum of lipids in a cellular/organismal lipidome could be obtained to explore the alterations of lipid redistribution, remodeling, and degradation under pathological conditions.

The definition of lipidome refers to "the entire collection of chemical distinct lipid species in a cell, an organ, or a biological system" [76]. According to the analogy to other "omics" disciplines, lipidomics is an emerging research field based on analytical chemistry, studying lipidomes in a large scale and at the levels of intact molecular species [5]. Lipidomic research focus on the structures and functions of the global lipids (profiles) in a certain cell or organism, and their interactions among cellular components, which includes (1) accurately identify the structures of cellular lipid classes and their species including the number of atoms, the number and location of double bonds, the core structures and head groups, individual fatty acyl chains, and the regional specificity of each isomer, etc.; (2) accurately quantify individual lipid species to explore the underlying mechanisms that are involved in cellular signaling/pathway and discover associated biomarker signatures; (3) reveal the interactions of individual molecular species among lipid classes, proteins, and metabolites; and (4) disclose the nutritional or therapeutic status related to disease prevention or intervention [5].

Lipidomics has been widely applied in many research fields and developed subdiscipline categories, e.g., molecular/structural lipidomics, functional lipidomics, nutritional lipidomics, dynamic lipidomics, oxidized lipidomics, mediator lipidomics, neurolipidomics, sphingolipidomics, fatty acidomics, etc., focusing on particular fields ([5] and therein references). The dynamic alterations in lipids during cellular physiological or pathological processes are also revealed by the analysis of lipid structures, cell functions, and their interactions in a spatial and temporal manner. The cellular lipidomes research has provided many new insights into disease conditions through the detailed quantitation of cell's lipidome (e.g., lipid classes, subclasses, and their molecular species) of diseases, study on the lipid metabolic kinetics and the interactions between lipids and cellular proteomes (e.g., Hazen et al. [77]; Han et al. [78–81]). In short, lipidomics exerts a critical role in revealing the underlying mechanisms of lipid-associated diseases by identifying alterations in cellular lipid signaling, metabolism, trafficking, and homeostasis.

1.3.2 Mass Spectrometry for Lipidomics

Unlike other biological molecules, lipids are not characterized by a certain individual structure. The unique chemical structure of most lipid molecular species consists of linear combinations of a small number of building blocks including backbones, head groups, and aliphatic chains [82, 83]. Lipidomics analysis presents a challenge due to the diversities in the structure and characteristics of lipids. Over the past years, a variety of conventional separation technologies for comprehensive analysis of lipids in complex samples has been applied such as thin-layer chromatography, gas chromatography, liquid chromatography (LC), supercritical fluid chromatography, and capillary electrophoresis. Recently, mass spectrometry (MS), nuclear magnetic resonance, and other spectroscopic approaches have been introduced and become powerful in lipid analysis due to their advantages. The two major platforms used for lipidomic analysis include the direct infusion approach (i.e., shotgun lipidomics) and chromatographic separation coupled to mass spectrometry.

Mass spectrometry is an analytical discipline that uses mass spectrometers to study the mass-to-charge (*m/z*) ratio of individual analytes for structural elucidation and quantitation including steps of molecular ions and related fragment generation, ion separation according to their *m/z*, signal detection, and the intensity of individual ion measurement [5]. A mass spectrometer usually consists of an ion source, a mass analyzer system, a detector, and a data processing system (Fig. 1.2).

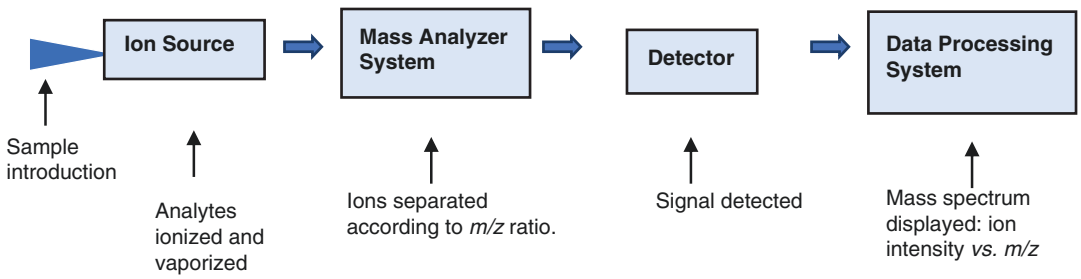

Fig. 1.2 Mass spectrometer composition. A mass spectrometer typically consists of an ion source, a mass analyzer system, a detector, and a data processing system

1.3.2.1 Ionization Source

An ion source is the part of a mass spectrometer where analytes are ionized. The resulting ions are then transmitted to the mass analyzer. Yang and Han [3] have summarized the ionization techniques that are usually used in modern mass spectrometry for lipidomics.

- *Electrospray ionization (ESI)*: a soft ionization technique used in mass spectrometry. A fine aerosol spray was generated through the desolation effect by applying a strong electric field on a capillary tube which is inside a stream of liquid flowing, and then the electrospray was further used in the mass analyzer.
- *Matrix-assisted laser desorption/ionization (MALDI)*: a soft ionization technique used in mass spectrometry. Specialized in analyzing large and/or labile molecules such as peptides, proteins, lipids, and polymers, etc., it also can perform MS imaging analysis of tissue or cell samples. By shining a pulsed laser onto the matrix embedded with analytes, the analyte molecules are ionized with the help of the matrix material by absorbing energy from the laser and form a hot plume of ablated gases and then utilized by the analyzer.
- *Atmospheric pressure chemical ionization*: a soft ionization technique that utilizes gas-phase ion-molecule reactions at atmospheric pressure. A corona discharge electrode was used to ionize the analyte, where either proton transfer/abstraction or adduct formation occurred between the relative proton affinities of the reactant gas ions (e.g., evaporated mobile phase or solvent in most cases) and the gaseous analyte molecules to generate the molecular ions.
- *Atmospheric pressure photoionization*: an alternative ionization technique when the analyte was not effectively ionized using ESI or atmosphere pressure chemical ionization method. In this method, a source of 10-eV photons is generated by a vacuum-ultraviolet lamp designed for photoionization detection in gas chromatography. The advantage of this technique is the high collision rate between the analyte and solvent molecules where the vapor of both is introduced into the photoionization region and the dopant photoions react to completion.
- *Secondary ion mass spectrometry*: the most sensitive surface analytical technique of analyzing the composition of thin films or solid surfaces. A primary ion beam (e.g., silver, or gold ion beam) is used to bombard the surface of the analyte to generate secondary ions ejected from the surface and later introduced to the mass analyzer.
- *Desorption ESI*: a combination of ESI and desorption ionization technique, an ambient ionization method. A charged electrospray mist is pneumatically directed to the sample surface where the analytes is desorbed, ionized, and subsequently carried by splashed droplets that travel in to the atmospheric pressure interface of the mass spectrometer.

The ESI and MALDI method are two of the most prominently used techniques in lipidomics among previously described ionization techniques. In lipid analysis, several remarkable advantages of ESI-MS are as follows: (1) Due to the charge propensity of certain lipid classes, the ion source could serve as a separation device to selectively ionize certain lipid or class of lipid of interest without prior LC separation; (2) high sensitivity and for low concentration of lipid detection fmol/μL (i.e., nM); (3) high quantitation quality and instrumentation response factors for individual molecular species in a polar lipid, when using low lipid concentration to avoid lipid aggregation and after 13C de-isotoping correction; (4) broad linear dynamic range between the ion peak intensity of a polar lipid species; and (5) an over 95% reproducibility for shotgun lipidomics which utilizes direct infusion for any lipid extraction with the presence of internal standards [5].

1.3.2.2 Mass Analyzer

From the ion source, the analyte is ionized and then transmitted to the mass analyzer which can separate ions by their *m/z*. Conventional mass analyzer like magnetic sector field analyzer is too large in size and has limited applications for lipi-

domics. Much more effective and easier to use analyzers such as quadrupole (Q), time of flight (TOF), and ion trap analyzers are often used for lipidomics study.

- *Quadrupole*: Quadrupole mass analyzers are relatively small and inexpensive. They utilize at least four parallel rods that could generate a radiofrequency quadrupole field to select ions by either stabilizing or destabilizing the ions that go through the path using oscillation electrical fields. They can only allow the ions within a certain range of m/z to pass through at a time. They can also allow a wide range of *m/z* to be swept quickly by swapping potentials on their rods. One of the most popular instruments in lipidomics is the triple quadrupole (QqQ) mass spectrometer. By setting three consecutive quadrupoles together, a QqQ-type is a powerful platform for both LC-MS and shotgun lipidomics. It offers high sensitivity, very good quantitation quality, and multiple scanning modes (e.g., selected/multiple reaction monitoring, neutral-loss scan, precursor-ion scan, and product-ion analysis) with broad linear dynamic range.
- *Time of flight (TOF)*: In a TOF analyzer, the ions were accelerated in the drift tube and fly toward the detector, and the time of their flight was recorded for the identification of different ions. TOF mass analyzers have been broadly used for lipid analysis in lipidomics ([5] and therein references).
- *Ion trap*: In a TOF analyzer, the ions were accelerated in the drift tube and fly toward the detector, and the time of their flight was recorded for the identification of different ions. TOF mass analyzers have been broadly used for lipid analysis in lipidomics ([5] and therein references). The three-dimensional ion trap also generates an electric field to select ions with its ring electrode and two end-cap electrodes and has the same principles as a quadrupole mass analyzer; only in ion traps the ions are trapped and sequentially ejected mainly by using an RF field. A linear quadrupole ion trap is similar to 3D ion trap, but it traps ions in a 2D quadrupole field. Ion trap is widely used in lipidomics ([5] and therein references).

1.3.2.3 Detector

The detector is the final component of a mass spectrometer. Detector records either the induced charge or the produced current when an ion passes by or hits a surface. To obtain more meaningful signal, some types of electron multipliers are usually used to amplify the signals.

1.3.2.4 Tandem Mass Spectrometry Techniques

Too much in-source fragmentation can trouble in mass analysis by introducing fragment ions of lipids, but soft ionization techniques (e.g., ESI and MALDI) yield minimal in-source fragmentation under appropriate experiment conditions. Even though in-source fragmentation can lead to complication of lipid analysis, if used properly, in-source fragmentation can also provide structural information. However, in-source fragmentation is rarely used for identification of lipid species in lipidomics compared to tandem mass spectrometry after collision-induced dissociation. There are four main MS/MS modes, including product ion analysis, neutral-loss scan, precursor ion scan, and selected/multiple reaction monitoring, that are particularly useful in lipidomics ([5] and therein references). Listed are the tandem mass spectrometric techniques.

- *Product ion scan*: In the first mass analyzer, a specific precursor is selected and then passed to be fragmented. The second mass analyzer is applied to record all the result ions of the selected precursor.
- *Neutral loss scan*: The first mass analyzer scans all the precursor ions, while the second mass analyzer scans the fragment ions set at an offset mass from the first mass analyzer. This neutral loss is related to the precursor structure; therefore, all the precursors that undergo the loss of the specified neutral fragment are monitored. The neutral loss scan mode is very useful in shotgun lipidomics to

detect a class or a group of lipids that structurally unique, for example, certain head group [66–69].

- *Precursor ion scan*: a tandem mass spectrometric technique, in which the first mass analyzer scans all the precursor ions while the second mass analyzer only monitors selected fragment ions. The selected fragment ions correspond to the common fragment ions of the precursor. Therefore, all precursors that produce specified fragment ion during fragmentation process are monitored. The precursor ion scan model has also been utilized to effectively detect a given class or a group of lipids that yield a given fragment ion after collision-induced dissociation [66–69].
- *Selected/multiple reaction monitoring*: This is a non-scanning technique for targeted analysis on QqQ-type instruments, and it uses two of the mass analyzers as static mass windows to perform the detection of certain fragmentation ions from selected precursor ion. From the precursor ion to the fragment ion generates a related *m/z* pair called "transition." Multiple reaction monitoring is used to indicate the parallel acquisition of multiple SRM transitions [3]. The selected/multiple reaction monitoring techniques have been widely used for quantitative analysis of individual lipid species in lipidomics when a mass spectrometer is coupled with LC [84–87].

1.3.3 Strategies of Mass Spectrometry-Based Shotgun Lipidomics

Shotgun lipidomics, originally described in 1994 [88], is a widely used and promising technology in the field of lipidomics research. Direct infusion is used to avoid the difficulties caused by concentration and ion-pairing changes and chromatographic anomalies to improve the resolution [89] and to achieve fast and accurate qualitative and quantitative analysis of lipid species. The mass spectrometry analysis of molecular ions of individual molecular species in targeted lipid and the alterations in fragmentation energies and reagent gases can be performed under the same infusion and constant concentration conditions. The characteristic allows shotgun lipidomics to achieve detailed tandem mass spectrometry with multiple fragmentation strategies including precursor ion scan, neutral loss scan, and a variety of other fragmentation techniques [89]. Currently, at least three platforms of shotgun lipidomics have been developed:

1.3.3.1 Tandem Mass Spectrometry-Based Shotgun Lipidomics

Neutral loss scan or precursor ion scan of a characteristic fragment of lipid class specifically detects individual species of this class. A unique building block of a certain kind of lipid class is usually involved. This method is very simple and efficient, yet the process and result of the technique is very accurate, easy to manage, and less expensive. Only in one MS/MS acquisition, all individual species in a particular class can be confirmed from a total lipid extract with any commercially available QqQ-type mass spectrometer. Neutral loss scan or precursor ion scan can also easily identify lipids that have a stable isotope which could give more insights to the kinetics of lipid turnover, biosynthesis, lipid trafficking and homeostasis, etc. [89].

1.3.3.2 High Mass Accuracy-Based Shotgun Lipidomics

This platform rapidly and efficiently acquires a product-ion spectrum of each protonated/deprotonated molecule ion with a Q-TOF or Q-Orbitrap mass spectrometer. The TOF or Orbitrap analyzer records numerous virtual precursor ion scan in parallel, and the high mass resolution and accuracy inherent in the instrument records the accurate mass of fragment ions (0.1 amu) to minimize any false-positive identifications [90]. Identification can be performed from bioinformatic reconstruction of the fragments from precursor ion scan or neutral loss scan. Quantitation can be achieved with a comparison of the sum of the intensities of extracted fragments of an ion to that of a preselected internal standard [89].

1.3.3.3 Multidimensional Mass Spectrometry-Based Shotgun Lipidomics (MDMS-SL)

MDMS-based shotgun lipidomics [83, 91, 92] is a well-known approach to analyze individual lipid species straightly in lipid extracts of biological samples, with which the collision-induced dissociation process depends on the chemical structure of each individual molecular species. The technology maximizes the utility of unique chemical methods inherent in discrete lipid classes to analyze lipids including the molecular species with low abundance. Different types of lipids have different structures and charge properties, which are used to selectively ionize under various experimental conditions to separate specific lipid classes from source. After collision-induced dissociation, the targeted lipid class has its unique fragment pattern, which can usually be predicted according to the covalent structures of these lipid classes. The informational fragment ions derived from the head group or the neutral loss of the head group are used to identify the target lipid class, while precursor ion scan or neutral loss scan of fatty acyl chains is used to identify the individual molecular species present in the lipid class. Additionally, diagnostic ions can be identified and quantified by comparing the peak intensity of a certain ion to that of the selected standard in the same mass spectrum after correction for isotopologue [93, 94]. For example, the presence of a primary amine in phosphoethanolamine-containing species is unique in the cellular lipidome and has been exploited to tag the phosphoethanolamine-containing lipid species with fluorenylmethoxylcarbonyl chloride [95]. The facile loss of fluorenylmethoxylcarbonyl from the tagged lipid species allows one to readily identify and quantify these species with unprecedented sensitivity at an amol/μL level [89, 95].

1.3.4 Techniques of LC-MS-Based Lipidomics

The typical lipidomic analyses for biological samples include sample preparation and lipid extraction, lipid separation, and data acquisition and processing.

1.3.4.1 The Sample Preparation and Lipid Extraction

Proper sample preparation, storage, and processing are required prior to analysis. The ideal methods for sample preparation should be fast, reproducible, and able to extract a wide range of lipids with different polarities and compatible with the instrumental technique [96]. In the field of lipidomics research, the commonly used sample-preparation methods with high lipid recovery for biological samples include liquid-liquid extraction, organic solvent precipitation, and solid-phase extraction [97]. Organic solvent precipitation methods are still commonly used for most lipidomics research. Their advantages and disadvantages are summarized in Table 1.3 [3]. Solid-phase extraction, a method of lipid separation, can be used for lipidomic analysis. It is highly recommended if specific lipid fractions interfere with LC-MS measurements or where in-depth characterizations of lipid classes are required in lieu of more comprehensive lipidomic profiling [96].

1.3.4.2 Lipid Separation by LC

LC could separate or concentrate different types of compounds according to their physical and chemical properties [89, 96], eliminate the interaction of many lipid species [89], and enables comprehensive analysis of complex samples with trace level species [3].

- *Reversed-phase LC*: This type of LC, which separates lipid based on their hydrophobicity, is the most widely used for the analysis of complex lipids, although the retention time varies with lipids. The short (50–150 mm; typically 100 mm) microbore columns (1–2.1 mm I.D.) with sub-2-μm or 2.6–2.8-μm (fused-core) particle size and C18 or C8-modified sorbents are usually used in lipidomics research [96].
- *Normal-phase LC*: This type of LC, which separates lipids based on their polarities, rep-

Table 1.3 Advantages and disadvantages of organic solvent precipitation methods for lipid extraction

Methods	Solvents	Advantages	Disadvantages
Modified Bligh and Dyer	Chloroform/methanol/H_2O (1:1:0.9, v:v:v)	Well-established; Widely used	Hazardous solvents used; difficult to avoid aqueous component contaminants
Modified Folch	Chloroform/methanol (2:1, v:v), 0.9% NaCl (0.2 volume)	Well-established; Widely used	Hazardous solvents used; difficult to avoid aqueous component contaminants
Methyl tert-butyl ether	Methyl tert-butyl ether (MTBE)/ methanol/water (5:1.5:1.45, v:v:v)	Feasible for high throughput and automation	Contains aqueous component contaminants
Butanol/ methanol	Butanol/methanol (3:1, v:v), heptane/ethyl acetate (3:1, v:v), 1% acetic acid	Less water-soluble contaminants	The butanol component in the organic phase is difficult to evaporate

resents a separation mechanism complementary to reversed-phase LC. Highly nonpolar solvents with low ionization capacity are used during normal-phase LC. Normal-phase LC is good for the separation of phospholipids, particularly PA. The analysis time in normal-phase LC is typically longer (30–60 min), which can separate on long columns with large particle-size sorbents. Normal-phase LC can completely separate lipid classes [96].

- *Hydrophilic interaction chromatography*: This type of LC, which compromises the physical properties of both reversed-phase and normal-phase columns to a certain degree, provides better reproducibility and robustness and is more compatible with MS [98].
- *Supercritical fluid chromatography*: Using columns packed with sub-2-μm particles, this type of setting is a newly emerging technique that can be used for fast lipid profiling (<20 min) [96].
- *Two-dimensional liquid chromatography*: This type of setting, combining two different types of columns described above, separates lipids according to two independent molecular properties. It can optimize the separation conditions in both dimensions, which enables the characterization of further complex lipidomes [99]. However, it is more laborious and more time-consuming [96].

1.3.4.3 Electrospray Ionization Using Mass Spectrometry (MS) and Data Processing

The LC-MS techniques include selected ion extraction, spectra acquisition using selected or multiple reaction monitoring or full mass spectra acquisition, and data-dependent analysis [89]. The ion extraction approach is usually used for global lipid analysis, in which mass spectra are continuously acquired during the elution of the chromatographic column and then targeted ions are extracted from the acquired data array after chromatographic separation [89]. The ionization modes used in LC-MS based lipidomics differ among lipid classes. ESI positive mode is the most common mode in LC-MS, because it can effectively ionize a variety of lipids [96], while negative mode is usually used for specific lipid classes, including PI, PS, and PA [100].

The monitoring of selected or multiple reaction would be specific, if the monitored fragment ions are precursor-specific to the combination of LC separation and on interfering transition [89]. The data analysis elucidates the structures of detected lipid ions [89]. The data processing usually includes a couple of steps: (1) filtering which processes the raw signal to remove the noise and/or baselines; (2) feature detection which is used to identify the true ion signals and avoid detecting false-positive signals, and (3) normalization.

Data normalization eliminates the confounding factors that cause systematic bias in ion intensities during measurements and/or sample-preparation procedures. Finally, the three-dimensional data will be merged into a two-dimensional data matrix [96].

1.3.5 Lipidomics in Cancer Research

Lipidomics is a rapidly developing field of study that focuses on the identification and quantitation of various lipid species in the lipidome. Additionally, as the boundary of cells and organelles, lipids directly expose to the biochemical changes of intra- and extracellular environments [101]. Therefore, alterations in the lipidome might reflect the biochemical changes of the cellular system in diseases such as cancer. Analysis of lipids for biological samples will help in the understanding of lipid-driven mechanisms and identifying lipid-based biomarkers in cancer [101].

Cancer initiation and progression is associated with specific alterations of cellular metabolism that are not only by-products of the disease but also drive the disease [102]. Warburg first reported the "Warburg effect," which is that cancer cells prefer to produce ATP by glycolysis, a less efficient pathway than oxidative phosphorylation [103]. Adaptive alterations, such as increased rates of glycolysis, amino acid, and lipid turnover, are known to be important features of the neoplastic process that promotes unlimited cell proliferation and tumor expansion [104]. Accordingly, altered metabolism of lipid is related to cellular metabolism in cancer and could be considered a hallmark characteristic of many malignancies [105]. Disturbances in lipid metabolism, such as changes in metabolite patterns, have been reported in ovarian cancer versus benign or healthy controls [106, 107] and in different cancer status, e.g., phospholipid levels reduce in plasma with stage III/IV disease relative to benign controls but increased in early-stage cancers [108].

Due to its rapid, accurate, and high throughput in data acquisition, lipidomics is expected to be a potential approach to identify the biochemical homeostasis and abnormalities caused by interference in early biochemical processes, such as early stage of cancer or cancer initiation [101]. For example, palmitoyl sphingomyelin in urine provides the strongest predictive power to differentiate bladder cancer from non-cancer samples. Urine arachidonate level is higher in bladder cancer versus non-cancer controls [109]. The metabolites involved in fatty acid β-oxidation, such as carnitine and acylcarnitines (carnitine, isovalerylcarnitine, glutarylcarnitine, octenoylcarnitine, and decanoylcarnitine) in urine, can clearly distinguish bladder cancer patients from control groups [110]. A prospective study on pre-diagnostic metabolic alteration and cancer risk observed that higher levels of LysoPCs, particular LysoPC (C18:0), are related to lower risks of breast, prostate, and colorectal cancer, but higher levels of PC (C30:0) are associated with increased cancer risk [111], indicating that lipidomics might precede the diagnosis of common malignancies by a couple of years [111].

Lipidomics could reflect the cancer progression. Eicosanoids and sphingolipids have been linked to inflammation associated with cancer development and progression, tumor growth, and maintenance for the cancers of colon, breast, and lung [112–114]. Many aggressive cancer cells exhibit decreased PC content and increased LysoPC content, which links with phospholipase A_2 activation, an initial rate-limiting enzyme in eicosanoid biosynthesis [115]. Overexpression of phospholipase A_2 is also associated with the malignant potential in human breast cancer [116]. It has been observed that carnitine and acetylcarnitine in serum decreased in remission and increased in relapse in multiple

myeloma patients, indicating that mitochondrial β-oxidation is altered at different cancer stages [117]. A longitudinal lipidomics approach could monitor the cancer progression, although the longitudinal lipidomics could be affected by various confounding factors such as treatment. On the other hand, the longitudinal lipidomics might provide a window into designing targeted therapies and monitoring.

1.4 Application of MS-Based Lipidomics in Cancer Research

Lipids are involved in all of the basic processes essential for tumor development [2]. One of the roles of lipidomics is to identify the molecular mechanism(s) responsible for the altered lipid levels induced by a stimulus [5]. For example, using lipidomics analysis, it has been found that epithelial cell cyclooxygenase-2-dependent eicosanoids might mediate tumor development [118]. Application of lipidomics, particularly the MS-based shotgun lipidomics strategies, in cancer research has been reportedly widespread in past decades from cancer cellular remodeling, metabolism, and function alterations to attempts in biomarkers for cancer diagnosis and progression.

1.4.1 Lipid Profiling and Cellular Remodeling in Cancer

Lipids, particular phospholipids, constitute vital components of cells. Therefore, changed cell number in tumor proliferation might lead to alterations in lipid profiles. Dynamic remodeling in tumor was also reported in a study using global LC-MS-based lipidomics [119]. This study on prostate cancer included 76 prostatic cancer patients and 19 benign prostatic hyperplasia patients. Global lipidomics profiling was applied for qualitative and quantitative characterization of lipidome in prostatic tissue samples [119]. The investigators analyzed 350 lipid species including fatty acyls, sterol lipids, sphingolipids, glycerolipids, glycerophospholipids, etc. About 140 distinct alterations of lipid species in prostatic cancer patients were found. PC, PE, PG, PI, Cer, diglyceride, cholesteryl ester, and NEFA significantly increased in prostatic cancer tumor by 1.65–15.87-fold. Relative composition of lipidome in prostatic cancer was also found in the study. Diacyl-PC and diacyl-PE percentages increased in prostatic cancer, whereas ether-linked PCs (alkyl/acyl-PCs, PC with alkyl substituent) and PEs [alkenyl/acyl-PEs, ethanolamine plasmalogens (pPE)] decreased; percentages of free mono- and polyunsaturated fatty acids elevated, while the percentage of free saturated fatty acids reduced. In NEFA species composition, SFA% was significantly attenuated by 20%, whereas the percentages of mono- and polyunsaturated fatty acids were enhanced by 40–50%. In the categories of phospholipids (PC, PE, PI, PS, and PG), monounsaturated fatty acids -acyl residues increased by 10–40%, while polyunsaturated fatty acids -acyl and ether-linked chains were reduced by 10–20% and 20–40%, respectively. These results of lipidomic analysis indicated that dynamic remodeling exists in prostatic cancer tumor.

Another example is the study on the relationship among oxidative stress, aberrant lipid metabolism, and pro-inflammatory cytokines in systemic lupus erythematosus (SLE) using MDMS-SL [120]. SLE is a chronic inflammatory autoimmune disease characterized by dysfunction of immunocytes and genetic and/or environmental factors. Oxidative stress associated with cardiovascular disease [121] is a major causal factor for the morbidity and mortality in SLE [122]. Increase in very-low-density lipoprotein cholesterol and TAG and decrease in high-density lipoprotein cholesterol are dyslipoproteinemia characteristics in SLE [123]. Using MDMS-SL, Hu et al. [120] analyzed the serum lipid species and their metabolites in SLE female patients aged 20–55 years. They found that the levels of pPE species in SLE patients were significantly lower than those of controls by 27%. The most reduced serum pPE species in SLE patients were 16:0–20:4 (by ~40 mol% reduction) and 18:0–20:4 (by ~38 mol% reduction). The LysoPE (~46 mol%)

level in SLE patients increased significantly. Plasmenylcholine (pPC) (18:0–18:0, 16:0–18:2), usually low abundance in human plasma, was detected in SLE patients using MDMS-SL. It was found in the study that serum pPC levels decreased in SLE by ~21 mol%. The results suggested that lipid peroxidation might exist in SLE patients. Since the majority of PE species possess polyunsaturated fatty acid chains at *sn − 2* position, increase in LysoPE species level strongly suggests a molecular mechanism that leads to breakdown of *sn* − 1 aliphatic chains in PE species, which leads to reduction of pPE [120].

The lipid profile alterations contributing to cellular signal transduction were reported by the same research team [124]. Although no alternations were found in serum phosphatidylethanolamine (dPE), PI, SM, and Cer levels, the profiles of their species in serum were significantly different between SLE patients and healthy controls. The serum dPE species of 16:0–18:2, 18:0–18:2, 16:0–22:6, and 18:0–22:6 increased in SLE by ~34%, 18%, 54%, and 39%, respectively. PI (18:0–18:2) increased by ~16%. However, the dPE and PI species containing 20:4 fatty acyl chain at the *sn-2* position appeared to reduce to a certain degree, e.g., dPE (18:0/20:4) reduced by~26%, dPE (20:0/20:4) reduced by~66%, and PI (18:1/20:4) reduced significantly. Cer (N22:0 and N23:0) reduced by ~10%, whereas Cer (N24:1) increased by 26% [124]. In general, PUFA (e.g., arachidonic acid) incorporations are one the most common alterations in glycerophospholipid remodeling. The PUFA species in PE, such as arachidonic acid, linoleic acid, eicosapentaenoic acid, and docosahexaenoic acid, are major sources of lipid mediators and endocannabinoids [114]. The acyl moieties of PE species are remodeled by the action of phospholipases and lysophospholipid acyltransferases, which contributes to PE diversity and lipid mediator production. The dPE species containing arachidonic acid remodeling was observed in SLE. The arachidonic acid-derived eicosanoids, such as prostaglandins and leukotrienes, are generated to involve in inflammation, allergy, and immune responses in SLE. For example, a series of these reactions resulted in dPE (18:0–20:4) reduction. Elevation of dPE (16:0–18:2, 18:0–18:2, 16:0–22:6, and 18:0–22:6) may compensate for the reduction in dPE (18:0–20:4) [124]. Meanwhile, the glycerophospholipids containing docosahexaenoic acid were synthesized in the remodeling pathway. They increase membrane fluidity and regulate the biophysical properties of the membrane to maintain the cellular functions and also act as the reservoir for producing anti-inflammatory mediators such as resolvins and protectins [125–127]. The cellular lipid remodeling is consistent with and might contribute to the inflammatory and oxidative stress characteristics observed in SLE patients.

It is noteworthy that the dynamic remodeling might associate with the regulation of metabolism and biomolecular functions to a certain degree and in turn be involved in pathophysiological process in cancer. The MDMS-SL technology reveals the changes of lipid profiles, e.g., oxidative stress-related lipids, such as plasmalogens, lysophospholipids, and 4-hydroxynonenal species, and then discloses the underlying mechanism(s) of the alterations through the analysis on lipid species.

1.4.2 Alterations of Lipids Involved in the Metabolic Pathway in Cancer

The rapid cell proliferation and growth, one of the characteristics of cancer cells, can affect the demand and metabolism in all lipid classes. The different lipid classes play different roles. First, lipids act as components of cellular construction. For instance, lipid biosynthesis and remodeling request NEFAs as basic building blocks. Cellular and plasma membrane are composed largely by cholesterol, glycerophospholipids, and sphingolipids. TAG, along with acyl-CoA and acylcarnitine, contributes to energy storage, energy metabolism, and ATP generation [5]. Second, lipids act as second messengers and/or hormone involving cell signaling. Lysophospholipids and oxidized lipids are involved in cancer cell proliferation, survival, and migration [56]. The hydrolysis products of phosphatidylinositol and

its phosphorylated derivatives activate PI_3K/AKT signaling pathway [58], contributing to chemotherapy and radiotherapy for human cancers [58].

Hu et al. [120] investigated the relationship among oxidative stress, abnormal lipid metabolism, and pro-inflammatory cytokines in SLE to explore the underlying mechanisms, pathogenesis, and development in SLE. In addition to the presence of lipid peroxidation suggested by the alterations in pPE, LysoPE, they found that the levels of 4-hydroxyalkenals, a sensitive indicator of lipid peroxidation to evaluate oxidative stress, and 4-hydroxynonenal, an indicator of oxidative stress, increased significantly in SLE patients. Given that 4-hydroxy-2(E)-nonenal and 4-hydroxy-2(E)-hexenal are the products of lipid peroxidation of $n-6$ and $n-3$ polyunsaturated fatty acids [128, 129], Hu's study indicates a high oxidative stress existing in SLE patients. The pro-inflammatory cytokines including IL-6, IL-10, and TNF-α in serum were found increased in SLE patients. A positive correlation was found among IL-10 and some clinical indicators such as the systemic inflammation marker, autoantibody titers, and complement component C3 [130]. It is interesting that serum IL-10 was significantly correlated among pPE species in SLE patients. In this study, the pPE species could predict 95.9% of the variability of IL-10 level. Lipid species including pPE (16:0–20:4, 16:1–20:4, 18:1–20:4, 18:0–20:4, 18:0–22:5, and 18:0–22:4), LysoPE (20:4), and 4-hydroxynonenal also significantly predicted IL-10 level by 60% in SLE patients. Hu's study suggested that peroxisomal dysfunction, phospholipase A_2 activation, and peroxidation-mediated degradation might result in pPE species reduction in SLE, indicating that accumulation of *sn* − 2 acyl-containing LysoPE and 4-hydroxynonenal supported an increased oxidative stress at SLE. The subsequent lipid peroxidation was the underlying mechanism that leads to the pPE reduction in SLE, because plasmalogens function as endogenous antioxidant reagents. The oxidation products, reactive aldehydes, can spread oxidative stress to other intracellular organelles through the bloodstream. With shotgun lipidomics approach, the findings in lipid redistribution and remodeling might reveal the SLE pathogenesis including the following: (1) The elevated 4-hydroxyalkenals suggest the presence of oxidative stress and severe oxidized injury in SLE; (2) as one of the endogenous antioxidants, the pPE reduction in SLE serum suggests that blood cells and apolipoprotein are severely damage in SLE; (3) hydroxyalkenals are stable and can escape from the cells, thereby attacking nucleophilic targets far away from the original event site through the bloodstream [129]; (4) lysolipids generated from plasmalogen peroxidation are very toxic and could lead to cell death and cellular dysfunction [131–133]. The alterations in pPE species and the peroxidation products may serve as novel biomarkers for diagnosis, progression, and effectiveness of therapy in SLE [120].

As aforementioned, the upregulation of fatty acid biosynthetic pathway and reprogrammed composition in membrane phospholipids has been observed in prostatic tumors [119]. For example, the key genes in de novo lipogenesis and genes encoding fatty acid transporter protein, plasma membrane fatty acid-binding protein, scavenger receptor class B type I and phospholipase A_2 were found elevated in tumor tissues, consistent with the aforementioned result of overall lipid abundance upregulated and polyunsaturated fatty acid accumulation [119].

1.4.3 Biomarker Identification for Cancer

MS-based lipidomics approach enables reliable and accurate characterization of lipid structure and quantitation in given biological samples. The alterations of lipid metabolism in cancer have been identified including down- or upregulation of lipid abundance, lipid redistribution, phospholipid composition and remodeling, etc. In order to explore the potential biological characteristics of pathogenesis of cancer, the correlation between lipid alterations revealed by MS-based lipidomics and the progression of cancer has been investigated [119, 120, 124, 134], e.g., focused on identification of novel molecular biomarker(s) which is extremely important for early diagnosis, prognosis, and treatment of cancer.

With the information of global lipidomics profiling and quantitative characterization of lipidome in prostatic tissue samples [119], analysis of the relationship between tumor lipidome and cancer diagnosis and progression revealed that cholesteryl ester species (20:1, 24:5, 24:4, 18:1, and 22:6), Cer species (d18:2–20:0 and d18:1–20:1), NEFA species (22:3), and TAG species (58:1) showed a high discriminant power in distinguishing prostatic cancer tumor from non-tumor [119]. Cholesteryl ester exhibited greatest expression increase by 10.5–45.5-fold in prostatic cancer tumor [119], indicating that the accumulation of cholesteryl oleates could be a potential biomarker for prostatic cancer diagnosis and progression.

The multivariate and multiple regression analysis on the difference of the serum lipid profile between SLE patients and healthy volunteers indicated that the lipid species pattern including pPE (18:0–20:4, 16:0–20:4, 18:0–22:4, 18:0–22:5, and 18:1–22:4), 4-hydroxynonenal, and LysoPE (18:0) could be a potential biomarker for SLE [120].

Lipid species profiling could be used as plasma biomarkers for diagnosis of breast cancer [135, 136]. Using a normal-phase/reversed-phase two-dimensional LC-mass spectrometry method, Yang et al. conducted comprehensive lipid profiling that included 512 lipid species in human plasma from 6 benign breast tumor patients, 5 breast cancer patients, and 9 healthy controls. It was found that lipid species of PI (16:0–16:1) and PI (18:0–20:4) in plasma could be utilized as potential breast cancer biomarkers, whereas PI (16:0–18:1), PG (36:3), and glucosylceramide (d18:1–15:1) in plasma were demonstrated to be potential biomarkers to evaluate the level of malignancy of breast tumor [136].

Using triple quadrupole liquid chromatography electrospray ionization tandem mass spectrometry, Chen et al. [135] analyzed plasma lipid profiling that included 367 lipid species from 13 classes of phospholipids as well as cholesterol esters in 84 patients with early-stage breast cancer (stage 0–II) and 110 patients with benign breast disease. A lipid species pattern of combination of 15 lipid species was reported having diagnostic value according to the predictive model including LysoPC (18:3, 20:2, 20:1, and 20:0), cholesterol esters (C19:1, C19:1, and C20:0), PC (32:1, 34:4, 38:3, 40:5, 40:3, and 44:11), and ether-containing PC (32:2, 38:3). The sensitivity, specificity, positive predictive value, and negative predictive value of the combination of these 15 lipid species were 83.3%, 92.7%, 89.7%, and 87.9%, respectively, in training set, and were 81.0%, 94.5%, 91.9%, and 86.7%, respectively, in validation set. The area under receiver operating characteristic curve was 0.926 in training set and 0.938 in the validation set [135]. The results suggested that the pattern of lipid profile is a potential biomarker for diagnosis of breast cancer.

Using ultra-performance LC-electrospray ionization-QTOF-MS, combined with multivariate data analysis, Zhang et al. [137] studied plasma lipid profile in 27 ovarian cancer and 27 benign gynecological tumor patients and 11 healthy women. Higher LysoPC, lower PC, and TAG with specific fatty acid chains in plasma were found in ovarian cancer patients. The lipid species pattern of combination of LysoPC (14:0, 16:0, 18:1, 20:3, 20:4, and 22:6), PC (16:0–18:1, 16:0–18:2, 16:0–20:3, 16:0–20:4, 18:0–18:1, 18:0–18:2, 18:0–20:5, and 18:2–18:2), and TAG (18:2–18:2–16:0) was identified to be potential biomarkers for distinguishing ovarian cancer from healthy population [137].

Using LC-MS, Buas et al. [108] performed global (untargeted) and targeted metabolite profiling of plasmas isolated from 50 serous ovarian carcinoma and 50 benign controls. Global lipidomics analysis identified 34 metabolites (of 372 assessed) differing significantly between cases and controls in both training and testing sets of analysis. Compared to the controls, 17 glycerolipids and glycerophospholipids decreased in abundance in ovarian carcinoma plasmas including PS (O-18:0–0:0, O-18:0–16:1, O-20:0–0:0), cholesteryl ester (18:3), TAG (16:0–16:1–16:1, 16:1–16:1–16:1, 17:2–17:2–20:5, 16:1–17:2–17:2, 16:1–17:1–17:2, 16:0–16:0–16:1, 16:1–17:0–17:2), PG (P-20:0–12:0), PE (18:1–20:3), PE (18:1–20:3), etc. Four of the most abundant lipids were selected and conducted multivariate modeling analysis, and then compared to CA125,

one of the tumor markers. The estimated specificity at 95% sensitivity was ~35% in four lipid metabolites, 87% in CA125 alone, and 91% in combination of four lipid metabolites with CA125. The authors also assessed the classification accuracy of a multi-marker model between CA125 alone and the combination of four lipid metabolites with CA125 (hybrid model). A significant higher level of estimated specificity was found in hybrid model (~43%) versus CA125 alone (~10%). This study provides insight into lipid metabolic alterations that are potentially associated with ovarian tumor. Lipid metabolites in plasma might be used to differentiate cancer from benign and/or healthy controls.

Low-invasive or noninvasive samples, such as plasma, serum, and urine, are usually applied in the above biomarker-related studies. Body fluids such as whole blood or blood cells, plasma, and serum are worthy exploring to be a source of biomarker(s) for early diagnosis and progression of cancers, as their lipid profiles reflect the general condition of the whole organism [5]. These samples are also considered as one of the choices for cancer monitoring as they offer minimal patient discomfort and can justifiably be taken from healthy population as a control.

1.5 Conclusion

Lipids are the crucial component of cellular membrane, which constitutes an impermeable barrier of cellular compartments, and play important roles in numerous cellular processes including cell growth, proliferation, differentiation, and signaling. Cancer cells undergo profound alterations in lipid homeostasis from remodeling, reprogramming, and metabolism to signaling. This offers new diagnostic and therapeutic strategies that could be explored by high-throughput lipidomics approach. MDMS-SL improves resolution and achieves rapid and accurate qualitative and quantitative analysis. Direct infusion is used to avoid difficulties from alterations in concentration, chromatographic anomalies, and ion-pairing alterations in MDMS-SL. Due to the diversities in the structures and characteristics of lipid classes and their species, it is promising that MDMS-SL has been widely exploited in cancer research from metabolism(s) exploration to biomarker(s) or biomarker signature(s) recognition and identification.

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References

1. Wang M, Han X. Multidimensional mass spectrometry-based shotgun lipidomics. Methods Mol Biol. 2014;1198:203–20.
2. Santos CR, Schulze A. Lipid metabolism in cancer. FEBS J. 2012;279(15):2610–23.
3. Yang K, Han X. Lipidomics: techniques, applications, and outcomes related to biomedical sciences. Trends Biochem Sci. 2016;41(11):954–69.
4. Fahy E, et al. A comprehensive classification system for lipids. J Lipid Res. 2005;46(5):839–61.
5. Han X. Lipidomics: comprehensive mass spectrometry of lipids. Hoboken, NJ: Wiley; 2016.
6. Bach D, Wachtel E. Phospholipid/cholesterol model membranes: formation of cholesterol crystallites. Biochim Biophys Acta. 2003;1610(2):187–97.
7. Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu Rev Biochem. 1994;63:451–86.
8. Kuzuyama T, Seto H. Diversity of the biosynthesis of the isoprene units. Nat Prod Rep. 2003;20(2):171–83.
9. Drosatos K, Schulze PC. Cardiac lipotoxicity: molecular pathways and therapeutic implications. Curr Heart Fail Rep. 2013;10(2):109–21.
10. Lelliott C, Vidal-Puig AJ. Lipotoxicity, an imbalance between lipogenesis de novo and fatty acid oxidation. Int J Obes Relat Metab Disord. 2004;28(Suppl 4):S22–8.
11. Shevchenko A, Simons K. Lipidomics: coming to grips with lipid diversity. Nat Rev Mol Cell Biol. 2010;11(8):593–8.
12. Unger RH, Orci L. Lipotoxic diseases of nonadipose tissues in obesity. Int J Obes Relat Metab Disord. 2000;24(Suppl 4):S28–32.
13. Christie WW, Han X. Lipid analysis: isolation, separation, identification and lipidomic analysis. Bridgewater: Oily Press; 2010. p. 448.
14. Haimovitz-Friedman A, Kolesnick RN, Fuks Z. Ceramide signaling in apoptosis. Br Med Bull. 1997;53(3):539–53.
15. Obeid LM, et al. Programmed cell death induced by ceramide. Science. 1993;259(5102):1769–71.

16. Venable ME, et al. Role of ceramide in cellular senescence. J Biol Chem. 1995;270(51):30701–8.
17. Pettus BJ, et al. Ceramide 1-phosphate is a direct activator of cytosolic phospholipase A2. J Biol Chem. 2004;279(12):11320–6.
18. Gomez-Munoz A, et al. Ceramide-1-phosphate promotes cell survival through activation of the phosphatidylinositol 3-kinase/protein kinase B pathway. FEBS Lett. 2005;579(17):3744–50.
19. Gomez-Munoz A, et al. Ceramide-1-phosphate blocks apoptosis through inhibition of acid sphingomyelinase in macrophages. J Lipid Res. 2004;45(1):99–105.
20. Hinkovska-Galcheva V, et al. Ceramide 1-phosphate, a mediator of phagocytosis. J Biol Chem. 2005;280(28):26612–21.
21. Smith ER, et al. Effects of sphingosine and other sphingolipids on protein kinase C. Methods Enzymol. 2000;312:361–73.
22. Spiegel S, Milstien S. Sphingosine-1-phosphate: an enigmatic signalling lipid. Nat Rev Mol Cell Biol. 2003;4(5):397–407.
23. Bektas M, Spiegel S. Glycosphingolipids and cell death. Glycoconj J. 2004;20(1):39–47.
24. Irvine RF. Inositol lipids in cell signalling. Curr Opin Cell Biol. 1992;4(2):212–9.
25. Nishizuka Y. Protein kinase C and lipid signaling for sustained cellular responses. FASEB J. 1995;9(7):484–96.
26. Prokazova NV, et al. Lipid second messengers and cell signaling in vascular wall. Biochemistry (Mosc). 2007;72(8):797–808.
27. D'Arrigo P, Servi S. Synthesis of lysophospholipids. Molecules. 2010;15(3):1354–77.
28. Hung ND, Kim MR, Sok DE. 2-polyunsaturated acyl lysophosphatidylethanolamine attenuates inflammatory response in zymosan A-induced peritonitis in mice. Lipids. 2011;46(10):893–906.
29. Hung ND, Sok DE, Kim MR. Prevention of 1-palmitoyl lysophosphatidylcholine-induced inflammation by polyunsaturated acyl lysophosphatidylcholine. Inflamm Res. 2012;61(5):473–83.
30. Sevastou I, et al. Lysoglycerophospholipids in chronic inflammatory disorders: the PLA(2)/LPC and ATX/LPA axes. Biochim Biophys Acta. 2013;1831(1):42–60.
31. Carracedo A, Cantley LC, Pandolfi PP. Cancer metabolism: fatty acid oxidation in the limelight. Nat Rev Cancer. 2013;13(4):227–32.
32. Esslimani-Sahla M, et al. Increased expression of fatty acid synthase and progesterone receptor in early steps of human mammary carcinogenesis. Int J Cancer. 2007;120(2):224–9.
33. Piyathilake CJ, et al. The expression of fatty acid synthase (FASE) is an early event in the development and progression of squamous cell carcinoma of the lung. Hum Pathol. 2000;31(9):1068–73.
34. Swinnen JV, et al. Overexpression of fatty acid synthase is an early and common event in the development of prostate cancer. Int J Cancer. 2002;98(1):19–22.
35. Kapur P, et al. Fatty acid synthase expression in cutaneous melanocytic neoplasms. Mod Pathol. 2005;18(8):1107–12.
36. Kuhajda FP. Fatty-acid synthase and human cancer: new perspectives on its role in tumor biology. Nutrition. 2000;16(3):202–8.
37. Innocenzi D, et al. Fatty acid synthase expression in melanoma. J Cutan Pathol. 2003;30(1):23–8.
38. Liu Y. Fatty acid oxidation is a dominant bioenergetic pathway in prostate cancer. Prostate Cancer Prostatic Dis. 2006;9(3):230–4.
39. Vazquez-Martin A, et al. Overexpression of fatty acid synthase gene activates HER1/HER2 tyrosine kinase receptors in human breast epithelial cells. Cell Prolif. 2008;41(1):59–85.
40. Lingwood D, Simons K. Lipid rafts as a membrane-organizing principle. Science. 2010;327(5961):46–50.
41. Hager MH, Solomon KR, Freeman MR. The role of cholesterol in prostate cancer. Curr Opin Clin Nutr Metab Care. 2006;9(4):379–85.
42. Eberle D, et al. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie. 2004;86(11):839–48.
43. Horton JD. Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis. Biochem Soc Trans. 2002;30(Pt 6):1091–5.
44. Cully M, et al. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat Rev Cancer. 2006;6(3):184–92.
45. Lasuncion MA, et al. Post-lanosterol biosynthesis of cholesterol and cancer. Curr Opin Pharmacol. 2012;12(6):717–23.
46. Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol. 2000;1(1):31–9.
47. Talebi A, Dehairs J, Swinnen JV. De novo lipogenesis and membrane remodeling in cancer. Biomed Res India. 2012;23:49–53.
48. Swinnen JV, Dehairs J, Talebi A. Membrane lipid remodeling takes center stage in growth factor receptor-driven cancer development. Cell Metab. 2019;30(3):407–8.
49. Bi J, et al. Oncogene amplification in growth factor signaling pathways renders cancers dependent on membrane lipid remodeling. Cell Metab. 2019;30(3):525–38. e8
50. Gupta GP, et al. Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. Nature. 2007;446(7137):765–70.
51. Nomura DK, et al. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell. 2010;140(1):49–61.
52. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
53. Effert PJ, et al. Metabolic imaging of untreated prostate cancer by positron emission tomography with 18fluorine-labeled deoxyglucose. J Urol. 1996;155(3):994–8.
54. Zha S, et al. Peroxisomal branched chain fatty acid beta-oxidation pathway is upregulated in prostate cancer. Prostate. 2005;63(4):316–23.

55. Desvergne B, Michalik L, Wahli W. Transcriptional regulation of metabolism. Physiol Rev. 2006;86(2):465–514.
56. Murph M, et al. Liquid chromatography mass spectrometry for quantifying plasma lysophospholipids: potential biomarkers for cancer diagnosis. Methods Enzymol. 2007;433:1–25.
57. Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. Nat Rev Cancer. 2003;3(8):582–91.
58. Fresno Vara JA, et al. PI3K/Akt signalling pathway and cancer. Cancer Treat Rev. 2004;30(2):193–204.
59. Albini A, Sporn MB. The tumour microenvironment as a target for chemoprevention. Nat Rev Cancer. 2007;7(2):139–47.
60. Currie E, et al. Cellular fatty acid metabolism and cancer. Cell Metab. 2013;18(2):153–61.
61. Bi J, et al. Targeting cancer's metabolic co-dependencies: a landscape shaped by genotype and tissue context. Biochim Biophys Acta Rev Cancer. 2018;1870(1):76–87.
62. Lunt SY, Fendt SM. Metabolism—a cornerstone of cancer initiation, progression, immune evasion and treatment response. Curr Opin Syst Biol. 2018;8:67–72.
63. Elia I, et al. Organ-specific cancer metabolism and its potential for therapy. Handb Exp Pharmacol. 2016;233:321–53.
64. Muir A, Danai LV, Vander Heiden MG. Microenvironmental regulation of cancer cell metabolism: implications for experimental design and translational studies. Dis Model Mech. 2018;11(8):dmm035758.
65. Li X, et al. Navigating metabolic pathways to enhance antitumour immunity and immunotherapy. Nat Rev Clin Oncol. 2019;16(7):425–41.
66. Al-Khami AA, et al. Exogenous lipid uptake induces metabolic and functional reprogramming of tumor-associated myeloid-derived suppressor cells. Onco Targets Ther. 2017;6(10):e1344804.
67. Herber DL, et al. Lipid accumulation and dendritic cell dysfunction in cancer. Nat Med. 2010;16(8):880–6.
68. Zech T, et al. Accumulation of raft lipids in T-cell plasma membrane domains engaged in TCR signalling. EMBO J. 2009;28(5):466–76.
69. Haghikia A, et al. Dietary fatty acids directly impact central nervous system autoimmunity via the small intestine. Immunity. 2015;43(4):817–29.
70. Yang W, et al. Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. Nature. 2016;531(7596):651–5.
71. Geeraerts X, et al. Macrophage metabolism as therapeutic target for cancer, atherosclerosis, and obesity. Front Immunol. 2017;8:289.
72. Dennis EA, et al. Phospholipase A2 enzymes: physical structure, biological function, disease implication, chemical inhibition, and therapeutic intervention. Chem Rev. 2011;111(10):6130–85.
73. Mills EL, O'Neill LA. Reprogramming mitochondrial metabolism in macrophages as an anti-inflammatory signal. Eur J Immunol. 2016;46(1):13–21.
74. Han X, Gross RW. Shotgun lipidomics: electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples. Mass Spectrom Rev. 2005;24(3):367–412.
75. Pike LJ. Lipid rafts: bringing order to chaos. J Lipid Res. 2003;44(4):655–67.
76. Kishimoto K, et al. Nondestructive quantification of neutral lipids by thin-layer chromatography and laser-fluorescent scanning: suitable methods for "lipidome" analysis. Biochem Biophys Res Commun. 2001;281(3):657–62.
77. Hazen SL, et al. Isolation of a human myocardial cytosolic phospholipase A2 isoform. Fast atom bombardment mass spectroscopic and reverse-phase high pressure liquid chromatography identification of choline and ethanolamine glycerophospholipid substrates. J Clin Invest. 1993;91(6):2513–22.
78. Han X, et al. Diabetes-induced changes in specific lipid molecular species in rat myocardium. Biochem J. 2000;352(Pt 1):79–89.
79. Han X, et al. Substantial sulfatide deficiency and ceramide elevation in very early Alzheimer's disease: potential role in disease pathogenesis. J Neurochem. 2002;82(4):809–18.
80. Han X, Gross RW. Quantitative analysis and molecular species fingerprinting of triacylglyceride molecular species directly from lipid extracts of biological samples by electrospray ionization tandem mass spectrometry. Anal Biochem. 2001;295(1):88–100.
81. Han X, et al. Alterations in individual molecular species of human platelet phospholipids during thrombin stimulation: electrospray ionization mass spectrometry-facilitated identification of the boundary conditions for the magnitude and selectivity of thrombin-induced platelet phospholipid hydrolysis. Biochemistry. 1996;35(18):5822–32.
82. Han X. Neurolipidomics: challenges and developments. Front Biosci. 2007;12:2601–15.
83. Han X, Gross RW. Shotgun lipidomics: multidimensional MS analysis of cellular lipidomes. Expert Rev Proteomics. 2005;2(2):253–64.
84. Bielawski J, et al. Simultaneous quantitative analysis of bioactive sphingolipids by high-performance liquid chromatography-tandem mass spectrometry. Methods. 2006;39(2):82–91.
85. Merrill AH Jr, et al. Sphingolipidomics: high-throughput, structure-specific, and quantitative analysis of sphingolipids by liquid chromatography tandem mass spectrometry. Methods. 2005;36(2):207–24.
86. Mesaros C, Lee SH, Blair IA. Targeted quantitative analysis of eicosanoid lipids in biological samples using liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2009;877(26):2736–45.

87. Quehenberger O, et al. Lipidomics reveals a remarkable diversity of lipids in human plasma. J Lipid Res. 2010;51(11):3299–305.
88. Han X, Gross RW. Electrospray ionization mass spectroscopic analysis of human erythrocyte plasma membrane phospholipids. Proc Natl Acad Sci U S A. 1994;91(22):10635–9.
89. Han X, Yang K, Gross RW. Multi-dimensional mass spectrometry-based shotgun lipidomics and novel strategies for lipidomic analyses. Mass Spectrom Rev. 2012;31(1):134–78.
90. Ekroos K, et al. Quantitative profiling of phospholipids by multiple precursor ion scanning on a hybrid quadrupole time-of-flight mass spectrometer. Anal Chem. 2002;74(5):941–9.
91. Han X, et al. Toward fingerprinting cellular lipidomes directly from biological samples by two-dimensional electrospray ionization mass spectrometry. Anal Biochem. 2004;330(2):317–31.
92. Yang K, et al. Automated lipid identification and quantification by multidimensional mass spectrometry-based shotgun lipidomics. Anal Chem. 2009;81(11):4356–68.
93. DeLong CJ, et al. Molecular species composition of rat liver phospholipids by ESI-MS/MS: the effect of chromatography. J Lipid Res. 2001;42(12):1959–68.
94. Han X. Characterization and direct quantitation of ceramide molecular species from lipid extracts of biological samples by electrospray ionization tandem mass spectrometry. Anal Biochem. 2002;302(2):199–212.
95. Han X, et al. Shotgun lipidomics of phosphoethanolamine-containing lipids in biological samples after one-step in situ derivatization. J Lipid Res. 2005;46(7):1548–60.
96. Cajka T, Fiehn O. Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry. Trends Analyt Chem. 2014;61:192–206.
97. Wolf C, Quinn PJ. Lipidomics: practical aspects and applications. Prog Lipid Res. 2008;47(1):15–36.
98. Novakova L, Vlckova H. A review of current trends and advances in modern bio-analytical methods: chromatography and sample preparation. Anal Chim Acta. 2009;656(1–2):8–35.
99. Sandra K, Sandra P. Lipidomics from an analytical perspective. Curr Opin Chem Biol. 2013;17(5):847–53.
100. Seppanen-Laakso T, Oresic M. How to study lipidomes. J Mol Endocrinol. 2009;42(3):185–90.
101. Stephenson DJ, Hoeferlin LA, Chalfant CE. Lipidomics in translational research and the clinical significance of lipid-based biomarkers. Transl Res. 2017;189:13–29.
102. Armitage EG, Southam AD. Monitoring cancer prognosis, diagnosis and treatment efficacy using metabolomics and lipidomics. Metabolomics. 2016;12:146.
103. Warburg O, The metabolism of carcinoma cells. Cancer Res. 1925;9:148–63.
104. O'Connell TM. Recent advances in metabolomics in oncology. Bioanalysis. 2012;4(4):431–51.
105. Pakiet A, et al. Changes in lipids composition and metabolism in colorectal cancer: a review. Lipids Health Dis. 2019;18(1):29.
106. Denkert C, et al. Mass spectrometry-based metabolic profiling reveals different metabolite patterns in invasive ovarian carcinomas and ovarian borderline tumors. Cancer Res. 2006;66(22):10795–804.
107. Jones CM, et al. Metabolomic serum profiling detects early-stage high-grade serous ovarian cancer in a mouse model. J Proteome Res. 2015;14(2):917–27.
108. Buas MF, et al. Identification of novel candidate plasma metabolite biomarkers for distinguishing serous ovarian carcinoma and benign serous ovarian tumors. Gynecol Oncol. 2016;140(1):138–44.
109. Wittmann BM, et al. Bladder cancer biomarker discovery using global metabolomic profiling of urine. PLoS One. 2014;9(12):e115870.
110. Jin X, et al. Diagnosis of bladder cancer and prediction of survival by urinary metabolomics. Oncotarget. 2014;5(6):1635–45.
111. Kuhn T, et al. Higher plasma levels of lysophosphatidylcholine 18:0 are related to a lower risk of common cancers in a prospective metabolomics study. BMC Med. 2016;14:13.
112. Cook JA. Eicosanoids. Crit Care Med. 2005;33(12 Suppl):S488–91.
113. Lamour NF, et al. Ceramide kinase regulates the production of tumor necrosis factor alpha (TNFalpha) via inhibition of TNFalpha-converting enzyme. J Biol Chem. 2011;286(50):42808–17.
114. Serhan CN, Chiang N, Van Dyke TE. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol. 2008;8(5):349–61.
115. Yamashita H, et al. Differential expression of lysophosphatidic acid receptor-2 in intestinal and diffuse type gastric cancer. J Surg Oncol. 2006;93(1):30–5.
116. Yamashita S, Yamashita J, Ogawa M. Overexpression of group II phospholipase A2 in human breast cancer tissues is closely associated with their malignant potency. Br J Cancer. 1994;69(6):1166–70.
117. Lodi A, et al. Proton NMR-based metabolite analyses of archived serial paired serum and urine samples from myeloma patients at different stages of disease activity identifies acetylcarnitine as a novel marker of active disease. PLoS One. 2013;8(2):e56422.
118. Jiao J, et al. Targeted deletion and lipidomic analysis identify epithelial cell COX-2 as a major driver of chemically induced skin cancer. Mol Cancer Res. 2014;12(11):1677–88.
119. Li J, et al. Integration of lipidomics and transcriptomics unravels aberrant lipid metabolism and defines cholesteryl oleate as potential biomarker of prostate cancer. Sci Rep. 2016;6:20984.
120. Hu C, et al. Oxidative stress leads to reduction of plasmalogen serving as a novel biomarker for systemic lupus erythematosus. Free Radic Biol Med. 2016;101:475–81.

121. Lee R, et al. Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations. Curr Med Chem. 2012;19(16):2504–20.
122. Trager J, Ward MM. Mortality and causes of death in systemic lupus erythematosus. Curr Opin Rheumatol. 2001;13(5):345–51.
123. Reichlin M, et al. Autoantibodies to lipoprotein lipase and dyslipidemia in systemic lupus erythematosus. Arthritis Rheum. 2002;46(11):2957–63.
124. Lu L, et al. Shotgun lipidomics revealed altered profiles of serum lipids in systemic lupus erythematosus closely associated with disease activity. Biomol Ther. 2018;8(4):105.
125. Ariel A, Serhan CN. Resolvins and protectins in the termination program of acute inflammation. Trends Immunol. 2007;28(4):176–83.
126. Gorjao R, et al. Comparative effects of DHA and EPA on cell function. Pharmacol Ther. 2009;122(1):56–64.
127. Serhan CN, Petasis NA. Resolvins and protectins in inflammation resolution. Chem Rev. 2011;111(10):5922–43.
128. Singhal SS, et al. Antioxidant role of glutathione S-transferases: 4-Hydroxynonenal, a key molecule in stress-mediated signaling. Toxicol Appl Pharmacol. 2015;289(3):361–70.
129. Uchida K. Role of reactive aldehyde in cardiovascular diseases. Free Radic Biol Med. 2000;28(12):1685–96.
130. Yin Z, et al. Serum level of eight cytokines in Han Chinese patients with systemic lupus erythematosus using multiplex fluorescent microsphere method. Cent Eur J Immunol. 2014;39(2):228–35.
131. Birgbauer E. Lysophospholipids in retinal axon guidance: roles and cell signaling. Neural Regen Res. 2015;10(7):1067–8.
132. Brugger B, et al. Quantitative analysis of biological membrane lipids at the low picomole level by nano-electrospray ionization tandem mass spectrometry. Proc Natl Acad Sci U S A. 1997;94(6):2339–44.
133. Kihara Y, Mizuno H, Chun J. Lysophospholipid receptors in drug discovery. Exp Cell Res. 2015;333(2):171–7.
134. Wymann MP, Schneiter R. Lipid signalling in disease. Nat Rev Mol Cell Biol. 2008;9(2):162–76.
135. Chen X, et al. Plasma lipidomics profiling identified lipid biomarkers in distinguishing early-stage breast cancer from benign lesions. Oncotarget. 2016;7(24):36622–31.
136. Yang L, et al. Comprehensive lipid profiling of plasma in patients with benign breast tumor and breast cancer reveals novel biomarkers. Anal Bioanal Chem. 2015;407(17):5065–77.
137. Zhang Y, et al. High resolution mass spectrometry coupled with multivariate data analysis revealing plasma lipidomic alteration in ovarian cancer in Asian women. Talanta. 2016;150:88–96.

2 Applications of Lipidomics in Tumor Diagnosis and Therapy

Yuping Wang

Abstract

Lipids have many critical biological functions in cancer. There are characteristic changes of lipid metabolism and metabolites in different physiological and pathological processes. Lipidomics is an emerging discipline of metabolomics for systematic analysis of lipids in organisms, tissues, or cells and the molecules that interact with them. With the development of new analytical techniques, especially the application and development of mass spectrometry techniques, the determination of lipids can be carried out quickly and accurately and has a high throughput. A large number of studies have shown that abnormal lipid metabolism is closely related to the occurrence and development of tumors. The application of lipidomics technology can reveal changes in lipids and relative abnormal metabolic pathways associated with tumors. Moreover, it shows a wide range of application prospects in the identification of tumor lipid biomarkers, early tumor diagnosis, and the discovery of antitumor drug targets. This chapter mainly introduces the application and development direction of lipidomics in the diagnosis and therapy of different tumors.

Keywords

Lipidomics · Lipid · Tumor · Diagnosis Therapy

Lipids are a class of essential biomolecules that are involved in many critical cellular processes. Because of their hydrophobicity, lipids are the main components of biofilms (Fig. 2.1). They are, therefore, the physical basis of all organisms because they provide the ability to separate organisms from the natural environment. Lipids not only provide energy for cells [1], but they are also involved in both extracellular and intracellular signaling processes in which lipids conduct signals and amplify regulatory cascade reactions.

Clinical lipidomics is a novel extension of lipidomics that investigates lipid profiles, pathways, and networks by characterizing and quantifying complete lipid molecules in patient cells, biopsy tissues, or body fluids. It is expected to be more stable during treatment, more sensitive to changes, and targeted to disease and to enable more efficient data analysis and more standardized measurements to meet clinical needs [2]. Lipidomics is projected to become a more critical method in clinical application and an important

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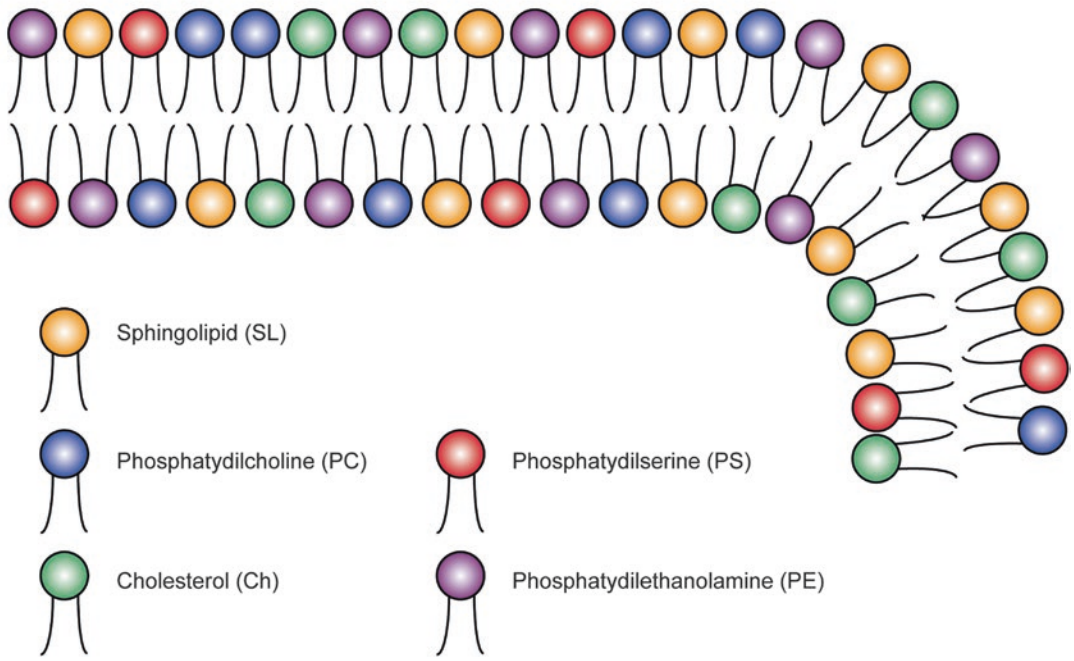

Fig. 2.1 Schematic representation of the cell membrane of phospholipids in the bilayer

tool for the early diagnosis and evaluation of disease progression of cancers (Fig. 2.2).

Because of the role of lipid molecules in cell structure, energy, and signal transduction, the characterization of cellular and extracellular lipid composition changes is critical for understanding cancer biology. Moreover, several mass spectrometry-based analyses and imaging studies have shown that lipid molecules may help enhance existing biochemical and histopathological approaches for cancer diagnosis, staging, and prognosis [3]. Therefore, the analysis of lipid metabolic changes associated with cancer cells and tumor tissues is useful for both basic and translational research. In the field of tumor lipidomics, scientists mainly focus on the applications in the diagnosis and treatment of tumors, which will be overviewed in this chapter.

2.1 Tumor Diagnosis

Lipids undergo subtle metabolic changes during the early stages of tumorigenesis. Accordingly, capturing the signals of the changes in these molecular profiles will greatly benefit the early diagnosis of cancer. Most clinical serum biomarkers for cancer detection were established in the early 1980s when the Nobel Prize in Physiology or Medicine was awarded for "discovering the principles of monoclonal antibody production." Using this "Nobel" technique, various monoclonal antibodies were developed, and the ligands on the surface of cancer cells were characterized. Abnormal sugar chain structures and abnormal sugar chain-associated glycoproteins have been identified as standard features of cancer cell surface through specific interactions with monoclonal antibodies. Subsequently, sugar-related biomarkers were detected in the serum of cancer patients and developed into serum biomarkers such as CA125, CA153, CA195, CA199, CA242, and CA724, which are popular in clinical use today [4].

Lipid metabolic reprogramming is an essential marker of tumorigenesis and development. Alterations in the tumor metabolism, including the accumulation of metabolites, lead to local immunosuppression of the tumor microenvironment. Hao et al. conducted a systematic analysis

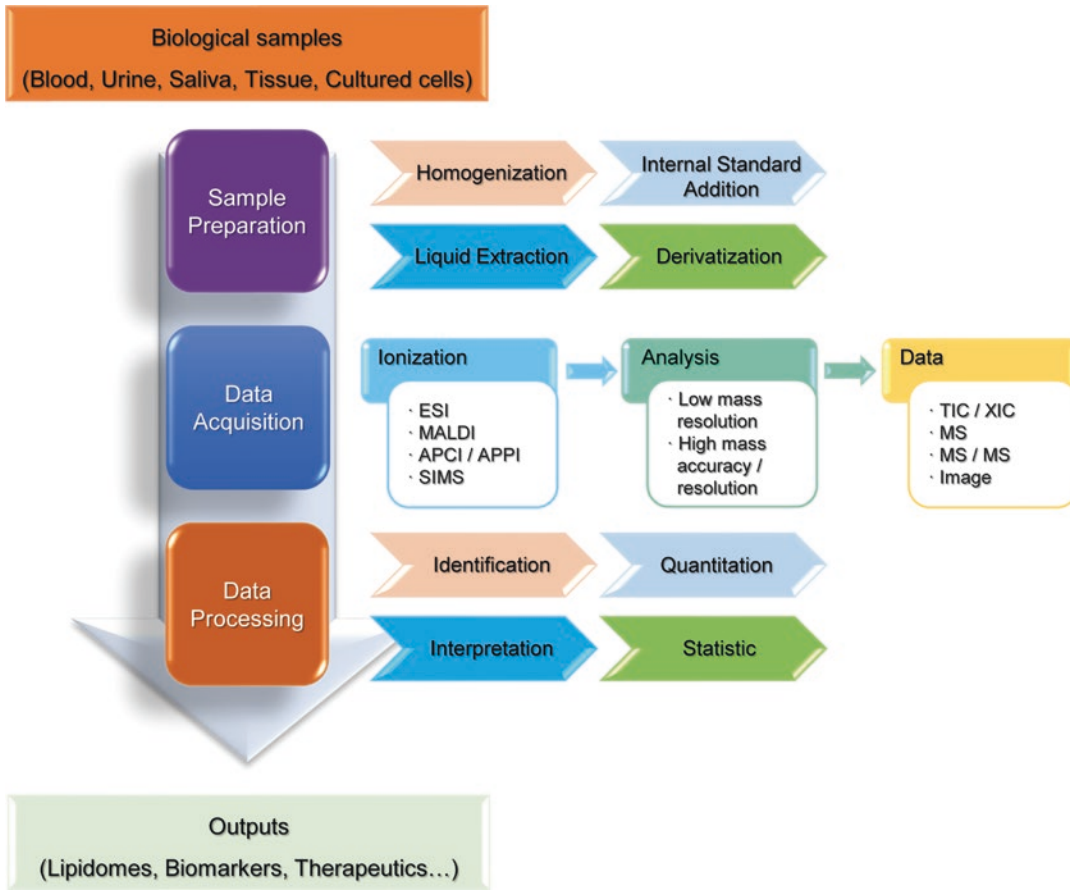

Fig. 2.2 Clinical lipidomics workflow, including all basic steps from samples to biological results

of The Cancer Genome Atlas (TCGA) multiple-omics data and found that the most widely altered lipid metabolic pathways in pan-cancer are fatty acid metabolism, arachidonic acid metabolism, cholesterol metabolism, and PPAR signaling [5].

Recent reports about lipidomics in tumor diagnosis have covered most organs of the human body, which will be discussed below (Fig. 2.3).

2.1.1 Lung Cancer

Lung cancer is the leading cause of cancer death worldwide [6]. Therefore, lipidomics studies are relatively centered on the diagnosis of lung cancer. Small-cell lung cancer (SCLC) is a type of aggressive lung cancer with low survival rates. Although kinases commonly play a crucial role in tumorigenesis, very few kinases are currently known to promote SCLC development. Cristea et al. reported that MEK5 and ERK5 are necessary for optimal survival and amplification of SCLC cell lines in vitro and in vivo. In-depth lipidomics analysis suggests that the loss of MEK5/ERK5 disrupts several lipid metabolic pathways, including the mevalonate pathway that controls cholesterol synthesis [7].

Preliminary data from recent studies suggest that lipid profiling has high specificity for evaluating the stage, severity, subtype, and drug response in lung cancer. The heterogeneity of lipid profiles and lipid metabolism may be part of the heterogeneity of lung cancer and leads to drug resistance [8]. Malignant pleural effusion (MPE) is an essential marker of advanced metastasis of lung cancer. However, current

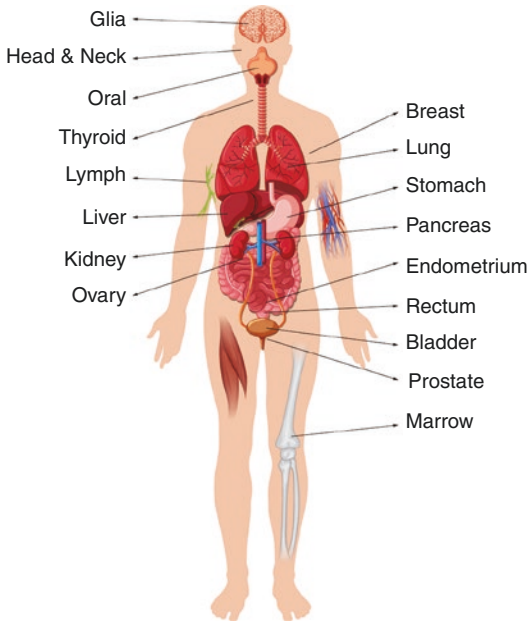

Fig. 2.3 Reported lipidomics diagnosis of human tumors (anatomy vector is from Vecteezy)

diagnostic methods entail a tedious process to distinguish between malignant pleural effusions and benign pleural effusions (BPE). Yang et al. conducted a global metabolomics and lipidomics analysis based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) to characterize the metabolic characteristics of lung cancer MPE and identify the potential metabolite biomarkers diagnostic of MPE. During MPE, 25 ether lipids, including phosphatidylcholine (PC), lysophosphatidylcholines (LPC), and phosphatidylethanolamine (PE), were significantly downregulated. This supported the diagnostic potential of the upregulated expression of oxidized polyunsaturated fatty acids (PUFAs) in MPE [9].

Noreldeen HAA et al. reported other lipidomics methods based on ultrahigh-performance liquid chromatography other than intended targets plus quadrupole time-of-flight mass spectrometry (UHPLCQ-TOF/MS). Two machine learning methods (genetic algorithm and binary logical regression) were also used to screen candidates for different lipids and to establish a combined lipid biomarker to distinguish between women with non-small-cell lung cancer (NSCLC) and healthy controls. The results showed that fatty acids (FA) (20:4), FA (22:0), and LPE (204) could be used as a combination biomarker to distinguish NSCLC from healthy tissues in women, with excellent sensitivity and specificity [10]. Klupczynska et al. conducted targeted lipid group screening to select potential molecules for the early detection of lung cancer. Of the lipids tested, there were significant differences between the PC group and the lysophosphatidylcholines (lysoPC) group in NSCLC patients and healthy controls, especially a C26:0; lysoPC a C26:1; PC aa C42:4; and PC aa C34:4 [11].

Yu et al. used MS to analyze the lipids of 390 individuals from 44 plasma samples obtained from the training lung cancer cohort. C18:2 cholesterol ester and sphingomyelin 22:0 as lipid markers were identified to be useful for distinguishing between squamous cell lung carcinoma (SqCC) patients and high-risk individuals, with 95.5% sensitivity, 90.9% specificity, and 95.2% accuracy [12]. Using UHPLCQ-TOF/MS through targeted lipid profiling, Chen et al. identified PCs and phosphatidylethanolamine (PEs) as biomarkers of early-stage NSCLC. The levels of PCs and PEs were abnormal during glycerophospholipid metabolism, which is the most altered pathway in early NSCLC [13].

2.1.2 Breast Cancer

Breast cancer (BC) is a heterogeneous malignancy. It is the most frequent malignancy and the leading cause of cancer-related death in American women. Compared with other major BC subtypes, triple-negative breast cancer has a lower survival rate and a higher metastasis rate, thus highlighting the need for more sensitive and specific methods for early-stage TNBC (ES-TNBC) detection. However, early diagnosis remains challenging because of the high pathological level, and thus the survival rate remains relatively low. Eghlimi et al. reported that LC-tandem MS can detect lipids with high specificity and sensitivity. Two diagnostic biomarker panels were proposed for TNBC/ES-TNBC [14]. Terao et al. evaluated all-trans retinoic acid (ATRA)-treated BC cell lines and found that ATRA disrupted the

homeostasis of many lipids, the most significant of which was in the mitochondrial intima and those involved in the oxidative phosphorylation of cardiac phospholipids. ATRA can reduce the level of cardiac phospholipid, and this can inhibit the growth activity of retinoid. ATRA exerts its antiproliferative activity by reducing tumor cell respiration and energy balance, thus its important role in breast cancer [15].

Kang et al. investigated the role of lipid metabolic alterations in malignant phenotypes of BC. They found significant homeostatic interference of various complex lipid substances (including ceramide, sphingomyelin, ether-linked phosphatidylcholine, and ether-linked phosphatidylethanolamine) in the mesenchymal state of cancer cells. The polyunsaturated fatty acid composition in spherical cells was significantly reduced, and SCD, ACOX3, and FADS1 were upregulated. Meanwhile, PTPLB, PECR, and ELOVL2 were downregulated. The ratio of C226n3 (docosahexaenoic acid, DHA) to C225n3 was significantly reduced in globular cells, like ELOVL2 downregulation. ELOVL2 expression is associated with a malignant phenotype and appears to be a novel prognostic biomarker in breast cancer [16]. Zhao et al. investigated the toxic effects of bisphenol F (bp F) in BC xenografts and the potential mechanisms for tumor metastasis-related tissues (e.g., in the liver and kidney). They found that BPF exposure disrupts the metabolic and lipid groups in the liver and kidney. Exposure induces reprogramming of glutathione (GSH) biosynthesis and glycolysis metabolism by activating glycine, serine, cysteine, glutamine, lactate, and pyruvate in the liver and kidney tissues. This also interferes with the biosynthesis and degradation of glycerol phospholipids (GPs) and glycerol phospholipids (GLs), resulting in abnormal renal tissue membrane homeostasis and cellular function [17].

Reprogramming of lipid metabolism is a hallmark of many cancers and has been shown to promote BC progression. Purwaha et al. showed that higher sphingomyelin levels were significantly associated with better disease-free survival in patients with TNBC [18]. LC-MS and environmental mass spectrometry imaging (MSI) have been shown to be robust and reproducible diagnostic techniques for BC. Silva et al. investigated whether the lipid features observed in cancer tissues via desorption electrospray ionization (DESI)-MSI correspond to those detected in LC-MS plasma samples. A comparison of the plasma and tissue lipid profiles suggests that each matrix studied (e.g., blood or tumor) has its particular molecular characteristics [19]. Nishida-Aoki et al. performed an extensive targeted quantitative lipid group analysis of cells and extracellular vesicles (EV) from high-metastatic and low-metastatic TNBC cell lines using supercritical fluid chromatography rapid scanning tripolar mass spectrometry. They confirmed that EV between different lipid components is associated not only with their origin cells but also with high- and low-metastatic cell lines. Moreover, compared with those of low-metastatic cells, the EV of high-metastatic BC cells accumulated unsaturated diacylglycerol (DGs) and did not increase in the cells. DG enrichment of EVs activates protein kinase D signaling pathways in endothelial cells, leading to angiogenesis stimulation [20].

2.1.3 Colorectal Cancer

Colorectal cancer (CRC) is the third leading cause of cancer-related death worldwide. Reliable biomarkers for early CRC diagnosis are crucial for reducing mortality. Liu et al. used the combined lipid group method to study the differences in blood lipid profiles between 101 CRC patients and 52 healthy volunteers. A total of 11 lipid species, including glycerophosphoethanolamine, ethanolamine plasmalogens, plasmanyl glycerophosphatidylethanolamine, fatty acids, fatty acid ester of hydroxyl fatty acid, and diacylglycerophosphates, were identified to distinguish healthy controls at an early stage [21]. Bestard-Escalas et al. described the characteristics of membrane lipid groups and their EV in five commercial colonic cell lines. Moreover, the results showed that both cells and EV lipid groups could be separated according to the degree of cell malignancy. Furthermore, the effects of all CRC lines on ether

lipids were specific and significantly homogeneous [22].

Solid tumors are characterized by overall metabolic alterations in their growth and progression. Wang et al. measured the molar abundance of 342 species of 20 lipids in biopsy-matched CRC and adjacent normal mucosa samples. Compared with the findings of previous reports, CRC samples showed a large amount of preserved lipid composition similar to that in the normal colonic mucosa samples. Significant exceptions include increased levels of phosphatidylinositol in CRC and decreased phosphatide abundance in late CRC [23]. Serafim et al. examined patients with stage I–III CRC, patients with adenomatous polyps, and individuals who underwent routine colonoscopy. All patients underwent peripheral blood lipid extraction, and the lipids of the samples were identified via MALDI-MS technology. The polyketide group (810.1) is the lipid represented in the tumor, and the polyp and control group are mostly represented. We observed differences in the lipid profile between patients with lymph node invasion (N1–2) and those without lymph node infiltration (N) in CRC patients [24].

Kitamura et al. studied the level of lysophospholipids in colorectal cancer tissues and found that lysophosphatidylinositol and lysophosphatidylserine levels were significantly higher than those in normal tissues. Meanwhile, lysophosphatidic acid levels were significantly lower than those in normal tissues. The fatty acid analysis showed that lysophospholipids 18:0 and 18:0 were the dominant lipids in colon cancer [25]. Choi et al. used MS to analyze the lipid groups of colon cancer stem cells (CSCs) and large cancer cells (BCCs) and reported that CSCs contain a unique lipid profile. The free MUFA was higher in CSCs than that in BCCs, whereas the levels of free SFA were lower. In addition, all identified MUFAs containing phosphatidylethanolamine had high levels in CSCs. Interestingly, low phosphatidyl-serine (18:1/18:0), phosphatidyl-choline (PC; p-18:0/18:1), and sphingomyelin (SM; d18:1/20:0 or d16:1/22:0) levels in CSCs were observed. The specific PC, SM, and MUFAs in CSCs can be increased rapidly. Collectively, these results suggest that these specific lipid components are essential for the maintenance of CSCs [26].

2.1.4 Gastric Cancer

Malignant tumor growth is characterized by significant changes in metabolites. Sun et al. found that palmitic acid (PA) was significantly downregulated in gastric carcinoma. Cell proliferation in gastric cancer (GC) cell lines, such as AGS, SGC-7901, and MGC-803, was inhibited by the high concentration of PA in vitro, impairing cell invasiveness and migration ability. In addition, sterol regulatory element-binding protein 1 (SREBP-1c) is activated in human GC, promoting the expression of various genes such as SCD1 and FASN, which are associated with fatty acid synthesis. SREBP-1c downregulation rescued migration and invasion defects of AGS and SGC-7901 GC cells [27]. Based on a breakthrough in genomics, TCGA recently proposed an integrated genome analysis approach wherein GC is divided into four subtypes according to the chromosomal instability (CIN) states. Hung et al. collected GC tissue specimens and noncancer tissue specimens from cancer patients and conducted an analysis following TCGA classification. They identified 409 oncogene and tumor suppressor gene sequences, and the samples were divided into CIN and non-CIN types. Using LC-MS, the authors identified the lipid profiles of GC samples and adjacent noncancerous tissue samples. Compared with adjacent noncancerous tissues, gas chromatography samples showed distinct features of lysophospholipid, phosphocholine, phosphatidylethanolamine, phosphatidylinositol, phosphoserine, sphingomyelin, ceramide, and triglycerides. The levels of GPs (choline phosphate, phosphatidylethanolamine, and phosphatidylinositol) increased by 1.4–2.3 times in the CIN group compared with those in the non-CIN group ($P < 0.05$). These changes in the glycerol and glycerophospholipid pathways indicated GC progression to CIN [28].

2.1.5 Prostate Cancer

EVs of non-tumorigenic cells in prostate cancer (PCa) patients are rich in fatty acids, glycolipids, and precursor oils. In contrast, EVs of tumorigenic or metastatic cells are abundant in glycolipids, sphingolipids, and glycerol phospholipids [29]. Zhou et al. compared PCa with benign prostate tissue (BPT). The results showed that the total fatty acid content, monounsaturated fatty acid content, polyunsaturated fatty acid content, and $n-6$ total fatty acid content of the PCa group were significantly higher than those of the BPT group. A significantly higher PCa $n-6$FFA and $n-3$FFA concentration of most fatty acid parameters was associated with Gleason grade and clinical stage [30]. However, the fatty acids associated with the occurrence, progression, and ethnic differences between African American (AA) and Caucasian American (CA) populations as well as the fatty acids that are differentially expressed remain unclear. Kregel et al. observed that both bromine-containing and external (BET) degraders inhibited PCa cell growth in vivo and in vitro. These drugs preferentially affect AR-positive PCa cells (22 Rv1, LNCaP, VCaP) rather than AR-negative cells (PC3 and DU145). The increase in PUFAs and thioredoxin-interacting proteins (TXNIP) indicate their potential as pharmacological biomarkers for targeting BET proteins [31].

2.1.6 Endometrial Carcinoma

In endometrial cancer, preoperative biomarkers for identifying patients with low risk of disease progression can help establish the appropriate degree of surgery required and avoid possible complications from radical surgery. Using electrospray ionization tandem mass spectrometry, Knific et al. conducted a quantitative analysis of 163 metabolites in 126 plasma samples from 61 patients with endometrial cancer and 65 controls. Three levels of single phosphatidylcholine decreased significantly in patients with endometrial cancer [32]. Cummings et al. discussed the changes of epithelial eicosane metabolism gene expression in endometrial carcinogenesis. These were combined with eicosane-like profiles in matched clinical specimens. The expression of candidate eicosane metabolic enzymes, that is, low HPGD combined with high ALOX5 expression, was associated with worse overall survival and progression-free survival, emphasizing that HPGD and ALOX5 are potential therapeutic targets for invasive EC subtypes [33].

2.1.7 Bladder Cancer

Bladder cancer is an elusive disease because of its rapid recurrence and drug resistance. The prognosis of BC patients with recurrence and hyperthermia is extremely poor. Lee et al. conducted a lipid group comparison analysis of two homogeneous human T24 bladder cancer cell lines. Ultrahigh-performance liquid chromatography-mass spectrometry (UPLC-MS) analysis of 1864 lipids identified differentially expressed lipid levels suspected to be associated with cisplatin resistance [34]. Vantaku et al. used the NIST MS metabolomics outline and lipid blast MS/MS library to identify 519 metabolites and 19 lipids differentially expressed between low- and high-grade bladder cancer, respectively. They identified metabolic features of high-grade bladder cancer by integrating unbiased metabolomics, lipidomics, and transcriptomics to predict patient survival and identify novel therapeutic targets [35].

2.1.8 Ovarian Cancer

Cheng et al. reported the findings from the protein and lipid group analyses of exosomes from ovarian cancer cells (SKOV-3) and ovarian surface epithelial cells (HOSEPiC). A total of 1433 proteins and 1227 lipids were identified from the exocrine derived from both cell lines. The exocrine extracted from the SKOV-3 was more abundant than the ChE and ZyE species extracted from

the HOSEPiC. V collagen chains (COL5A2) and lipoprotein lipase (LPL) in the exocrine from SKOV-3 sources were significantly higher [36]. Wefers et al. analyzed 109 lipid mediators of ascites in patients with ovarian cancer and found that the lipid involved in ascites inhibition was different from that in normal peritoneal fluid. In addition, there were lipid intermediates in the ascites of ovarian cancer patients, which is consistent with T cell dysfunction [37].

2.1.9 Pancreatic Cancer

Pancreatic cancer is one of the most aggressive malignancies. Early diagnosis of pancreatic cancer is difficult, leading to its poor prognosis. Tao et al. evaluated possible prognostic or diagnostic metabolite biomarkers in serum exocrine of pancreatic cancer patients and found that 270 of the 20 lipids showed significant abnormalities. Of them, 61 were verified in a larger sample size. LysoPC 22:0, PC (P-14:0/22:2), and PE (16:0/18:1) were associated with tumor stage, CA19–9 expression, CA242 expression, and tumor diameter. PE (16:0/18:1) was also significantly correlated with overall survival [38]. Arnoletti et al. collected portal vein plasma samples during the intraoperative period from 29 patients undergoing pancreaticoduodenectomy and used multidimensional mass spectrometry-based shotgun lipid histology to analyze lipid changes. The unique characteristic analysis of 20 lipids and 235 lipids was found to reliably identify PDAC (stage I–IV), intraductal papillary mucosa (IPMN), and nonmalignant pancreatitis [39].

The carnitine palmitoyltransferase (CPT) family is essential for fatty acid oxidation. Guan et al. found that carnitine palmitoyltransferase 1C (CPT1C), one of the subtypes, plays an essential role in the aging of tumor cells. However, whether other subtypes (CPT1A, CPT1B, and CPT2) have the same effect on cellular senescence remains unclear [40]. CPT1C has the most significant effect on cell senescence. Using lipidomics analysis, we further found that the downregulation of CPTs alters lipid content involved in mitochondrial function and induces lipid accumulation.

2.1.10 Liver Cancer

The cellular heterogeneity of tumor tissue is one of the causes of tumor recurrence after chemotherapy. Thus, identification of specific tumor tissue cell subtypes is critical for precision medicine and prognostic prediction. Lipids, as structural and functional components of cells, are closely related to the apparent morphology of cells. They are biomarkers of potential cancer species that can be used to classify different cancer cell types. Wang et al. described a lipid spectrum analysis method based on nanostructured laser desorption/ionization mass spectrometry (NALDI-MS). This method can classify five HCC cell lines and distinguish the subtypes of mixed cells and tumor tissues. The molecular structures of these biomarkers were classified into two categories as phosphatidylcholine (i.e., PE, PI, PG, PA, and PS) and phospholipids (i.e., LacCer, ST) [41].

2.1.11 Glial Tumor

Isocitrate dehydrogenase (IDH)1 mutation is a highly frequent event in low-grade gliomas and secondary glioblastomas. Zhou et al. found marked changes in glycolysis and lipid metabolism in IDH1 mutant glioma tissues compared with IDH1 wild-type glioma through comprehensive metabolic studies on clinical IDH1 mutant glioma specimens. More pyruvate was found to enter the TCA cycle in IDH1 mutant gliomas presenting with reduced triglycerides and sphingolipids [42].

2.1.12 Thyroid Cancer

The difference between papillary thyroid carcinoma and benign thyroid lesions is of great significance for clinical management. Histopathological classification can be supported by molecular biomarkers, including lipid group features, identified using high-throughput mass spectrometry techniques. Wojakowska et al. used a high-resolution MALDI-Q-Ion mobility-TOF-MS technique to analyze lipid groups in

formalin-fixed thyroid tissue samples. Multiple phosphatidylcholine (32:0, 32:1, 34:1, and 36:3), sphingomyelin (34:1 and 36:1), and phosphatidic acid (36:2 and 36:3) were detected in cancer tissues. Moreover, they were significantly more abundant in cancer tissue than in noncancerous tissue [43].

2.1.13 Head and Neck Cancer

Fanconi anemia (FA) gene mutations are common in sporadic head and neck squamous cell carcinoma (HNSCC). We have previously demonstrated that FA pathway deletion stimulates invasion of HNSCC cell lines. Zhao et al. used a systematic approach to define FA pathway-dependent lipid metabolism and to extract lipid-based features and invasive effectors in FA defective cells. The most notable element in the lipid profile results was the consistent elevation of glycolipid, especially ganglioside accumulation. In contrast, such lipids were inhibited with genetic correction of HNSCC cells from FA patients [44].

2.1.14 Myeloma

Multiple myeloma (MM) is a blood malignancy characterized by clonal expansion of malignant plasma cells. Although long-term palliative treatment is possible, MM is incurable and most patients develop recurrence. Mohamed et al. evaluated the feasibility of simultaneous lipidomics and proteomics analysis of plasma cells. The results showed that PCs were significantly downregulated in recurrent MM. PC, ceramide, cardiac phospholipid, arachidonic acid, and cholesterol metabolic pathways were significantly correlated only in patients with recurrence, but not in those who were newly diagnosed [45].

2.1.15 Oral Cancer

Metabolic recombination as one of the characteristics of cancer is beneficial to rapid energy production, biosynthetic ability, and therapeutic resistance. We previously found that balsam pear extract (BME) could prevent carcinogen-induced oral cancer in mice. RNA sequence analysis of the mouse tongue showed that compared with other cancers, BME significantly regulated the metabolic process by altering glycolysis and lipid metabolic pathways in oral cancer [46]. Bednarczyk et al. compared the usefulness of proteome and lipidome components to distinguish between oral cancer cells and normal mucosa. The tumor regions were more heterogenous than the normal epithelium, and the distribution of peptide components was more uneven than that of lipid components. Furthermore, there were significant differences in the abundance of peptide components and lipid components between the tumor and the normal epithelium (for peptide and lipid components, the median effect of Cohen was 0.49 and 0.31, respectively). In addition, compared to normal epithelial cells, a multicomponent cancer classifier was detected using tissue samples from three patients and then validated with tissue samples from the fourth patient. The weighted accuracy of cancer classification for peptide-based signature and lipid-based signature was 0.85 and 0.69, respectively. Although the molecular differences between cancerous and normal mucosa were higher in the proteome domain than in the analyzed lipidome subdomain, imaging of lipidome components can also distinguish between oral cancer and normal epithelium. Therefore, both tumor proteome and lipidome are promising sources of biomarkers for oral malignancies [47].

2.1.16 Renal Carcinoma

The clear cell carcinoma (ccRCC) subtype of renal cell carcinoma (RCC) is characterized by lipid accumulation and metabolic alterations. However, data on ccRCC metabolic alterations are limited. Schaeffeler et al. assessed metabolic alterations and lipid composition of RCC subtypes and ccRCC-derived metastases. They found differences in lipid synergistic regulatory

networks between ccRCC and chromophobe RCC (chRCC) except for lysophospholipids and sphingolipids [48].

2.2 Tumor Therapy

The above content indicates that lipidomics and changes in lipid molecules have high potential for application in early tumor diagnosis. Tumor diagnosis is easier to establish through direct detection and analysis of clinical samples. However, owing to the standardized management of clinical research and clinical observation of long-term curative effects, there are fewer studies on the application of lipidomics in tumor treatment. Therefore, most tumor treatment-related research still stays at the stage of intervening tumor cells in the laboratory.

2.2.1 Lung Cancer

Zhang et al. identified CCL3 metabolic-related genes or inflammation-related genes in lung adenocarcinoma and small lung cancer cells, respectively. Palmitic acid C16:0 or stearic acid C18:0 upregulated ACSL5 or CSF2 expression in a time- and dose-dependent manner. Deletion of both genes resulted in cell insensitivity. By altering intracellular energy, the target lipid increased the expression of PDK4 genes and inhibited cell proliferation [49]. Bergqvist et al. compared the effects of microsomal prostaglandin E synthase (mPGES)-1 inhibitor complex III (ciii) with those of cyclooxygenase (COX)-2 inhibitor NS-398 on interleukin (IL)-1-induced cellular protein and lipid profiles in lung cancer. Compared to the NS-398 that activate these pathways, CIII downregulated eIF2, eIF4/P70S6K, and mTOR signals. There are nine phospholipid changes between the two inhibitors. Compared with CIII, NS-398 lysophospholipid (LPC) shows more profound changes in living cell imaging systems. We also found that CIII reduced cell proliferation and enhanced the cytotoxic effects of cisplatin, etoposide, and vincristine [50].

The recent introduction of targeted therapy and immunotherapy for NSCLC offers new hope for NSCLC patients. However, not all patients respond to these drugs, and the complete response is low. New therapeutic targets and novel antitumor drugs are still urgently needed in NSCLC. Sphingomyelin kinase 2 (SphK2) is one of the critical enzymes in sphingomyelin metabolism. Positive SphK2 expression predicts poor survival in NSCLC patients and is associated with gefitinib resistance. Dai et al. explored the NSCLC activity of ABC294640, which is the only oral SphK2 inhibitor. The results showed that ABC294640 could induce NSCLC apoptosis, cell cycle arrest, and tumor growth inhibition both in vitro and in vivo [51]. Lipotoxicity, caused by intracellular lipid accumulation, accelerates the degenerative process of cellular senescence, which is crucial in cancer development and treatment. CPT1C, a mitochondrial enzyme that catalyzes carnitinylation of fatty acids, has been found to be a key regulator of cancer cell senescence. Analysis of the LC/MS lipid groups of PANC-1, MDA-MB-231, HCT-116, and A549 cancer cells after the deletion showed significant changes in lipid groups of cpt1c depleted cells, including fatty acids, diacylglycerols, triacylglycerols, oxidized lipids, cardiac phospholipids, phosphatidylglycerols, phosphatidylcholine-phosphatidylethanolamine ratios, and sphingomyelins [52].

2.2.2 Prostate Cancer

Androgen deprivation therapy (ADT) is a primary treatment strategy in patients with metastatic PCa. ADT is associated with various metabolic disorders, including impaired glucose tolerance, insulin resistance, and weight gain, thus increasing the risk of diabetes and cardiovascular death. ADT exerts its therapeutic effect through several mechanisms. First, ADT treatment reduces steroid synthesis, which is reflected in lower androgen sulfate and other steroid hormones. Second, ADT consistently reduces 3-hydroxybutyric acid and ketone formation. Third, ADT reduces many

acylcarnitines, thus affecting fatty acid metabolism. Fourth, ADT is reduces 3-formylindole (also called indole-3-carboxaldehyde) [53]. Clendinen et al. used multi-platform (NMR + LC-MS) metabolomics to study PCa recurrence and preoperative metabolic changes. Lipid histology experiments showed that many classes of lipids, including triglycerides, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, diglyceride, acylcarnitine, and ceramide, are highly abundant in patients with recurrence [54].

2.2.3 Ovarian Cancer

Lipidomics is a promising approach to identify lipid profiles in malignant phenotypic cells. Using MS, Cadoni et al. revealed quantitative differences in phospholipid composition between cisplatin-resistant and cisplatin-sensitive model cancer cell lines. Further, in CCRF-CEM cisplatin-sensitive cells, phosphocholines PC P-34:0, PC 34:1, PC 20:2_16:0, LPC 18:1 and LPC 16:0 PLs were found treated with 200-400 μM cisplatin, but not in cisplatin-resistant A2780 cells. Similarly, the PC 34:1, LPC 18:1, and LPC 16:0 of cisplatin-reactive A2780 increased in cells, whereas cisplatin-resistant A2780 cells PC 20:2_16:0 downregulated. The development of lipid entities and therapeutic resistance shown in MS may be helpful for molecular diagnosis and provide a potential complementary cancer biomarker [55].

2.2.4 Colorectal Cancer

Lipidome technique is a promising antigen delivery technique in cancer immunotherapy. The phospholipid content of the lipid group may act as immunostimulatory molecules in tumor immunotherapy [56]. The DOTAP and DOPE lipid groups (F1 lipid groups) stimulated a mixed immune response in Th1 and Th2 colon cancer mice without tumor-specific antigens.

2.2.5 Bone Marrow Tumor

Although the proteasome inhibitor bortezomib (BTZ) has shown excellent results in MM, a small number of patients experienced severe adverse events or did not respond to the drug. In addition, BTZ-induced peripheral neuropathy (BiPN) is a common side effect, thus limiting its application. Maekawa et al. identified 385 lipid metabolites in patients' serum and found that low levels of glycerophospholipids, sphingolipids, and cholesterol esters are associated with adverse therapeutic responses. Metabolites associated with platelet-activating factor biosynthesis and cholesterol metabolism appear to be particularly relevant. In addition, several lysophosphatidylcholines, phosphatidylcholine, ceramide, neutral lipids, and oxidized fatty acids were significantly increased or decreased in BiPN patients with grade G0–G3 disease [57].

2.2.6 Lymphoma

Monocarboxylic acid transporter 1 (MCT1) is a regulator of cell metabolism and a therapeutic target for cancer therapy. Beloueche-Babari et al. evaluated the effects of MCT1 inhibitor AZD3965 critical determinant of tumor biological function on tumor metabolism and immune cell infiltration in an MCT1-dependent model of lymphoma. Tumor growth was inhibited, and tumor choline was reduced in mice with severe combined immunodeficiency Raji xenograft tumors treated with AZD3965 [58].

2.2.7 Other

In the field of cancer treatment, lipid molecules are not only involved in antitumor effects through metabolism but can also affect the vitality of tumor cells through immune responses. Treatment with immune checkpoint inhibitors (ICI) requires the production of appropriate amounts of IL-6 and

TNF-cells to clear tumor cells. IL-6- and TNF-activated phospholipases induce the release of PUFAs in cell membrane phospholipid pools. PUFAs as a precursor of pro-inflammatory and anti-inflammatory eicosane can inhibit excess production of IL-6 and TNF. PUFAs can also selectively kill tumor cells by enhancing the production of free radicals and the accumulation of toxic lipid peroxides in tumors rather than in normal cells [59].

Bone marrow-derived suppressor cells (MDSCs) play an essential role in tumorigenesis; accordingly, their inhibition is key to the success of tumor immunotherapy. MDSCs induce oxidative phosphorylation resulting from glycolysis to fatty acid oxidation (FAO) and lipid accumulation in tumors through metabolic reprogramming. The increased uptake of exogenous fatty acids by tumor MDSCs enhances its immunosuppressive activity against T cells, thereby promoting tumor progression [60].

2.3 Conclusion and Remarks

Traditional studies on cancer cell metabolism mostly focus on glutamine decomposition and glycolysis. However, in the past decade, with the continuous development of lipidomics technology, new knowledge and new theories have deepened the understanding of the relationship between lipid metabolism and cancer biology [61, 62]. Recent studies have shown that the reprogramming of cell lipid metabolism is directly involved in the malignant transformation and progression of cells [63, 64]. For example, lipids synthesized de novo can provide phospholipid components for proliferation to form plasma membranes and organelle membranes of newly dividing cells [65]. In addition, the upregulated expression of mitochondrial microglobulin helps tumor cells maintain energy metabolism and redox homeostasis. Lipid-derived messenger molecules can regulate related signal pathways and coordinate immune suppression [66, 67]. Therefore, lipid metabolism is involved in various oncogenic processes, including proliferation, differentiation, migration, invasion, and drug resistance [68, 69]. However, whether we can safely and effectively regulate cancer treatment through lipid metabolism, the underlying mechanism remains unclear [70].

In addition to peripheral blood as a commonly used sample for early tumor diagnosis, other easily accessible body fluids have also received increasing research attention. Human saliva as a biological fluid is increasingly used for diagnosing diseases, monitoring systemic disease status, and predicting disease progression. The discovery of biomarkers in saliva provides a unique opportunity to assess patient health by using oral fluids, avoiding invasive blood collection. Salivary fluids are clinically relevant because their components can be found in plasma. Salivary lipids are one of the most important cellular components in human saliva, and thus they have high potential as biomarkers. Lipid components in cells and tissues change with physiological changes, and lipid components in normal tissues are different from those affected by disease. Lipid imbalance is strongly associated with many lifestyle-related diseases, such as atherosclerosis, diabetes, metabolic syndrome, systemic cancer, neurodegenerative diseases, and infectious diseases. Therefore, lipid biomarkers can be useful to diagnose disease and evaluate disease status and treatment response. However, whether saliva can be used as a substitute for serum lipid profiles requires further investigation as developing reliable diagnostic and salivary disease surveillance tests requires identifying saliva biomarkers using a high-sensitivity method with low detection limits [71]. The continuous development of mass spectrometry (MS) and the introduction of high-precision and high-resolution mass spectrometry detectors in recent years have also significantly improved lipidomics methods.

References

1. Züllig T, Trötzmüller M, Köfeler HC. Lipidomics from sample preparation to data analysis: a primer. Anal Bioanal Chem. 2020;412(10):2191–209.
2. Zhang L, Han X, Wang X. Is the clinical lipidomics a potential goldmine? Cell Biol Toxicol. 2018;34(6):421–3.

3. Islam SR, Manna SK. Lipidomic analysis of cancer cell and tumor tissues. Methods Mol Biol. 2019;1928:175–204.
4. Tang Y, Cui Y, Zhang S, et al. The sensitivity and specificity of serum glycan-based biomarkers for cancer detection. Prog Mol Biol Transl Sci. 2019;162:121–40.
5. Hao Y, Li D, Xu Y, et al. Investigation of lipid metabolism dysregulation and the effects on immune microenvironments in pan-cancer using multiple omics data. BMC Bioinformatics. 2019;20(Suppl 7):195.
6. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30.
7. Cristea S, Coles GL, Hornburg D, et al. The MEK5-ERK5 kinase axis controls lipid metabolism in small-cell lung cancer. Cancer Res. 2020;80(6):1293–303.
8. Zhang L, Zhu B, Zeng Y, et al. Clinical lipidomics in understanding of lung cancer: opportunity and challenge. Cancer Lett. 2020;470:75.
9. Yang Z, Song Z, Chen Z, et al. Metabolic and lipidomic characterization of malignant pleural effusion in human lung cancer. J Pharm Biomed Anal. 2020;180:113069.
10. Noreldeen HAA, Du L, Li W, et al. Serum lipidomic biomarkers for non-small cell lung cancer in nonsmoking female patients. J Pharm Biomed Anal. 2020;185:113220.
11. Klupczynska A, Plewa S, Kasprzyk M, et al. Serum lipidome screening in patients with stage I non-small cell lung cancer. Clin Exp Med. 2019;19(4):505–13.
12. Yu Z, Chen H, Zhu Y, et al. Global lipidomics reveals two plasma lipids as novel biomarkers for the detection of squamous cell lung cancer: a pilot study. Oncol Lett. 2018;16(1):761–8.
13. Chen Y, Ma Z, Shen X, et al. Serum lipidomics profiling to identify biomarkers for non-small cell lung cancer. Biomed Res Int. 2018;2018:5276240.
14. Eghlimi R, Shi X, Hrovat J, et al. Triple negative breast cancer detection using LC-MS/MS lipidomic profiling. J Proteome Res. 2020;19:2367.
15. Terao M, Goracci L, Celestini V, et al. Role of mitochondria and cardiolipins in growth inhibition of breast cancer cells by retinoic acid. J Exp Clin Cancer Res. 2019;38(1):436.
16. Kang YP, Yoon JH, Long NP, et al. Spheroid-induced epithelial-mesenchymal transition provokes global alterations of breast cancer lipidome: a multi-layered omics analysis. Front Oncol. 2019;9:145.
17. Zhao C, Xie P, Wang H, et al. Liquid chromatography-mass spectrometry-based metabolomics and lipidomics reveal toxicological mechanisms of bisphenol F in breast cancer xenografts. J Hazard Mater. 2018;358:503.
18. Purwaha P, Gu F, Piyarathna DWB, et al. Unbiased lipidomic profiling of triple-negative breast cancer tissues reveals the association of sphingomyelin levels with patient disease-free survival. Meta. 2018;8(3):41.
19. Silva AAR, Cardoso MR, Rezende LM, et al. Multiplatform investigation of plasma and tissue lipid signatures of breast cancer using mass spectrometry tools. Int J Mol Sci. 2020;21(10):3611.
20. Nishida-Aoki N, Izumi Y, Takeda H, et al. Lipidomic analysis of cells and extracellular vesicles from high- and low-metastatic triple-negative breast cancer. Meta. 2020;10(2):67.
21. Liu T, Tan Z, Yu J, et al. A conjunctive lipidomic approach reveals plasma ethanolamine plasmalogens and fatty acids as early diagnostic biomarkers for colorectal cancer patients. Expert Rev Proteomics. 2020;17(3):233–42.
22. Bestard-Escalas J, Maimó-Barceló A, Lopez DH, et al. Common and differential traits of the membrane lipidome of colon cancer cell lines and their secreted vesicles: impact on studies using cell lines. Cancers. 2020;12(5):1293.
23. Wang Y, Hinz S, Uckermann O, et al. Shotgun lipidomics-based characterization of the landscape of lipid metabolism in colorectal cancer. Biochim Biophys Acta Mol Cell Biol Lipids. 1865;2020(3):158579.
24. Serafim PVP, Figueiredo AG Jr, Felipe AV, et al. Study of lipid biomarkers of patients with polyps and colorectal câncer. Arq Gastroenterol. 2019;56(4):399–404.
25. Kitamura C, Sonoda H, Nozawa H, et al. The component changes of lysophospholipid mediators in colorectal cancer. Tumour Biol. 2019;41(5):1010428319848616.
26. Choi S, Yoo YJ, Kim H, et al. Clinical and biochemical relevance of monounsaturated fatty acid metabolism targeting strategy for cancer stem cell elimination in colon cancer. Biochem Biophys Res Commun. 2019;519(1):100–5.
27. Sun Q, Yu X, Peng C, et al. Activation of SREBP-1c alters lipogenesis and promotes tumor growth and metastasis in gastric cancer. Biomed Pharmacother. 2020;128:110274.
28. Hung CY, Yeh TS, Tsai CK, et al. Glycerophospholipids pathways and chromosomal instability in gastric cancer: global lipidomics analysis. World J Gastrointest Oncol. 2019;11(3):181–94.
29. Brzozowski JS, Jankowski H, Bond DR, et al. Lipidomic profiling of extracellular vesicles derived from prostate and prostate cancer cell lines. Lipids Health Dis. 2018;17(1):211.
30. Zhou X, Mei H, Agee J, et al. Racial differences in distribution of fatty acids in prostate cancer and benign prostatic tissues. Lipids Health Dis. 2019;18(1):189.
31. Kregel S, Malik R, Asangani IA, et al. Functional and mechanistic interrogation of BET bromodomain degraders for the treatment of metastatic castration-resistant prostate cancer. Clin Cancer Res. 2019;25(13):4038–48.
32. Knific T, Vouk K, Smrkolj Š, et al. Models including plasma levels of sphingomyelins and phosphatidylcholines as diagnostic and prognostic biomarkers of endometrial cancer. J Steroid Biochem Mol Biol. 2018;178:312–21.

33. Cummings M, Massey KA, Mappa G, et al. Integrated eicosanoid lipidomics and gene expression reveal decreased prostaglandin catabolism and increased 5-lipoxygenase expression in aggressive subtypes of endometrial cancer. J Pathol. 2019;247(1):21–34.
34. Lee MY, Yeon A, Shahid M, et al. Reprogrammed lipid metabolism in bladder cancer with cisplatin resistance. Oncotarget. 2018;9(17):13231–43.
35. Vantaku V, Dong J, Ambati CR, et al. Multi-omics integration analysis robustly predicts high-grade patient survival and identifies CPT1B effect on fatty acid metabolism in bladder cancer. Clin Cancer Res. 2019;25(12):3689–701.
36. Cheng L, Zhang K, Qing Y, et al. Proteomic and lipidomic analysis of exosomes derived from ovarian cancer cells and ovarian surface epithelial cells. J Ovarian Res. 2020;13(1):9.
37. Wefers C, Duiveman-de Boer T, Zusterzeel PLM, et al. Different lipid regulation in ovarian cancer: inhibition of the immune system. Int J Mol Sci. 2018;19(1):273.
38. Tao L, Zhou J, Yuan C, et al. Metabolomics identifies serum and exosomes metabolite markers of pancreatic cancer. Metabolomics. 2019;15(6):86.
39. Arnoletti P, Wang J, Litherland S, et al. Lipidomics analysis of alterations in portal vein plasma lipids in pancreatic cancer patients. HPB. 2018;20:S537.
40. Guan L, Chen Y, Wang Y, et al. Effects of carnitine palmitoyltransferases on cancer cellular senescence. J Cell Physiol. 2019;234(2):1707–19.
41. Wang T, Chen X, Luan C, et al. High throughput lipid profiling for subtype classification of hepatocellular carcinoma cell lines and tumor tissues. Anal Chim Acta. 2020;1107:92–100.
42. Zhou L, Wang Z, Hu C, et al. Integrated metabolomics and lipidomics analyses reveal metabolic reprogramming in human glioma with IDH1 mutation. J Proteome Res. 2019;18(3):960–9.
43. Wojakowska A, Cole LM, Chekan M, et al. Discrimination of papillary thyroid cancer from non-cancerous thyroid tissue based on lipid profiling by mass spectrometry imaging. Endokrynol Pol. 2018;69(1):2–8.
44. Zhao X, Brusadelli MG, Sauter S, et al. Lipidomic profiling links the Fanconi anemia pathway to glycosphingolipid metabolism in head and neck cancer cells. Clin Cancer Res. 2018;24(11):2700–9.
45. Mohamed A, Collins J, Jiang H, et al. Concurrent lipidomics and proteomics on malignant plasma cells from multiple myeloma patients: probing the lipid metabolome. PLoS One. 2020;15(1):e0227455.
46. Sur S, Nakanishi H, Flaveny C, et al. Inhibition of the key metabolic pathways, glycolysis and lipogenesis, of oral cancer by bitter melon extract. Cell Commun Signal. 2019;17(1):131.
47. Bednarczyk K, Gawin M, Chekan M, et al. Discrimination of normal oral mucosa from oral cancer by mass spectrometry imaging of proteins and lipids. J Mol Histol. 2019;50(1):1–10.
48. Schaeffeler E, Büttner F, Reustle A, et al. Metabolic and lipidomic reprogramming in renal cell carcinoma subtypes reflects regions of tumor origin. Eur Urol Focus. 2019;5(4):608–18.
49. Zhang L, Lv J, Chen C, et al. Roles of acyl-CoA synthetase long-chain family member 5 and colony stimulating factor 2 in inhibition of palmitic or stearic acids in lung cancer cell proliferation and metabolism. Cell Biol Toxicol. 2020; https://doi.org/10.1007/s10565-020-09520-w.
50. Bergqvist F, Ossipova E, Idborg H, et al. Inhibition of mPGES-1 or COX-2 results in different proteomic and lipidomic profiles in A549 lung cancer cells. Front Pharmacol. 2019;10:636.
51. Dai L, Smith CD, Foroozesh M, et al. The sphingosine kinase 2 inhibitor ABC294640 displays anti-non-small cell lung cancer activities in vitro and in vivo. Int J Cancer. 2018;142(10):2153–62.
52. Zhang H, Wang Y, Guan L, et al. Lipidomics reveals carnitine palmitoyltransferase 1C protects cancer cells from lipotoxicity and senescence. J Pharm Anal. 2020; https://doi.org/10.1016/j.jpha.2020.04.004.
53. Chi JT, Lin PH, Tolstikov V, et al. Metabolomic effects of androgen deprivation therapy treatment for prostate cancer. Cancer Med. 2020;9:3691.
54. Clendinen CS, Gaul DA, Monge ME, et al. Preoperative metabolic signatures of prostate cancer recurrence following radical prostatectomy. J Proteome Res. 2019;18(3):1316–27.
55. Cadoni E, Vanhara P, Valletta E, et al. Mass spectrometric discrimination of phospholipid patterns in cisplatin-resistant and -sensitive cancer cells. Rapid Commun Mass Spectrom. 2019;33(1):97–106.
56. Nikpoor AR, Jaafari MR, Zamani P, et al. Cell cytotoxicity, immunostimulatory and antitumor effects of lipid content of liposomal delivery platforms in cancer immunotherapies. A comprehensive in-vivo and in-vitro study. Int J Pharm. 2019;567:118492.
57. Maekawa K, Ri M, Nakajima M, et al. Serum lipidomics for exploring biomarkers of bortezomib therapy in patients with multiple myeloma. Cancer Sci. 2019;110(10):3267–74.
58. Beloueche-Babari M, Casals Galobart T, Delgado-Goni T, et al. Monocarboxylate transporter 1 blockade with AZD3965 inhibits lipid biosynthesis and increases tumour immune cell infiltration. Br J Cancer. 2020;122(6):895–903.
59. Das UN. Can bioactive lipids augment anti-cancer action of immunotherapy and prevent cytokine storm? Arch Med Res. 2019;50(6):342–9.
60. Yan D, Adeshakin AO, Xu M, et al. Lipid metabolic pathways confer the immunosuppressive function of myeloid-derived suppressor cells in tumor. Front Immunol. 2019;10:1399.
61. Yang K, Han X. Lipidomics: techniques, applications, and outcomes related to biomedical sciences. Trends Biochem Sci. 2016;41(11):954–69.
62. Ma X, Chong L, Tian R, et al. Identification and quantitation of lipid C=C location isomers: a shotgun lipi-

domics approach enabled by photochemical reaction. Proc Natl Acad Sci U S A. 2016;113(10):2573–8.
63. De Berardinis RJ, Chandel NS. Fundamentals of cancer metabolism. Sci Adv. 2016;2(5):e1600200.
64. Beloribi-Djefaflia S, Vasseur S, Guillaumond F. Lipid metabolic reprogramming in cancer cells. Oncogenesis. 2016;5:e189.
65. Zalba S, Ten Hagen TL. Cell membrane modulation as adjuvant in cancer therapy. Cancer Treat Rev. 2017;52:48–57.
66. Keckesova Z, Donaher JL, De Cock J, et al. LACTB is a tumour suppressor that modulates lipid metabolism and cell state. Nature. 2017;543(7647):681–6.
67. Jeon SM, Chandel NS, Hay N. AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. Nature. 2012;485(7400):661–5.
68. Pascual G, Avgustinova A, Mejetta S, et al. Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature. 2017;541(7635):41–5.
69. Viswanathan VS, Ryan MJ, Dhruv HD, et al. Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. Nature. 2017;547(7664):453–7.
70. Park JK, Coffey NJ, Limoges A, et al. The heterogeneity of lipid metabolism in cancer. Adv Exp Med Biol. 2018;1063:33–55.
71. Agatonovic-Kustrin S, Morton DW, Smirnov V, et al. Analytical strategies in lipidomics for discovery of functional biomarkers from human saliva. Dis Markers. 2019;2019:6741518.

Overview: Lipid Metabolism in the Tumor Microenvironment

3

Kaili Ma and Lianjun Zhang

Abstract

The tumor microenvironment represents the dynamic network consisting of tumor cells, stromal cells, and multiple lineages of immune subsets. It is well recognized that metabolic crosstalk within the tumor microenvironment (TME) greatly shapes both the composition and functionality of the infiltrated immune cells and therefore critically regulate the antitumor immunity. In general, most solid tumors are considered as lipid-enriched environment, which is beneficial for tumor cell growth and immune escape. Here we briefly summarize the effects of accumulated lipids on tumor cells and immune cells. We also discuss the possibility of targeting lipid metabolism within the TME and potential strategies for optimizing cancer treatment.

Keywords

Tumor microenvironment · Lipid metabolism
Immunosuppression · Antitumor immunity
T cells

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3.1 Accumulation of Lipids Within the TME

Cancer cell progression depends on a specialized niche termed the tumor microenvironment (TME), which contains extracellular matrix and numerous cell types including cancer cells, endothelial cells, stromal fibroblasts, and diverse subsets of immune cells [1]. The nutrient conditions such as glucose, amino acids, and fatty acids (FAs) are varied in the TME due to the high needs of tumor cells for nutrients to sustain their energy production and biological functions. This nutrient competition within the TME in turn leads to metabolic reprogramming of both cancer cells and immune cells, which may have profound effects on the composition/density of infiltrated immune cell types, or directly modulate their functionality to facilitate tumor growth and immunosuppression [2, 3]. Recently, numerous efforts have been devoted to understanding the metabolic crosstalk between cancer cells and immune cells with the aim to improve the immunotherapeutic efficacy against tumor [4–6].

Lipids are categorized into multiple subtypes according to their structural/biological features, including phospholipids, FAs, triglycerides, cholesterol, cholesterol esters, sphingolipids, etc. As one of the crucial energy sources and critical components of cellular membranes, lipid metabolism is tightly related to cell proliferation and function in the TME [6, 7]. Certain lipids such

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as sphingosine-1-phosphate, prostaglandins, diacylglycerol, and phosphatidylinositol phosphates and some derivatives of cholesterol can act as signaling molecules to regulate cell growth and function [8–11]. Numerous studies have shown that free FAs are abundant across several human tumor tissues. Despite the critical role of FAs to support energy production *via* FA β-oxidation (FAO) for proliferative cancer cells, enhancement of FAO in certain immune cells such as regulatory T cells (Tregs) and tumor-associated macrophages (TAMs) is recognized to be important for their survival and suppressive function in progressing tumors [12, 13]. In addition to FAs, cholesterol, an essential component of the plasma membrane, is also correlated to cancer growth and metastases [14]. Excess free cholesterol is esterified by cholesterol acyltransferase-1 (ACAT1) to form cholesteryl esters for storage in lipid droplets [15]. Cholesteryl esters were found in multiple tumor tissues such as breast cancer, glioma, and prostate cancer, while they are usually undetectable in the corresponding healthy tissues [16–18].

The mechanisms underlying lipid accumulation in TAMs are not clear. Xiang et al. demonstrated that monoacylglycerol lipase (MGLL) deficiency results in lipid overload in TAMs from colon cancer models. Furthermore, myeloid-specific deletion of MGLL could promote tumor growth, which is largely attributed to macrophage-mediated immunosuppression and decreased $CD8^+$ T cell functionality. On the other hand, overexpression of MGLL stimulates proinflammatory cytokine production and M1 polarization and superior antitumor effects [19]. With the rapid development of detection techniques, more powerful assays of high-throughput measurements of lipid species and quantity within the TME are needed. Yet, it remains to be determined how lipids were generated and accumulated in diverse cell subsets, and also it is important to understand their biological consequences in tumor progression and metastasis.

3.2 Lipid Metabolism in Tumor Metastasis

Consistent with high requirement for lipids, the lipogenesis and lipid uptake are also increased in a variety of cancers, which is recognized as a hallmark of malignancy [6, 7]. Cancer cells utilize acetyl-coenzyme A (AcCoA) that is mainly from citrate tricarboxylic acid (TCA) cycle for de novo lipid synthesis [20]. The key regulators of lipogenesis such as FA synthase (FASN), SREBPs, stearoyl-CoA desaturase 1 (SCD1), and acetyl-CoA carboxylase (ACC) are significantly upregulated in various human cancers [7, 21–23]. In addition to de novo lipid synthesis, mammalian cells can uptake lipid directly from the exogenous environment. For instance, CD36 is responsible for transporting FAs into the cell and increases lipid metabolism that would promote tumor cell growth and metastasis [24–26]. Similarly, mammalian cells can obtain cholesterol from the uptake of low-density lipoprotein (LDL) except for de novo synthesis. Binding of LDL with the membrane-bound LDL receptor (LDLR) is internalized and transported to late endosomes/lysosomes, where lysosomal acid lipase (LAL) could digest targets and lead to free cholesterol release [27]. In addition, accumulating evidences from in vivo and vitro experiments have shown that cancer-associated fibroblasts (CAFs) could transfer lipid to the TME which serve as a hub of FAs to support cancer cell growth (Fig. 3.1) [28, 29]. Moreover, ovarian cancer cells can even induce metabolic alterations in neighboring adipocytes to release free FAs to the microenvironment, which thus provide energy supply to support cancer cell proliferation [30].

As mentioned above, cancer cells tend to use FAO to survive from the nutrient-deprived microenvironment. Angiogenesis inhibitor treatment-induced hypoxia and nutrient-deprived condition were found to promote cancer cell proliferation by increasing lipid metabolic reprogramming [31]. Therefore, targeting carnitine palmitoyltrans-

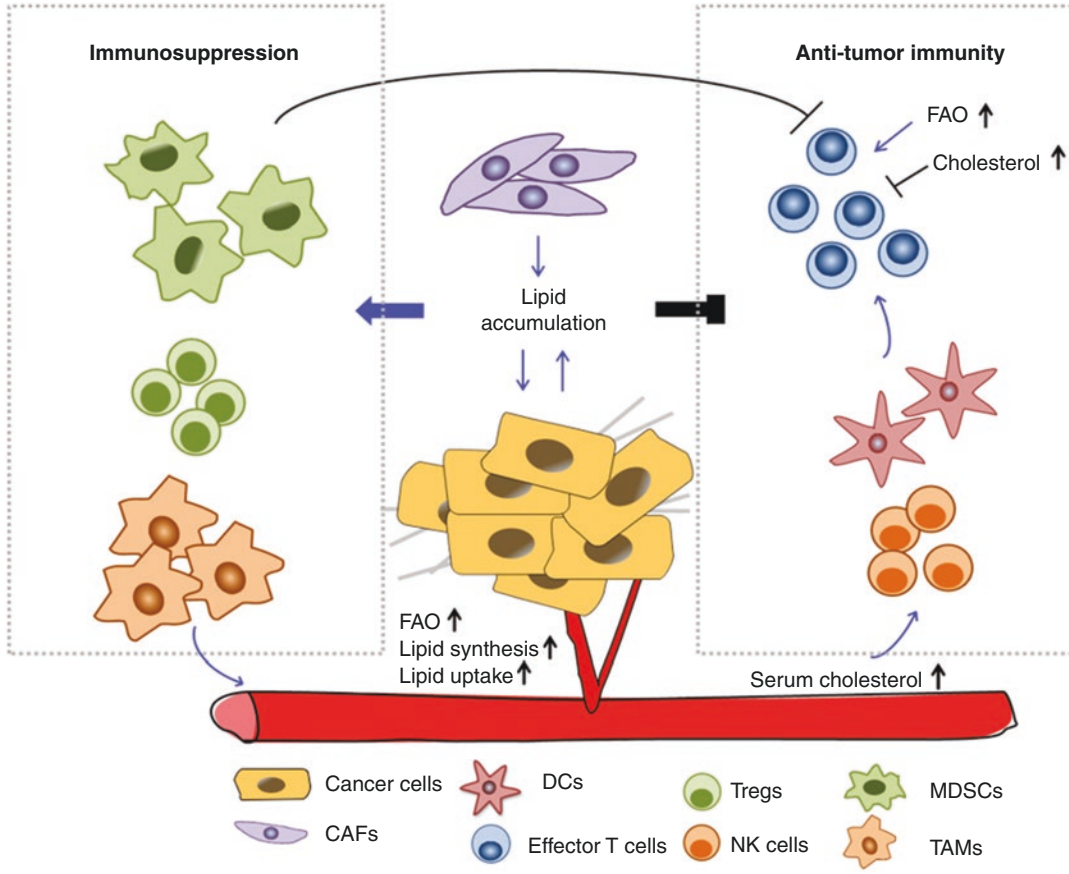

Fig. 3.1 Reprogramming of lipid metabolism in the TME. Cancer cells evolve to adapt to nutrient-deprived microenvironment by utilizing lipid catabolism to supply energy. Together with cancer-associated fibroblasts (CAFs) transferring lipid to the TME, increased lipid synthesis within cancer cells may cause lipid accumulation in the TME, which further modulates the immune response. MDSCs, TAMs, and Tregs benefit from enhanced lipid and increased FAO to support their immunosuppression and pro-tumoral functions. Of note, excessive lipids could be detrimental for effector functions of certain immune cells, which lead to impaired antitumor immunity. For instance, accumulation of FAs inhibits NK cell cytotoxicity and tumor-associated antigen-presenting capacity of DCs, and superfluous cholesterol can induce $CD8^+$ T cell exhaustion

ferase 1α (CPT1α), a key factor in β-oxidation in charge of FAs shuttling into mitochondria to initial β-oxidation, acts as an effective strategy to improve anticancer effects in various animal models [31–33]. Given that significantly upregulated lipogenesis and lipid uptake in cancer cells, targeting these pathways has been proved to be effective for treatment of some cancers. For instance, the effects of lipogenic enzyme FASN inhibitor, TVB-2640, are actively under clinical investigation in patients with solid tumors [34]. The frequency of CD36-positive cells in human oral carcinomas, melanoma, and luminal A breast cancer is more predisposed to initiate metastasis than that with low level of CD36 expression, which is consistent with clinical prognosis [24]. Further blocking CD36 on tumor cells by antibodies completely inhibits metastasis in both

immunodeficient and immunocompetent orthotopic mouse models of human oral cancer [24]. Likewise, targeting key components of cholesterol synthesis pathway has shown promising efficacy with preclinical mouse models, and the efficacy of statins combined with chemotherapeutic agents is also actively explored in clinical trials [35, 36]. Of note, excess intracellular free cholesterol is cytotoxic to cells due to higher ER stress, which may induce subsequent apoptosis. Therefore, inhibiting cholesterol esterification is proposed as a novel strategy for treating certain metastatic cancers [37].

3.3 Lipid Metabolism in Vascular Endothelial Cells and Angiogenesis

In contrast to most differentiated cells which rely more on mitochondrial oxidative phosphorylation, endothelial cells (ECs) are highly glycolytic and thus accumulate large amounts of lactate, even under sufficient oxygen [38]. VEGF could further increase glycolysis via enhanced glucose uptake and activation of phosphofructokinase-2/fructose-2,6-bisphosphatase 3 (PFKFB3). Consistently, silencing PFKFB3 results in impaired angiogenesis [39]. Intriguingly, inactivation of PFKFB3 activity in ECs leads to tumor vessel normalization, which impairs metastasis and promotes chemotherapy response rate [40]. FAO is needed for EC proliferation during vessel sprouting. Of note, quiescent ECs show higher FAO rate than proliferating ECs. Interestingly, increased FAO within quiescent ECs is not required for energy homeostasis or biomass synthesis. Instead, quiescent ECs rely heavily on FAO for NADPH regeneration and maintenance of redox balance [41]. Notch signaling activation triggers CPT1α transcription for FAO. Specific loss of CTP1α in ECs triggers impaired de novo nucleotide synthesis for DNA replication, with energy production and redox homeostasis unaffected [42].

3.4 Lipid Metabolism in Immunosuppression

In addition to alternations of lipid metabolism within tumor cells, the metabolic restrictions within the TME might also shape the distribution profiles of the infiltrating immune cells and their functionality (Fig. 3.1). TAMs represent the major fraction of the myeloid infiltrates in the TME to support tumor growth and metastasis through promoting immunosuppression and angiogenesis [43–45]. For instance, both mouse and human TAMs were found to increase lipid uptake due to elevated CD36 expression, which is required for TAM differentiation and function [46]. In addition, recent evidence suggests that extracellular FAs, particularly unsaturated FAs, can induce the polarization of pro-tumoral TAMs from infiltrating monocytes (Fig. 3.1), and disrupting lipid droplet-derived FAs in myeloid cells reduces tumor progression [47]. Moreover, phospholipid metabolism was also found to modulate the TME and thus tumor progression. For instance, a recent study demonstrated that the level of lysophosphatidic acid was decreased in colorectal carcinoma as compared with paracarcinoma regions, consistent with increased expression of 1-acylglycerol-3-phosphate O-acyltransferase 4 (Agpat4). Interestingly, Agpat4 deficiency decreases M2 whereas promotes M1-like macrophage polarization, which further boost T cell function and antitumor immunity [48]. Genetic deficiency of receptor-interacting protein kinase 3 (RIPK3) in TAMs of hepatocellular carcinoma leads to enhanced FAO and pro-tumoral M2 polarization due to PPAR activation [49].

With a mouse model of metastatic ovarian cancer, Goossens et al. clearly demonstrate that increased cholesterol efflux in TAMs critically modulates their tumor-promoting function, which is associated with increased IL-4 signaling and inhibition of IFN-γ transcriptional programs [50]. Therefore, preventing cholesterol efflux in TAMs represents a potentially novel antitumor therapeutic strategy [50]. Similarly,

previous studies show that FA uptake and FAO are also increased in tumor-infiltrating MDSCs (T-MDSCs) which inhibit antitumor immune response (Fig. 3.1) [51]. Intriguingly, one recent study elegantly demonstrated that CD36 was selectively increased in Tregs which represents another major barrier to antitumor immunity. As a result, these Treg cells exhibit enhanced FA uptake and lipid metabolism to support their survival and function. Treg-specific deletion of CD36 leads to decreased intratumoral Treg cells and delayed tumor growth, which is accompanied by enhanced infiltration of TILs and antitumor immunity [52]. Further targeting CD36 of intratumoral Treg cells by α-CD36 antibody leads to impaired Treg cell function and reinforces antitumor immunity [52]. Altogether, elevated CD36 expression on either TAMs or intratumoral Tregs leads to enhanced FAO and immunosuppression (Fig. 3.1). Targeting lipid uptake or selective inhibition of FAO within those immunosuppressive cells might serve as a potential therapeutic strategy of human cancer treatment.

3.5 Lipid Metabolism in T Cell/NK Antitumor Immunity

Among tumor-infiltrated immune cells, $CD8^+$ T cells play a central role in antitumor immunity however their effector functions are largely suppressed in the TME [53]. Considering that activated $CD8^+$ T cell mainly relies on aerobic glycolysis to supply energy for productive growth and cytokine production [54], it is reasonable to speculate that tumor-infiltrating $CD8^+$ T cells undergo functional decline (exhaustion) in response to hypoxia and low levels of glucose and/or other nutrients within the TME. Indeed, enhancing FA catabolism by PPARα agonist fenofibrate leads to enhanced $CD8^+$ T cell tumor-killing functions and significantly strengthens antitumor efficacy when synergizing with PD-1 blockade therapy in vivo [55]. However, increased lipid content in dendritic cells (DCs) was found to reduce capacity to present tumor-associated antigens [56]. Likewise, natural killer (NK) cells, another subset of cytotoxic immune cells in tumor immunosurveillance, display functional inhibition state in response to PPARα-mediated increased FA [57]. Lipid accumulation in NK cells inhibited trafficking of the cytotoxic machinery to the NK cell-tumor synapse and impaired immunosurveillance (Fig. 3.1). Blocking PPARα/δ signaling or the transport of lipids into mitochondria reversed NK cell cytotoxicity and reduced tumor growth [57]. Moreover, the accumulation of cholesterol in NK cells that was induced by high serum levels of cholesterol could stimulate their effector functions to protect against hepatoma cells in mouse model [58]. Of note, the effects of FA metabolism in modulating $CD8^+$ T cell or NK cell functionality/exhaustion is still in its infancy, which deserves further investigations.

Additionally, T cell-specific deletion of the key cholesterol esterification enzyme ACAT1 leads to enhanced $CD8^+$ T cell proliferation and superior effector function and in vivo antitumor immunity. In addition, pharmacological inhibition of ACAT1 with avasimibe shows better efficacy against mouse melanoma progression when combined with anti-PD1 blockade [59]. On the other hand, it was also shown that cholesterol was increased in the TME and elevated cholesterol level led to $CD8^+$ T cell exhaustion. Indeed, cholesterol was found to upregulate expression of co-inhibitory molecules such as PD-1 and 2B4 upregulation on $CD8^+$ T cells via triggering ER stress [5, 55]. Consistently, cholesterol inhibits IL-9-producing $CD8^+$ T cell (Tc9) differentiation and their antitumor activity, and reducing cholesterol levels could enhance Tc9 cell in vivo persistence and antitumor immunity in an IL-9-dependent manner [60]. Yet, it remains largely unknown how tumor-derived cholesterol metabolically reprograms $CD8^+$ T cell exhaustion. Development of strategies that reduce the cholesterol content within the TME may represent promising therapeutic targets that could restore the impaired functionality of TILs (Fig. 3.1).

3.6 Conclusions and Perspectives

Cancer cells' adaptive metabolic alterations caused lipid-enriched TME shapes both the composition and functionality of the infiltrated immune cells that finally protect tumor growth from immune response. Mitochondria is closely connected to FA metabolism, and it requires further characterization how alterations of lipid metabolism affect mitochondrial fitness (content and quality) regarding diverse cell types within the TME. Of note, given the possible opposite outcomes and the complexity of the TME, it remains challenging to target lipid metabolism specifically in a defined cell type and to achieve maximal antitumor effects when designing strategies for cancer immunotherapy.

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References

1. Egeblad M, et al. Tumors as organs: complex tissues that interface with the entire organism. Dev Cell. 2010;18(6):884–901.
2. Reina-Campos M, et al. Metabolism shapes the tumor microenvironment. Curr Opin Cell Biol. 2017;48:47–53.
3. Lyssiotis CA, Kimmelman AC. Metabolic interactions in the tumor microenvironment. Trends Cell Biol. 2017;27(11):863–75.
4. Binnewies M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med. 2018;24(5):541–50.
5. Ma X, et al. Cholesterol induces CD8(+) T cell exhaustion in the tumor microenvironment. Cell Metab. 2019;30(1):143–15.6.e5.
6. Anderson KG, et al. Obstacles posed by the tumor microenvironment to T cell activity: a case for synergistic therapies. Cancer Cell. 2017;31(3):311–25.
7. Cheng C, et al. Lipid metabolism reprogramming and its potential targets in cancer. Cancer Commun (Lond). 2018;38(1):27.
8. Cartier A, Hla T. Sphingosine 1-phosphate: lipid signaling in pathology and therapy. Science. 2019;366(6463):eaar5551.
9. Marra AN, et al. Prostaglandin signaling regulates renal multiciliated cell specification and maturation. Proc Natl Acad Sci U S A. 2019;116(17):8409–18.
10. Eichmann TO, Lass A. DAG tales: the multiple faces of diacylglycerol—stereochemistry, metabolism, and signaling. Cell Mol Life Sci. 2015;72(20):3931–52.
11. Marat AL, Haucke V. Phosphatidylinositol 3-phosphates-at the interface between cell signalling and membrane traffic. EMBO J. 2016;35(6):561–79.
12. Michalek RD, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. J Immunol. 2011;186(6):3299–303.
13. Huang SC, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol. 2014;15(9):846–55.
14. Silvente-Poirot S, Poirot M. Cancer. Cholesterol and cancer, in the balance. Science. 2014;343(6178):1445–6.
15. Qian H, et al. Structural basis for catalysis and substrate specificity of human ACAT1. Nature. 2020;581(7808):333–8.
16. de Gonzalo-Calvo D, et al. Intratumor cholesteryl ester accumulation is associated with human breast cancer proliferation and aggressive potential: a molecular and clinicopathological study. BMC Cancer. 2015;15:460.
17. Bemlih S, et al. Acyl-coenzyme A: cholesterol acyltransferase inhibitor Avasimibe affect survival and proliferation of glioma tumor cell lines. Cancer Biol Ther. 2010;9(12):1025–32.
18. Yue S, et al. Cholesteryl ester accumulation induced by PTEN loss and PI3K/AKT activation underlies human prostate cancer aggressiveness. Cell Metab. 2014;19(3):393–406.
19. Xiang W, et al. Monoacylglycerol lipase regulates cannabinoid receptor 2-dependent macrophage activation and cancer progression. Nat Commun. 2018;9(1):2574.
20. Currie E, et al. Cellular fatty acid metabolism and cancer. Cell Metab. 2013;18(2):153–61.
21. Rohrig F, Schulze A. The multifaceted roles of fatty acid synthesis in cancer. Nat Rev Cancer. 2016;16(11):732–49.
22. Santos CR, Schulze A. Lipid metabolism in cancer. FEBS J. 2012;279(15):2610–23.
23. Shimano H, Sato R. SREBP-regulated lipid metabolism: convergent physiology—divergent pathophysiology. Nat Rev Endocrinol. 2017;13(12):710–30.
24. Pascual G, et al. Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature. 2017;541(7635):41–5.
25. Yang P, et al. Dietary oleic acid-induced CD36 promotes cervical cancer cell growth and metastasis via up-regulation Src/ERK pathway. Cancer Lett. 2018;438:76–85.
26. Nath A, et al. Elevated free fatty acid uptake via CD36 promotes epithelial-mesenchymal transition in hepatocellular carcinoma. Sci Rep. 2015;5:14752.

27. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. Cell. 2015;161(1):161–72.
28. Lopes-Coelho F, et al. Breast cancer metabolic cross-talk: fibroblasts are hubs and breast cancer cells are gatherers of lipids. Mol Cell Endocrinol. 2018;462(Pt B):93–106.
29. Santi A, et al. Cancer associated fibroblasts transfer lipids and proteins to cancer cells through cargo vesicles supporting tumor growth. Biochim Biophys Acta. 2015;1853(12):3211–23.
30. Nieman KM, et al. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. Nat Med. 2011;17(11):1498–503.
31. Iwamoto H, et al. Cancer lipid metabolism confers antiangiogenic drug resistance. Cell Metab. 2018;28(1):104–117.e5.
32. Samudio I, et al. Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction. J Clin Invest. 2010; 120(1):142–56.
33. Liu PP, et al. Elimination of chronic lymphocytic leukemia cells in stromal microenvironment by targeting CPT with an antiangina drug perhexiline. Oncogene. 2016;35(43):5663–73.
34. Jones SF, Infante JR. Molecular pathways: fatty acid synthase. Clin Cancer Res. 2015;21(24):5434–8.
35. Ginestier C, et al. Mevalonate metabolism regulates basal breast cancer stem cells and is a potential therapeutic target. Stem Cells. 2012;30(7):1327–37.
36. Kuzu OF, et al. The role of cholesterol in cancer. Cancer Res. 2016;76(8):2063–70.
37. Li J, et al. Abrogating cholesterol esterification suppresses growth and metastasis of pancreatic cancer. Oncogene. 2016;35(50):6378–88.
38. Potente M, Carmeliet P. The link between angiogenesis and endothelial metabolism. Annu Rev Physiol. 2017;79:43–66.
39. De Bock K, et al. Role of PFKFB3-driven glycolysis in vessel sprouting. Cell. 2013;154(3):651–63.
40. Cantelmo AR, et al. Inhibition of the glycolytic activator PFKFB3 in endothelium induces tumor vessel normalization, impairs metastasis, and improves chemotherapy. Cancer Cell. 2016;30(6):968–85.
41. Kalucka J, et al. Quiescent endothelial cells upregulate fatty acid beta-oxidation for Vasculoprotection via redox homeostasis. Cell Metab. 2018;28(6):881–894.e13.
42. Schoors S, et al. Fatty acid carbon is essential for dNTP synthesis in endothelial cells. Nature. 2015;520(7546):192–7.
43. Gajewski TF, et al. Innate and adaptive immune cells in the tumor microenvironment. Nat Immunol. 2013;14(10):1014–22.
44. Vitale I, et al. Macrophages and metabolism in the tumor microenvironment. Cell Metab. 2019;30(1):36–50.
45. Ngambenjawong C, et al. Progress in tumor-associated macrophage (TAM)-targeted therapeutics. Adv Drug Deliv Rev. 2017;114:206–21.
46. Su P, et al. Enhanced lipid accumulation and metabolism are required for the differentiation and activation of tumor-associated macrophages. Cancer Res. 2020;80(7):1438–50.
47. Wu H, et al. Lipid droplet-dependent fatty acid metabolism controls the immune suppressive phenotype of tumor-associated macrophages. EMBO Mol Med. 2019;11(11):e10698.
48. Zhang D, et al. The Agpat4/LPA axis in colorectal cancer cells regulates antitumor responses via p38/p65 signaling in macrophages. Signal Transduct Target Ther. 2020;5:24.
49. Wu L, et al. RIPK3 orchestrates fatty acid metabolism in tumor-associated macrophages and hepatocarcinogenesis. Cancer Immunol Res. 2020;8(5):710–21.
50. Goossens P, et al. Membrane cholesterol efflux drives tumor-associated macrophage reprogramming and tumor progression. Cell Metab. 2019;29(6):1376–1389.e4.
51. Hossain F, et al. Inhibition of fatty acid oxidation modulates immunosuppressive functions of myeloid-derived suppressor cells and enhances cancer therapies. Cancer Immunol Res. 2015;3(11):1236–47.
52. Wang H, et al. CD36-mediated metabolic adaptation supports regulatory T cell survival and function in tumors. Nat Immunol. 2020;21(3):298–308.
53. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. Science. 2015;348(6230):74–80.
54. Raud B, et al. Fatty acid metabolism in CD8(+) T cell memory: challenging current concepts. Immunol Rev. 2018;283(1):213–31.
55. Zhang Y, et al. Enhancing CD8(+) T cell fatty acid catabolism within a metabolically challenging tumor microenvironment increases the efficacy of melanoma immunotherapy. Cancer Cell. 2017;32(3):377–391.e9.
56. Herber DL, et al. Lipid accumulation and dendritic cell dysfunction in cancer. Nat Med. 2010;16(8): 880–6.
57. Michelet X, et al. Metabolic reprogramming of natural killer cells in obesity limits antitumor responses. Nat Immunol. 2018;19(12):1330–40.
58. Qin WH, et al. High serum levels of cholesterol increase antitumor functions of nature killer cells and reduce growth of liver tumors in mice. Gastroenterology. 2020;158(6):1713–27.
59. Yang W, et al. Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. Nature. 2016;531(7596):651–5.
60. Ma X, et al. Cholesterol negatively regulates IL-9-producing CD8(+) T cell differentiation and antitumor activity. J Exp Med. 2018;215(6):1555–69.

Lipid Metabolism in Cancer Cells

4

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Abstract

Metabolic reprogramming is one of the most critical hallmarks in cancer cells. In the past decades, mounting evidence has demonstrated that, besides the Warburg Effect, lipid metabolism dysregulation is also one of the essential characteristics of cancer cell metabolism. Lipids are water-insoluble molecules with diverse categories of phosphoglycerides, triacylglycerides, sphingolipids, sterols, *etc*. As the major utilization for energy storage, fatty acids are the primary building blocks for synthesizing triacylglycerides. And phosphoglycerides, sphingolipids, and sterols are the main components constructing biological membranes. More importantly, lipids play essential roles in signal transduction by functioning as second messengers or hormones. Much evidence has shown specific alterations of lipid metabolism in cancer cells. Consequently, the structural configuration of biological membranes, the energy homeostasis under nutrient stress, and the abundance of lipids in the intracellular signal transduction are affected by these alterations. Furthermore, lipid droplets accumulate in cancer cells and function adaptively to different types of harmful stress. This chapter reviews the regulation, functions, and therapeutic benefits of targeting lipid metabolism in cancer cells. Overall, this chapter highlights the significance of exploring more potential therapeutic strategies for malignant diseases by unscrambling lipid metabolism regulation in cancer cells.

Keywords

Lipid metabolism · Cancer · Metabolic reprogramming · Cancer therapy

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4.1 Introduction

More and more oncology studies have revealed metabolic reprogramming as a hallmark of cancer [1, 2]. It has been shown that cancer cells in rapid proliferation exhibit high demands for macromolecule biosynthesis and energy consumption. The increased demands of glycolysis and glutamine consumption are even more [3–5]. For instance, the Warburg Effect decouples glycolysis from pyruvate oxidation in the glucose metabolism of cancer cells. Consequently, despite high oxygen availability in cancer cells, mitochondrial respiration cannot generate maximal ATP through the utilization of carbohydrates. A deeper understanding of these metabolic changes has accelerated new therapeutic approaches to cancer [3]. In recent years, though receiving less attention, lipid metabolic reprogramming in cancer cells has been increasingly recognized (Fig. 4.1). It has been widely accepted that lipid metabolic reprogramming is a critical molecular process in the progression of human malignancies [1, 6, 7]. By promoting the exogenous lipids' uptake or increasing endogenous lipid synthesis, cancer cells with active proliferation show high lipid avidity [8]. A growing body of studies has proved that, as most of the lipogenic enzymes are activated, cancer cells exhibit a shift in lipid metabolism [9]. For example, it has been reported that cancer cells cultured with a medium containing lipoprotein-deficient serum could significantly inhibit proliferation and increase cell death. However, supplementation of high-density lipoprotein (HDL), low-density lipoprotein (LDL), or very-low-density lipoprotein (VLDL) into the serum could partially restore the growth rate of transformed cells, suggesting the supportive role of lipoproteins in tumor growth [10]. Moreover, it has been reported that prostate cancer cells could significantly elevate the uptake of exogenous cholesterol and lipoproteins, leading to accumulation in lipid droplets (LDs) of cholesteryl ester (CE) and its storage depletion. This metabolic alteration reduces cancer proliferation, impairs its invasion capability, and suppresses tumor growth [11]. The LDs reserve excessive lipids and cholesterol in cancer cells. Therefore, high LDs and stored-CE content are now considered a hallmark of tumor aggressiveness [12]. All these discoveries emphasize the importance of lipid metabolism reprogramming, which plays a critical role in cancer cells.

4.2 Overview of Lipid Metabolic Reprogramming in Cancer Cells

Cancer happens in cells with disordered growth and proliferation, requiring nucleic acids, proteins, and lipids as building blocks. As sources

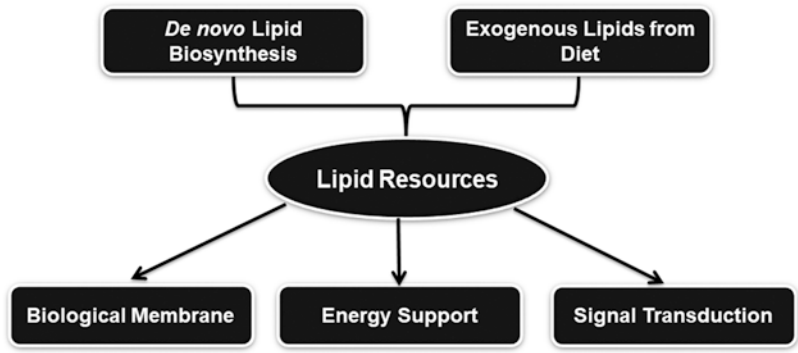

Fig. 4.1 Summary of the origin of lipids in mammalian cells. Lipid resources in cells are from de novo biosynthesis or the uptake of exogenous lipids. Due to the activation of oncogenic pathways, nutrition stress, or energy requirements for macromolecule biosynthesis in the transformed cells, genes regulating these two biological processes are expressed significantly. The abundance of lipids participates in membrane construction, energy support, signal transduction, etc. in cancer cells

of these building blocks, metabolic intermediates are often accumulated in cancer cells due to the disturbed metabolism. In the Warburg Effect, the most understood glucose metabolic disturbance in cancer cells, the carbon from glucose is used to build other molecules instead of complete oxidization to carbon dioxide [13]. During metabolism in normal cells, glucose undergoes glycolysis in the cytoplasm to produce pyruvate when the oxygen is sufficient. Pyruvate is then oxidized to acetyl-CoA after entering the mitochondria. Acetyl-CoA is a component of the Krebs cycle to reduce equivalents for oxidative phosphorylation. In the cytoplasm, excess pyruvate is fermented to lactate when the oxygen is limited. Due to the high efficiency, differentiated cells typically yield 36 ATP molecules with one glucose molecule undergoing the complete oxidative phosphorylation. Meanwhile, 2 ATP molecules are obtained from anaerobic glycolysis. The Warburg Effect fermentates pyruvate even in the presence of oxygen. It is characterized by lactate production, increased glucose uptake and consumption, and a decrease in oxidative phosphorylation. The increased glutamine metabolism marks another commonly observed alteration in cancer cells. By producing α-ketoglutarate to feed into the Krebs cycle, glutamine is a primary energy substrate in mammalian cells. α-ketoglutarate derived from glutamine contributes to citrate production by forwarding flux through the malic enzyme-dependent pyruvate and the Krebs cycle [14]. Through reversing the Krebs cycle reactions catalyzed by isocitrate dehydrogenase and aconitase, glutamine can also be converted to citrate. The citrate can then be used to generate acetyl groups for fatty acids (FAs) synthesis [15–17].

Lipid metabolism is altered in rapidly proliferative cells. The products of FAs are the hub in lipid metabolism. In the membrane, storage, or signaling lipids, incorporated intracellular FAs can be found. Otherwise, these FAs can be oxidized to carbon dioxide as an energy source [18–20]. In transformed cells from energy production, carbon is diverted to FAs for membranes and signaling molecules' biosynthesis. In addition to sterols and sphingolipids, many of the cell membrane lipids are phospholipids (PLs), including phosphatidylcholine (PC) and phosphatidylethanolamine (PE). All these lipids are derived partially from acetyl-CoA or contain FAs. Either exogenous sources or de novo FA synthesis constitutes the FA building blocks in cancer cells. Interestingly, normal cells and transformed cells have a distinct preference for the utilization of FAs. Most normal cells prefer to use exogenous sources of FAs, while tumor cells preferentially synthesize FAs de novo, and a shift toward FA synthesis is often seen in these cells [21, 22]. For the transfer to the active status, FAs are covalently modified by CoA via fatty acyl-CoA synthetases. Upon activation, FAs are esterified with glycerol or sterol backbones, thus producing TGs and sterol esters (SEs). Then they are stored in the LDs. Besides de novo FA synthesis pathways, it was also found that some cancer cells scavenge lipids from the environment, indicating that the FA uptake pathways might be a critical molecular event for the malignant behaviors in cancer cells. Due to the difference between in vitro and in vivo conditions for cell culture experiments, the exogenous uptake may be more important in some types of cancer cells. For example, fatty acid-binding protein 4 (FABP4), a lipid chaperone, is involved in providing FAs for tumor growth, chemo-resistance, and even cancer metastasis of ovarian cancer cells from surrounding adipocytes [23–25]. In the study of prostate cancer, the utilization of fatty acid synthase (FASN) or ATP citrate lyase (ACLY) inhibitors can only reduce tumor cell viability in cell culture medium deprived with lipoproteins, an exogenous lipid source [26]. And more importantly, a serial of studies in recent years showed that CD36 is related to multiple cancers' malignant behaviors. This molecule is a widely expressed transmembrane protein with multiple functions, including fatty acid uptake [27–30]. All the evidence indicates the reprogramming of lipid metabolism in cancer cells is in numerous dimensions (Fig. 4.2). Deeply understanding the undergoing mechanism for it would further extend our knowledge in cancer biology and help us explore more specific strategies to treat these malignant diseases.

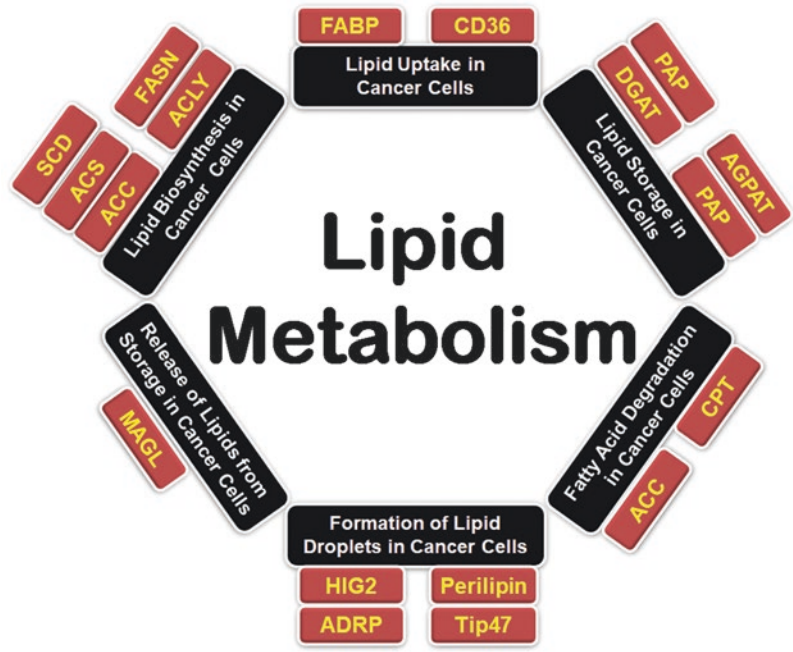

Fig. 4.2 Summary of some essential aspects of lipid metabolism in cancer cells. Some critical functional genes regulating lipid uptake, lipid biosynthesis, anabolic and catabolic metabolism of lipids, *etc*. are shown. All detailed functions and the full name of the genes' abbreviation will be described in the following context

4.2.1 Signaling Functions of Lipid Products in Cancer Cells

The stimulated biosynthesis of FAs and cholesterol and the mobilization of free FAs from triacylglycerides increase the lipids' level. As a result, the signaling functions of these lipid products contribute to different aspects of tumorigenesis. As a crucial component of lipid rafts, cholesterol can stimulate receptor-mediated signal transduction pathways [31]. Additionally, farnesyl-pyrophosphate, an intermediate of cholesterol synthesis, is required for protein prenylation. The addition of an isoprenoid chain modifies several vital proteins in the signal transduction. For example, geranyl-geranylation is essential for Rho, Rac, and cdc42 activity, while farnesylation is required to activate Ras and Rheb proteins [32]. Interestingly, suppressing the activity of retinoblastoma tumor suppressor RB causes senescence of cells by increasing the prenylation of N-Ras. This regulation is realized through the E2 transcription factor (TF)-dependent activation of sterol regulatory element-binding proteins (SREBP) [33]. All the evidence emphasizes the crucial role of lipid-mediated modification in cellular signal transduction.

For paracrine hormones and growth factors, including leukotrienes, prostaglandins, steroid hormones, and lysophosphatidic acid (LPA), lipids can also be a structural basis. The 20-carbon unit arachidonic acid is the source of leukotrienes and prostaglandins. And this acid is produced from phosphoglycerides by phospholipases A2 and C. Prostaglandin synthesized by the enzyme cyclooxygenase-2 (COX-2) has been implicated in the promotion of tumor growth, neovascularization, and metastatic cancer spread by activation of inflammatory responses [34]. LPA, a water-soluble phospholipid, comprises a phosphate group, a single fatty acid chain, and glycerol. It has been demonstrated that aberrantly high LPA activation can promote the initiation and progression of multiple cancers. Mechanistically, it promotes cancer cell survival, proliferation, and even migration through regulating the G-protein-coupled receptors [35]. In recent years, it has been demonstrated that the alteration of the free FAs level contributes to the progression and tumorigenicity of cancer.

The enzyme monoacylglycerol lipase (MAGL), highly expressed in aggressive cancer cell lines and primary tumors, catalyzes monoacylglycerides to free FAs and glycerol. It regulates a FA network promoting the survival, growth, invasion, and migration of tumor cells, enriched with oncogenic signaling lipids. In nonaggressive cancer cells, the overexpression of MAGL can recapitulate this FA network and enhance their pathogenicity phenotypes. A high-fat diet can rescue the impairments of growth in cancer cells lacking MAGL activity, implying the exogenous sources of FAs can contribute to MAGL-dependent malignancies [36]. Together, these findings reveal how a lipolytic enzyme can turn the cancer cells' lipogenic state into pro-tumor signals. Accumulative evidence has highlighted the importance of lipid products in the intracellular signal transduction of cancer cells.

4.2.2 Alterations in Lipid Metabolism in Cancer Cells

4.2.2.1 Deregulation of Lipid Metabolism in Cancer Cells

The survival and proliferation of many types of cancer require a supply of lipids. The primary pathway exploited by cancer cells for acquiring lipids is FA synthesis, especially the de novo FAs synthesis pathway activation. Boroughs et al. reported that most cultured cancer cells activate the de novo FAs synthesize pathway in the presence of abundant oxygen and extracellular nutrients. However, when there is metabolic stress, cancer cells will scavenge for extracellular lipids as a major adaptive mechanism to maintain viability and growth [1]. Yao *et al*. observed that rather than de novo synthesis, proliferating fibroblasts, and a serial of tumor cells prefer uptaking lipids directly from the extracellular environment [37]. Besides, many studies have suggested that to promote survival and proliferation, several cancer cells will utilize both lipogenic and lipolytic pathways to acquire lipids [6–9]. Together, these studies support how cancer cells obtain the lipids depend on the cell type and microenvironment. The obtaining approaches include de novo lipogenesis, uptaking from the environment, and hydrolysis of intracellular TG stored in LDs. They may play a crucial role in cancer initiation and progression. The link between cancer development and elevated level of lipid metabolism has been extensively featured [18]. The ways of lipid metabolism contributing to cancer progression are varied. One documented mechanism implicates the proliferation and growth of transformed cells by alterations in lipid metabolic pathways. These alterations can offer molecules for signaling transduction, substrates for phospholipid synthesis, and metabolic fuels through mitochondria oxidation [38]. Free FAs and cholesterols, the excess intracellular lipids, are esterified to TAG and CEs, respectively, and then incorporated into LDs [39]. In normal cells, the biology of LDs has also been extensively studied. For instance, after the fusion of smaller ones, LDs' size can vary and increase as their generation proceeds [40]. Moreover, adipose tissue can store abundant LDs in physiological situations [41]. Several studies have proved that cancer cells exhibit a significantly increased number of LDs and LD-related proteins, including adipose differentiation-related protein (ADRP) [42]. Therefore, LDs and ADRP are potential biomarkers of cancer. Also, in harmful situations like drug toxicity, endoplasmic reticulum (ER) stress, and reactive oxygen species (ROS), the increased number of LDs and LD-related proteins can play an adaptive and protective role. Therefore, LDs and LD-related proteins promote cancer cell proliferation and tumor growth.

4.2.2.2 Multiple Steps of Lipid Biosynthesis in Cancer Cells

Citrate is an important mediator to link FA metabolism with other metabolic networks [43]. For example, as an intermediate in the Krebs cycle, citrate is the keypoint of glucose metabolism feeding into the FA metabolism. The citrate's metabolic fate depends on its subcellular localization. The Krebs cycle is fed by mitochondrial citrate, and the FA synthesis is fed by cytoplasmic citrate. Citrate is transported by the

transport protein citrate carrier (CIC) across the mitochondria's inner membrane for utilization in the cytoplasm. It was found that elevated levels of CIC are correlated with poor prognosis in various cancers. Besides, inhibiting citrate transport by benzene-tricarboxylate analog (BTA) shows antitumor effects in xenograft mice with multiple tumor types [44]. In converting carbons from citrate to bioactive FAs, certain links are necessary, including ACLY, acetyl-CoA carboxylase (ACC), FASN, and acyl-CoA synthetases, also known as fatty acid-CoA ligase (ACS or ACSL). In recent years, a growing body of studies has demonstrated that the high level of some enzymes mentioned above is correlated with poor prognosis. Inhibiting them can have an antitumor effect in the established cancer models, especially in the model of decreased FA availability. When clinically applying these strategies, many enzymes' inhibitors have minimal impact on non-transformed cells. Here, we would like to describe them one by one in details as the following.

As a central metabolic enzyme, ACLY catalyzes the conversion of citrate to oxaloacetate and coenzyme A (CoA) to acetyl-CoA, both of which are ATP dependent. For cholesterol biosynthesis, FA metabolism, and protein prenylation and acetylation, acetyl-CoA is essential. Due to its relation to the proliferation activity of cancer cells, ACLY has been considered a target for anticancer drugs in many studies. For example, by converting the six-carbon citrate to precursors for FA synthesis, the four-carbon oxaloacetate, and two-carbon acetyl-CoA, ACLY bridges glucose and lipid metabolism. The knockdown of ACLY suppresses the ability of cancer cells to transfer glucose to lipids, which has been observed in murine lymphoid cells and adenocarcinoma cells [45, 46]. By genetic or pharmacological inhibition of ACLY, this metabolic change prevents tumorigenesis and impairs xenograft tumor formation in mice [46, 47]. More importantly, inhibiting acetyl-CoA production may influence other abnormal metabolic pathways in transformed cells, due to its essential role as a substrate for the acetylation of proteins and nucleic acids [48, 49]. ACC carboxylates acetyl-CoA to form malonyl-CoA, one of the most highly regulated enzymes in FA biosynthesis [50]. Citrate and glutamate can allosterically regulate ACC and activate its expression. Meanwhile, this enzyme is negatively and allosterically regulated by long- and short-chain fatty acyl-CoAs, such as palmitoyl-CoA. The AMP-activated protein kinase (AMPK) can inhibit ACC's activity by phosphorylation. ACC1 and ACC2 are the two isoforms of ACC in the human genome. ACC1 exists in lipogenic tissues in large amounts, while ACC2 is enriched in oxidative tissues. Due to their existence in different tissues, ACC1 and ACC2 have different functions in metabolism. Though malonyl-CoA is a common metabolite derived from both ACC1 and ACC2, the malonyl-CoA catalyzed by AAC1 is a substrate for FA synthesis, whereas the malonyl-CoA catalyzed by ACC2 inhibits CPT1 and prevents FA degradation. The inhibition of ACC1 induces apoptosis in prostate and breast cancer cells, but not in the non-transformed cells [51, 52]. The knockdown of both ACC1 and ACC2 by Soraphen-A in prostate cancer cells reveals similar results [51]. However, in breast cancer cells, where ACC is chemically inhibited by TOFA (5-(tetradecyloxy)-2-furoic acid), a contradictory result is reported [53]. Since TOFA has been observed to block the epidermal growth factor receptor (EGFR)-activated glioblastoma (GBM) cells, but not the EGFR-inactivated cell lines, we may explain the above findings with the role of EGFR signaling [54]. The observation further complicates the situation that, by promoting the NADPH-dependent redox balance, suppression of ACC1 or ACC2 can accelerate lung cancer growth [55]. Researchers still need to elucidate the other aspects of ACC roles in cancer cells. Importantly, it has been demonstrated that ACC activity is regulated in a phosphorylation-dependent manner. It is identified that AMPK phosphorylates ACC1 at Ser79 and ACC2 at Ser212. Thus the conversion of acetyl-CoA to malonyl-CoA can be inhibited [56–58]. Since AMPK can be activated by metformin, which is already widely used clinically, more preclinical experiments and clinical trials are needed to further explore the antitumor activity and therapeutic efficacy of metformin [59].

The successive condensation reactions catalyzed by FASN can form FA from malonyl-CoA and acetyl-CoA substrates, and the 16-carbon palmitate is the main product. In many types of cancer cells, the elevated level of FASN is potently correlated with enhanced FA synthesis and a poor prognosis [9]. It is reported that knockdown of FASN decreases TG and phospholipids levels, inhibits proliferation, and stimulates apoptosis of prostate cancer cells, while has no obvious effect on the viability and proliferation of non-transformed fibroblast cells [60]. More studies further confirmed the preferential killing of cancer cells by pharmacological inhibition of FASN [61]. Since most cancer cells depend on FASN-mediated de novo FA synthesis, whereas most non-transformed cells prefer uptake of exogenous FAs, FASN is a particularly appealing therapeutic target. However, data from the mechanistic experiments showed that FASN inhibitor might induce cell death due to the toxic accumulation of malonyl-CoA, rather than a lack of FAs [53].

ACS activates FAs to generate FA-CoA, thus entering the bioactive pools. The bioactive FAs also participate in protein palmitoylation, a crucial posttranslational modification in several cancers [62]. ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6 are the five isoforms of ACS genes in mammals. Among them, ACSL4 is upregulated in colon adenocarcinoma, and ACSL5 level is increased in GBM [63, 64]. Overexpression of ACSL4 promotes tumor cell survival by preventing apoptosis, likely through depletion of unesterified arachidonic acid, which yields a pro-apoptotic signal [63]. Chemical inhibition of ACS by Triacsin C (inhibitor targeting ACSL1, ACSL3, and ACSL4, but not ACSL5 or ACSL6) preferentially induces apoptotic cell death in lung, colon, and brain cancer cells [65–67]. Several thiazolidinediones (TZDs) can inhibit the activity of ACSL4 (not ACSL1 or ACSL5) by direct binding in vitro. TZDs, widely used in diabetes treatment, can stimulate the peroxisome proliferator-activated receptors (PPARs), especially PPARɣ. Strikingly, TZD utilization, in a PPARɣ-independent manner, is related to decreased incidence of several cancers [68]. Since different isoforms of ACS have different tissue specificities, responses to nutritional state, and preferred substrates, this is a noteworthy point when considering treatment through ACS inactivation [69]. Stearoyl-CoA desaturase (SCD) mainly catalyzes stearoyl-CoA to oleoyl-CoA by introducing the double bonds into short-chain FAs in the C9 position [70]. By changing the physical properties of FAs, this introduction has profound effects on lipid function. SCD1 and SCD5 are the two isoforms of SCD in human beings. The frequently increased SCD1 expression and activity and its importance for cancer biology are increasingly recognized [71]. By induction of unsaturated FAs, inhibiting SCD1 can promote the death of cancer cells [72]. Pharmacological inhibition of SCD1 limits tumor growth in preclinical cancer models without affecting the overall body weight [73, 74]. Interestingly, FAs are also substrates for sphingolipid synthesis. Sphingolipids such as ceramides and sphingosine-1-phosphate can actively suppress or promote tumor growth [75]. Furthermore, the accumulation of ceramides is involved in the therapeutic effects of various cancers [76–78].

4.2.3 The Contribution of Lipid Metabolism for the Malignant Behaviors in Cancer Cells

In recent years, accumulating evidence has demonstrated lipid metabolism influence in several aspects of cancer cells. Owing to the diversity of malignant behaviors in cancer cells, some of the potential roles and mechanisms of altered lipid metabolism on tumor growth, energy adaption, redox homeostasis, *etc*. are described below.

4.2.3.1 Altered Lipid Metabolism for Cancer Cell Proliferation and Tumor Growth

As the building blocks for biological membranes, lipids are primarily required for the highly proliferative cancer cells. In cultured mammalian cells, following Akt activation or interleukin-3 administration, lipid synthesis is also necessary for cell growth [79]. Researchers have also found

the essential role of SREBP in maintaining the cell and organ size of *Drosophila melanogaster*, indicating the conserved significance of lipogenesis for growth [80]. The lipogenic gene SREBP is activated during mitosis, thus affecting the cell cycle progression [81]. SCD, the target gene of SREBP, has been reported to be overexpressed in ontogenically transformed cells and in several human cancers [82–84]. SCD is essential for cell transformation in vitro and is associated with the genetic predisposition and growth of cancer in the mouse model [85]. The knockdown of SCD or interruption of the *Scd* gene inhibits lipid synthesis and enhances β-oxidation by activating AMPK in mice [83, 86, 87]. And the cell cycle progression can be blocked, and cell death can be induced by a chemical inhibitor of SCD1 in lung cancer cells [88].

4.2.3.2 Altered Lipid Metabolism for Energy Homeostasis in Cancer Cells

Compelling evidence has shown the necessity of de novo lipid synthesis in cancer cell proliferation. However, why the uptake of exogenous lipids fails to meet the enhanced lipid demand needs to be elucidated. Therefore, researchers reasonably hypothesize that lipid synthesis may be involved in the tumourigenic process. Cancer cells produce and secrete a high level of lactate due to their large glucose consumption for energetic and biosynthetic use [89]. Thus mechanisms equilibrating the intracellular pH are required, which leads to acidification of the tumor microenvironment [90]. In some cancer cells and conditions, lipid synthesis functions as a carbon sink to sequester excess pyruvate and avoid lactate production but maintains the glycolytic rate at a high level. Furthermore, this metabolic process may also participate in redox balance. When oxygen is not available, hypoxia-tolerant organisms use $NADP^+$, a metabolite of lipid synthesis, as an electron acceptor. And the hypoxic cancer cells may adopt a similar strategy, where lipid synthesis-derived $NADP^+$ can elevate the cytoplasmic NAD^+ level to maintain glycolysis [91]. The cytosolic $NADP^+$ is used by isocitrate dehydrogenase-1 to produce α-ketoglutarate since a mitochondria-cytosolic NADPH shuttle may exist [92]. After being transported to the mitochondria, this metabolite is converted back to isocitrate with $NADP^+$'s concomitant production. This recently described inverse reaction is catalyzed by isocitrate dehydrogenase-2 [16, 17, 93]. When there is not enough oxygen to maintain flux through the electron transport chain, the mitochondrial NADH/NAD^+ ratio goes up. In this way, the mitochondrial nicotinamide nucleotide transhydrogenase can take up the excess NADH to transfer a proton to $NAPD^+$ and generate NAD^+. Through the malate-aspartate or the glycerol phosphate shuttles, this product is available to maintain glycolysis [94]. Thus, lipid synthesis is involved in redox balance between the mitochondria and cytoplasm and plays a role in maximizing glycolysis.

4.2.3.3 Altered Lipid Metabolism for Resistance to Oxidative Stress in Cancer Cells

Oxidative stress is harmful to the survival and proliferation of mammalian cells. The resistance to oxidative stress is one of the critical characteristics of transformed cells. Much evidence has demonstrated the resistance to oxidative stress can be promoted by de novo lipid biosynthesis in cancer cells [95–97]. Due to the absence of desaturase, mammalian cells are incapable of synthesizing polyunsaturated FAs. Therefore, compared with those obtained through diet, a high de novo lipid synthesis rate can elevate the relative amount of monounsaturated and saturated FAs. Polyunsaturated acyl chains are more susceptible to peroxidation. Studies show that lipid synthesis inhibition makes cancer cells sensitive to death induced by oxidative stress or chemotherapy drugs, indicating a novel therapeutic target for cancer. However, this intriguing observation still needs further investigation.

4.2.3.4 Altered Lipid Metabolism for Resistance to Energy Stress in Cancer Cells

Most cancer cells have a high glucose utilization rate to meet the increased demands of energy and biosynthesis. In contrast, some cancer cells dis-

play a mounting dependence on lipid oxidation as their primary energy source [1]. For instance, prostate cancer cells exhibit a low glucose consumption rate in general. But the uptake of FAs, such as palmitate, is increased, and some β-oxidation enzymes are overexpressed in these cells [98]. The specialized metabolism of prostate epithelial cells, which secrete high levels of citrate into the prostatic fluid, may explain the above observation. And prostate cancer cells will reactivate the TCA cycle to oxidize the secreted citrate during transformation. Moreover, β-oxidation has been demonstrated to play a role in the proliferation and survival of leukemia cells. Under energy stress, the activation of β-oxidation plays a crucial role in supporting cancer cell viability [99]. The hematopoietic cells can be sensitized to withdraw glucose or growth factors through the constitutive activation of the PI3K/Akt pathway [100]. However, under these circumstances, activating β-oxidation alone is enough to maintain cell viability [101]. In GBM cells, β-oxidation has also been proved to participate in ATP production and oxidative stress resistance by providing substrates for glutathione and NADPH production, thus allowing cells to remove ROS [102].

4.2.4 Upstream Regulatory Mechanisms of Lipid Synthesis in Cancer Cells

Most of the FA and cholesterol synthesis enzymes are regulated by SREBPs, TFs of the helix-loop-helix leucine zipper family [103]. SREBP1a, SREBP1c, and SREBP2 are the three SREBP isoforms identified in mammalian cells. Among them, SREBP1a is the isoform with the greatest abundance in most cultured cells and mainly controls FA, phospholipid, and TG synthesis. Both SREBP1a and SREBP1c are alternatively spliced, and their levels vary across different tissues. Meanwhile, SREBP2 regulates the expression of genes associated with cholesterol synthesis [104]. The SREBPs' activity is closely related to the intracellular sterol concentration [105]. The SREBP/SCAP complex binds to COPII-coated vesicles when the sterol level is low and would be translocated to the Golgi apparatus. In this organelle, the transcriptionally active fragment of the 65-kDa N-terminal is released by a two-step proteolytic cleavage [106]. This mature protein enters the nucleus and binds to the promoter region of SREBP target genes with the sterol regulatory elements [107]. When the sterol level is saturated, the combination between SREBP/SCAP complex and COPII is blocked due to insulin-induced genes binding. Thus, the complex stays in the endoplasmic reticulum and cannot enter the nucleus. This classic sterol-dependent regulation termed "regulated intramembrane proteolysis," is mainly applicable to SREBP2 target genes. It is highly conserved from flies to mammals. However, Drosophila's SREBP processing is regulated by phosphatidylcholine and phosphatidylethanolamine rather than sterols [108]. Interestingly, even in the presence of cholesterol, the depletion of phosphatidylcholine in mammalian cells leads to SREBP1 accumulation in the nuclear, but not SREBP2. And SREBP1 accumulates through a SCAP-independent mechanism, indicating phospholipid level as the principal regulator of SREBP1 [109]. Besides proteolysis regulation, SREBPs' activity is regulated by their interaction with transcriptional coactivators, such as p300 [110]. To transcriptionally activate specific target genes, SREBP can also recruit and bind to cofactor or mediator complexes [111]. Furthermore, with a cdc4 phospho-degron motif, SREBPs can be phosphorylated by glycogen synthase kinase 3, leading to the polyubiquitination and degradation of mature proteins [112, 113]. The phosphoinositide 3-kinase/Akt/PKB signaling pathway is often stimulated in human cancer cells. By phosphorylating the ATP-citrate lyase, Akt can elevate the expression of several FA and cholesterol synthesis-related genes [114]. As critical downstream effector of Akt, the mammalian target of rapamycin complex I (mTORC1) is a multiprotein kinase implicating in certain metabolic processes [115]. Intriguingly, the accumulation of mature SREBP1 in the nucleus needs an activated mTORC1, a downstream target of Akt phosphorylation. The metabolically regulatory role of mTORC1 has also been proved in cells

lacking the tuberous sclerosis complex 1 or 2 genes, which are two negative regulators of mTORC1 [80]. mTORC1 also modulates the level of SREBP1 and is essential for stimulating hepatic lipogenesis [116]. In mammalian cells and the developing wing of *D. melanogaster*, SREBP is also required for the cell size control dependent on Akt activation. This finding explains the coordinated manner of the Akt/mTORC axis in regulating protein and lipid synthesis during cell growth [80]. SREBP is also found downstream of certain tumor-suppressive pathways. Downstream of the tumor suppressor liver kinase B1 (LKB1), AMPK can directly phosphorylate SREBP and inhibit its proteolytic function [117]. Through SREBP1 and SREBP2 induction, the retinoblastoma protein loss increases the expression of genes implicated in the isoprenylation of N-Ras [35]. Moreover, mutant p53 binds to SREBP at the promoter region of genes on the mevalonate pathway, promoting its expression. Through disturbing the tissue architecture and promoting breast cancer formation, this hyperactivation reveals the crucial role of SREBP-dependent lipogenesis during transformation [118]. Both SREBP1 and SREBP2 are upregulated in many cancers. Though independent of mTORC1, SREBP1 is activated by aberrant EGFR signaling in human GBM. Meanwhile, SREBP1-dependent induction of low-density lipoprotein receptors is critical for the survival of some cancer [119, 120]. These findings have verified the primary role of activated SREBP in oncogenic signaling pathways. The microenvironment around solid tumors is often hypoxic due to the increased tumor volume. Low oxygen level induces hypoxia-inducible factors (HIFs), two heterodimeric TFs formed by an a-subunit (HIF1-α or HIF2-α), and a β-subunit. In the presence of oxygen, the oxygen-sensitive prolylhydroxylases target and mark HIF-1α and HIF-2α. These two TFs are then degraded by the tumor suppressor, Von Hippel–Lindau (VHL)-dependent ubiquitination [121]. In renal cell carcinomas, VHL is frequently mutated, thus creating a pseudo-hypoxic state that stabilizes the level of HIF1α and HIF2α, even under normoxia [122]. HIFs can also be induced by tumorigenic pathways [123]. Interestingly, HIFs activity can also be attributed to metabolic activity. TCA cycle enzymes of fumarate, hydratase, and succinate dehydrogenase with inactivating mutations can lead to the collection of succinate, which blocks prolyl-hydroxylases and promotes the assembly of HIF1α [124]. By promoting the expression of vascular endothelial growth factor (VEGF), HIF activation can induce angiogenesis. And through a metabolic shift to anaerobic energy production, HIFs can also adapt to the hypoxic microenvironment [125, 126]. HIF also increases the expression of glucose transporter 1 (GLUT1) and other glycolytic enzymes [127, 128]. HIF also upregulates the level of pyruvate dehydrogenase kinase 1 (PDK1), a kinase that phosphorylates and suppresses pyruvate dehydrogenase, thus blocking the entry of pyruvate into the TCA cycle and the glucose-derived lipid synthesis [129]. However, it has been observed that in breast cancer cells, HIF1 increases the level of FASN, which is upregulated in the hypoxic tumor environment [130]. Since hypoxia attenuates the carbon flow from glucose to FAs, the FA synthesis under this condition requires other carbon sources. Indeed, the bidirectional enzyme Acetyl-CoA synthetase 2, which catalyzes acetyl-CoA synthesis from cytoplasmic acetate, is upregulated under this hypoxic condition to facilitate the survival of cancer cells [131]. More importantly, two back-to-back papers published in *Nature* have reported that when mitochondria are unfunctional, the primary carbon source for lipid synthesis falls in glutamine. Researchers found that by reductive carboxylation of glutamine-derived α-ketoglutarate in cancer cells, isocitrate dehydrogenase-1 can produce cytoplasmic citrate, which is an active metabolic phenotype in conditions of hypoxia and defective mitochondria [16, 17]. In different tissues, hypoxia has been demonstrated to have an inhibitory effect on β-oxidation. In the heart cells, ischemia inhibits β-oxidation by blocking the oxidation of NADH and FADH2. When the macrophages are exposed to hypoxic conditions, the storage of triacylglycerides is enhanced [132, 133]. By introducing hypoxia-inducible protein 2 implicated in the

deposition of neutral lipids into LDs, HIF1 can also promote lipid accumulation [134]. Through the induction of PPARγ, HIF1α can also enhance the uptake of free FAs and TG production in the liver and adipose tissue, respectively [134]. When researchers specifically knockout VHL in mouse liver, they observe steatosis with increased LD formation and downregulated β-oxidation genes. They have reported that HIF2α is responsible for these metabolic changes as well [135]. HIF2a has also been found to downregulate SREBP1c and its target genes in the liver. Interestingly, it has been reported that the accumulation of lipids is frequently occurring in renal cancer cells, where VHL is mutated and the HIF1α level is stabilized [136]. However, the exact role of LDs in promoting tumor cell proliferation and progression is not fully elucidated. Researchers have proposed that under intermittent hypoxia, the increased storage of triacylglycerides could be beneficial as a ready-to-use fuel source after reoxygenation.

4.2.5 Molecular Events of Fatty Acid Degradation in Cancer Cells

FA levels might be reduced in cancer cells due to their increased degradation, broken down by β-oxidation in the mitochondria. By carnitine palmitoyltransferase 1 (CPT1), FA-CoAs are converted to FA carnitines at the outer membrane of mitochondria after transported from the cytoplasm. In the mitochondria, acetyl-CoAs are produced following the repeat cleavage of FAs. The acetyl-CoAs can enter the TCA cycle and reduce equivalents for oxidative phosphorylation. Limitations on FA abundance by enhancing its oxidation could be theoretically beneficial, but experimental data have revealed mixed ideas. Carnitine palmitoyl transferase-1 (CPT1) participates in the rate-limiting and first step of FA transportation into mitochondria for oxidation to carbon dioxide, which is inhibited by malonyl-CoA. As the direct product derived from ACC, malonyl-CoA depletion can enhance the β-oxidation of FAs and inhibit the activity of ACC2. Therefore, the increased degradation of FAs may partially contribute to the slowed proliferation of cancer cells by inhibiting ACC. Whether the elevated FA oxidation will block the growth of cancer cells remains unclear. Depending on the energy needs and ACC isoforms, cancer types might differ in their clinical responses to the enhanced FA oxidation, diminishing the FA availability. Moreover, the CPT1 and FA oxidation inhibitors, etomoxir and ranolazine, may both kill cancer cells [102, 137]. Another significance of the increased FA oxidation rate lies in the elevated cellular ATP level, which provides energy for further cell proliferation. Indeed under energy stress, CPT1C, the brain isoform of CPT1, is vital for the existence of cancer cells [138]. PPARα is a central transcriptional regulator of FA oxidation. Extended PPARα activation causes liver cancer in mice and rats by an unclear mechanism that implicates cell cycle disturbance and ROS production [139]. However, PPARα agonists administered in humans have not caused similar cancers, and on the contrary, PPARα activation suppresses tumor growth in the established cancer models [140].

4.2.6 Diverting Fatty Acids to Storage in Cancer Cells

Once produced, FAs can be utilized for membrane lipid synthesis, be degraded, or be stored. Intriguingly, elevated storage of FAs belonging to neutral lipids, such as TGs or SEs, can decrease availability the FAs to be used as membrane building or signaling blocks, thus blocking cell proliferation. Most cells store TGs in the cytosolic LDs, the prominent lipid storage organelle [141]. The function of LDs in cancer cells remains still unclear. In many cancer cells, researchers have observed an increased number of LD and proposed them to be pathogenic. However, LD accumulation per se may not be the reason for cancer development but might reflect a cellular response to energy stress [42]. The location of LD accumulation, whether it occurs in cancer or surrounding cells, needs to be elucidated in future studies.

The major pathway of TG synthesis is known as the glycerol phosphate or Kennedy pathway.

It condenses FAs with enzymes of acylglycerol-phosphate acyltransferase (AGPAT), glycerol-3-phosphate acyltransferase (GPAT), diacylglycerol acyltransferase (DGAT), and phosphatidic acid phosphohydrolase (PAP). Except for the most distal enzyme (DGAT), all the other products will feed into the PL synthesis. Therefore, the PL production may be limited by blocking AGPAT, GPAT, and PAP, while activating DGAT might enhance the FA storage. Additionally, only with concomitant suppression of FA release can the potential benefits of improving FA storage be realized.

AGPAT esterifies lysophosphatidic acid (LPA) and an FA-CoA to form phosphatidic acid (PA). There are 11 members of AGPATs documented [142]. An elevated expression of AGPAT2 predicts a poor prognosis of ovarian cancer, and AGPAT2 inhibitors exhibit antitumor activity in the xenograft mouse model. Additionally, AGPAT9 and AGPAT11 have been reported to be upregulated in various cancers [143].

PAP, also known as Lipin, produces diacylglycerol (DG) by removing a PA's phosphate group. As one of the least studied enzymes in terms of cancer, it is still unclear how this step of lipid synthesis and storage influences cancer progression. However, PAP is implicated in regulating SREBP activity, a TF family modulating the expression of several enzymes participating in FA and cholesterol biosynthesis [144]. PAP is phosphorylated and then inactivated by mTORC1, leading to SREBP activation [145]. This discovery suggests that PAP may therefore have a significant effect on maintaining cellular lipid homeostasis.

DGAT encodes a multipass transmembrane protein that functions as a critical metabolic enzyme. It catalyzes the conversion of DG and FA-CoA to TG. DGAT1 and DGAT2 are the two isoforms of DGATs Mammals have. DGAT catalyzes the only dedicated step in TG conversion, resulting in decreased available lipids via increased lipid storage. In transformed human fibroblasts, increased TG, reduced phospholipids level, and inhibited cell growth and invasiveness can be observed due to DGAT1 overexpression [146]. Inhibiting DGAT1 may also facilitate the accumulation of its substrate DG in cancer cells, which is of great significance in the signal transduction [147]. These findings would give cautious concerns in treating cancers with DGAT1 inhibitors, which are under clinical trials for metabolic diseases.

4.2.7 Lipid Uptake in Cancer cells

Transformed cells require more lipids and would uptake more lipids than normal cells. One way of increasing the uptake is through the upregulation of receptors for plasma lipids on the cell surface. Among these receptors, the cluster of differentiation 36 (CD36) can facilitate lipid uptake from the extracellular environment [148]. In a recent study, Pascual et al. revealed that with lipid receptor CD36, cancer cells display a poor prognosis. It has also demonstrated that inhibition of CD36 impairs the metastasis of cancer cells. Along with other findings, the FA receptor CD36 has been identified as a metastasis-initiating marker and driver in a lipid metabolism-dependent manner [29].

Another approach to enhance lipid uptake in cancer cells is through the increased level of fatty acid-binding proteins (FABPs) [149]. In regulating lipid uptake and tumor development, different isoforms of FABPs expressed in various tissues may play distinctive roles. FABP3 and FABP7 have been shown to promote the uptake of extracellular FAs under hypoxia, thus forming an increased level of LDs [150]. Additionally, through activating the intracellular receptor PPARβ/δ, overexpression of FABP5 plays a critical role in promoting cell proliferation and tumor growth in prostate cancer [151]. These findings extend our knowledge of lipid metabolism, thus providing potential targets for novel antitumor therapeutics.

4.2.8 Release of Lipids from Storage in Cancer Cells

Specific lipases can release FAs from storage for utilization. By inhibiting lipolysis, the available pool of FA for cancer cell proliferation becomes

smaller. Lipolysis can also produce FAs to serve as precursors of essential signaling lipids [152]. In adipocytes, sequential reactions through adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MAGL) can fully hydrolyze one TG molecule to release three FAs in the LD. Moreover, each of these lipases also has specific functions in other tissues.

Currently, most studies addressing the lipases–cancer relation have focused on MAGL. Upon leaving the glycerol backbone, MG is hydrolyzed by MAGL to the final FA. Researchers have observed enhanced MAGL expression and activity in primary tumors and aggressive human cancer cells [36]. Chemically inhibiting MAGL by JZL184 can lower the free FA level and prevent melanoma and ovarian cancer cells' tumorigenicity both in vitro and in vivo. In contrast, the upregulation of MAGL showed the opposite phenotype. Interestingly, when the MAGL inhibitor suppresses the mouse model's tumor growth, a high-fat diet can reverse the phenotype. This observation raises whether a specific diet can influence the efficacy of targeting lipid metabolism as a cancer treatment [36]. Furthermore, MAGL also functions in regulating signaling lipids. For example, invasive tumors have increased levels of LPA and PGE2, which can be lowered by MAGL inhibitors.

4.2.9 Formation of Lipid Droplets in Cancer Cells

LDs are major energy storage organelles of excess cellular lipids in esterified form. Cancer cells contain remarkably more LDs than normal cells. The upregulation of certain LD-decorating proteins (i.e., hypoxia-inducible protein 2 (HIG2), Perilipin, ADRP, and Tip47) has been proved to facilitate the formation and accumulation of LDs in different types of cancer cell [153]. These proteins may participate in scavenging ROS, maintaining ER homeostasis, and drug resistance in cancer cells. LD density stimulation enhances the proliferation of colon cancer cells (CRC), whereas perilipin 2 (PLIN2) knockdown suppresses tumor growth [154]. Another research has reported that the HIF-2α/PLIN2/lipid storage axis is essential for ER homeostasis and resistance against cytotoxic ER stress [155]. It has also revealed that loss of HIF-2a/PLIN2-dependent lipid storage enhances sensitivity to ER stress. Moreover, suppressing lipid synthesis or silencing PLIN2 can noticeably attenuate CRC proliferation [156]. All of the studies above suggest LD-associated proteins as potential targets for cancer treatment. However, more studies are needed to explain further the LD-associated stress responses mechanically.

4.2.10 Lipid Scavenging and Fatty Acid Oxidation in Cancer Cells

Lipids, required for cell survival and growth, comprise a large fraction of mammalian cells' dry weight. With oxygen and abundant extracellular nutrients, most cancer cells choose the de novo synthesis to produce FAs. However, to maintain viability and proliferation, cancer cells have to scavenge extracellular lipids to adapt to metabolic stress. Scavenging, rather than synthesizing lipids, spare cells from the need to supply carbon. The oncogenic Ras stimulates the uptake and consumption of lysophospholipids, providing an intracellular lipid pool for tumor growth, which also occurs under hypoxia [157, 158]. As a result, cancer cells driven by KRas are resistant to the silencing of SCD1. This enzyme generally desaturates the de novo FAs synthesis before their incorporation into complex lipids [159]. Extracellular lipids, especially desaturated FAs, are also critical in cells with inactivated mTORC1 under hypoxia. In this context, protein synthesis is enhanced and lipid desaturation is decreased, resulting in activation of the unfolded protein response and cell death, but can be rescued by unsaturated FAs [160]. These findings have been repeated in renal carcinoma, GBM, and bladder cancer, suggesting solid tumors' dependence on the extracellular environment to uptake FAs. However, a subset of diffuse large B-cell lymphomas prefer FA oxidation as a fuel source even under nutritious conditions, which express high

levels of related enzymes [161]. Autophagy and related processes enable other cells to utilize FAs for fuel. In a genetic lung cancer mouse model, impaired autophagy leads to lipid accumulation, dysfunctional mitochondria, defective FA oxidation, and enhanced starvation sensitivity. Moreover, FA oxidation and other mitochondrial oxidative pathways are likely to enable cancer cells to survive through the regression period [162]. In a pancreatic cancer model driven by KRas, tumor regression induced by kinase inhibitors or knockdown of KRas produce a dormant population of cancer cells, which largely rely on mitochondrial respiration for survival. Inhibition of either autophagy or FA oxidation decreases the tumor-initiating potential of this population, thus mimicking a condition after an initial round of treatment and suggesting the importance of these catabolic pathways in enabling cancer cells to form tumors [163]. Another study has shown the coordinated mechanisms of stromal cells providing FAs to tumor cells as the fuel source, especially in ovarian cancer [28]. These tumor cells generally metastasize to the omentum, the large fold of fatty tissue in the abdomen. The co-culture of ovarian cancer cells with adipocytes has revealed that FAs transferred from adipocytes can activate AMPK and FA oxidation in the cancer cells, thus promoting cell proliferation. These findings raise many questions regarding the possibility of metabolite transfer between cells and the role of tumor microenvironment in promoting cell metabolism.

4.3 Conclusion and Perspective

Compelling evidence has revealed the critical role of lipid metabolism reprogramming in supporting transformed cells' malignant behaviors. They are obtained from lipidomics studies, established cancer models, and clinical trials. Disrupting lipid metabolism can induce the regression of tumors and inhibit their metastatic spread. The approaches include limiting lipids' origins, blocking lipid utilization, breaking down LDs, or deactivating certain enzymes involved in lipid metabolism. Based on these findings, the lipid metabolic pathways have been targeted to develop several cancer treatment drugs. However, many of the mechanisms involved have not been elucidated clearly. Certain inhibitors could suppress the proliferation and growth of cancer cells, but along with cytotoxicity in the normal cells. Thus, an all-around and in-depth understanding of lipid metabolism in cancer cells is necessary for future studies. The potential challenges are as the following: (1) identify different lipid metabolic processes and the hub genes involved in the initiation and progression of cancer, (2) avoid toxicity to normal cells while developing targeted drugs, and (3) clarify the relationship and crosstalk of lipid, glucose, protein, and energy metabolism. The oncogenic signalings and lipid metabolism are interwound with each other, and the lipids function in a broad spectrum at both cellular and organismal levels. These facts highlight the importance of targeting lipid metabolism in offering novel cancer treatment strategies in the future.

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References

1. Boroughs LK, DeBerardinis RJ. Metabolic pathways promoting cancer cell survival and growth. Nat Cell Biol. 2015;17(4):351–9.
2. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
3. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer. 2016;16(10):619–34.
4. Wise DR, Thompson CB. Glutamine addiction: a new therapeutic target in cancer. Trends Biochem Sci. 2010;35(8):427–33.
5. Dang CV, Le A, Gao P. MYC-induced cancer cell energy metabolism and therapeutic opportunities. Clin Cancer Res. 2009;15(21):6479–83.
6. Cao Y. Adipocyte and lipid metabolism in cancer drug resistance. J Clin Invest. 2019;129(8):3006–17.
7. Luo X, Cheng C, Tan Z, Li N, Tang M, Yang L, Cao Y. Emerging roles of lipid metabolism in cancer metastasis. Mol Cancer. 2017;16(1):76.

8. Beloribi-Djefaflia S, Vasseur S, Guillaumond F. Lipid metabolic reprogramming in cancer cells. Oncogenesis. 2016;5(1):e189.
9. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer. 2007;7(10):763–77.
10. Huang J, Li L, Lian J, Schauer S, Vesely PW, Kratky D, Hoefler G, Lehner R. Tumor-Induced hyperlipidemia contributes to tumor growth. Cell Rep. 2016;15(2):336–48.
11. Yue S, Li J, Lee SY, Lee HJ, Shao T, Song B, Cheng L, Masterson TA, Liu X, Ratliff TL, Cheng JX. Cholesteryl ester accumulation induced by PTEN loss and PI3K/AKT activation underlies human prostate cancer aggressiveness. Cell Metab. 2014;19(3):393–406.
12. de Gonzalo-Calvo D, López-Vilaró L, Nasarre L, Perez-Olabarria M, Vázquez T, Escuin D, Badimon L, Barnadas A, Lerma E, Llorente-Cortés V. Intratumor cholesteryl ester accumulation is associated with human breast cancer proliferation and aggressive potential: a molecular and clinicopathological study. BMC Cancer. 2015;15:460.
13. Warburg O. On the origin of cancer cells. Science. 1956;123(3191):309–14.
14. DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. Proc Natl Acad Sci U S A. 2007;104(49):19345–50.
15. Wise DR, DeBerardinis RJ, Mancuso A, Sayed N, Zhang XY, Pfeiffer HK, Nissim I, Daikhin E, Yudkoff M, McMahon SB, Thompson CB. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci U S A. 2008;105(48):18782–7.
16. Mullen AR, Wheaton WW, Jin ES, Chen PH, Sullivan LB, Cheng T, Yang Y, Linehan WM, Chandel NS, DeBerardinis RJ. Reductive carboxylation supports growth in tumour cells with defective mitochondria. Nature. 2011;481(7381):385–8.
17. Metallo CM, Gameiro PA, Bell EL, Mattaini KR, Yang J, Hiller K, Jewell CM, Johnson ZR, Irvine DJ, Guarente L, Kelleher JK, Vander Heiden MG, Iliopoulos O, Stephanopoulos G. Reductive glutamine metabolism by IDH1 mediates lipogenesis under hypoxia. Nature. 2011;481(7381):380–4.
18. Swinnen JV, Brusselmans K, Verhoeven G. Increased lipogenesis in cancer cells: new players, novel targets. Curr Opin Clin Nutr Metab Care. 2006;9(4):358–65.
19. DeBerardinis RJ, Thompson CB. Cellular metabolism and disease: what do metabolic outliers teach us? Cell. 2012;148(6):1132–44.
20. Santos CR, Schulze A. Lipid metabolism in cancer. FEBS J. 2012;279(15):2610–23.
21. Medes G, Thomas A, Weinhouse S. Metabolism of neoplastic tissue. IV. A study of lipid synthesis in neoplastic tissue slices in vitro. Cancer Res. 1953;13(1):27–9.
22. Ookhtens M, Kannan R, Lyon I, Baker N. Liver and adipose tissue contributions to newly formed fatty acids in an ascites tumor. Am J Phys. 1984;247(1 Pt 2):R146–53.
23. Gharpure KM, Pradeep S, Sans M, Rupaimoole R, Ivan C, Wu SY, Bayraktar E, Nagaraja AS, Mangala LS, Zhang X, Haemmerle M, Hu W, Rodriguez-Aguayo C, McGuire M, Mak CSL, Chen X, Tran MA, Villar-Prados A, Pena GA, Kondetimmanahalli R, Nini R, Koppula P, Ram P, Liu J, Lopez-Berestein G, Baggerly K, Eberlin LS, Sood AK. FABP4 as a key determinant of metastatic potential of ovarian cancer. Nat Commun. 2018;9(1):2923.
24. Nieman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Gutbrod R, Zillhardt MR, Romero IL, Carey MS, Mills GB, Hotamisligil GS, Yamada SD, Peter ME, Gwin K, Lengyel E. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. Nat Med. 2011;17(11):1498–503.
25. Mukherjee A, Chiang CY, Daifotis HA, Nieman KM, Fahrmann JF, Lastra RR, Romero IL, Fiehn O, Lengyel E. Adipocyte-induced FABP4 expression in ovarian cancer cells promotes metastasis and mediates carboplatin resistance. Cancer Res. 2020;80(8):1748–61.
26. Ros S, Santos CR, Moco S, Baenke F, Kelly G, Howell M, Zamboni N, Schulze A. Functional metabolic screen identifies 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 as an important regulator of prostate cancer cell survival. Cancer Discov. 2012;2(4):328–43.
27. DeFilippis RA, Chang H, Dumont N, Rabban JT, Chen YY, Fontenay GV, Berman HK, Gauthier ML, Zhao J, Hu D, et al. CD36 repression activates a multicellular stromal program shared by high mammographic density and tumor tissues. Cancer Discov. 2012;2:826–39.
28. Ladanyi A, Mukherjee A, Kenny HA, Johnson A, Mitra AK, Sundaresan S, Nieman KM, Pascual G, Benitah SA, Montag A, Yamada SD, Abumrad NA, Lengyel E. Adipocyte-induced CD36 expression drives ovarian cancer progression and metastasis. Oncogene. 2018;37(17):2285–301.
29. Pascual G, Avgustinova A, Mejetta S, Martín M, Castellanos A, Attolini CS, Berenguer A, Prats N, Toll A, Hueto JA, Bescós C, Di Croce L, Benitah SA. Targeting metastasis-initiating cells through the fatty acid receptor CD36. Nature. 2017;541(7635):41–5.
30. Watt MJ, Clark AK, Selth LA, Haynes VR, Lister N, Rebello R, Porter LH, Niranjan B, Whitby ST, Lo J, Huang C, Schittenhelm RB, Anderson KE, Furic L, Wijayaratne PR, Matzaris M, Montgomery MK, Papargiris M, Norden S, Febbraio M, Risbridger GP, Frydenberg M, Nomura DK, Taylor RA. Suppressing fatty acid uptake has therapeutic effects in preclinical models of prostate cancer. Sci Transl Med. 2019;11(478):eaau5758.

31. Simons K, Toomre D. Lipid rafts and signal transduction. Nat Rev Mol Cell Biol. 2000;1(1):31–9.
32. Sebti SM. Protein farnesylation: implications for normal physiology, malignant transformation, and cancer therapy. Cancer Cell. 2005;7(4):297–300.
33. Shamma A, Takegami Y, Miki T, Kitajima S, Noda M, Obara T, Okamoto T, Takahashi C. Rb Regulates DNA damage response and cellular senescence through E2F-dependent suppression of N-ras isoprenylation. Cancer Cell. 2009;15(4):255–69.
34. Gupta GP, Nguyen DX, Chiang AC, Bos PD, Kim JY, Nadal C, Gomis RR, Manova-Todorova K, Massagué J. Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. Nature. 2007;446(7137):765–70.
35. Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. Nat Rev Cancer. 2003;3(8):582–91.
36. Nomura DK, Long JZ, Niessen S, Hoover HS, Ng SW, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell. 2010;140(1):49–61.
37. Yao CH, Fowle-Grider R, Mahieu NG, Liu GY, Chen YJ, Wang R, Singh M, Potter GS, Gross RW, Schaefer J, Johnson SL, Patti GJ. Exogenous fatty acids are the preferred source of membrane lipids in proliferating fibroblasts. Cell Chem Biol. 2016;23(4):483–93.
38. Zaidi N, Lupien L, Kuemmerle NB, Kinlaw WB, Swinnen JV, Smans K. Lipogenesis and lipolysis: the pathways exploited by the cancer cells to acquire fatty acids. Prog Lipid Res. 2013;52(4):585–9.
39. Koizume S, Miyagi Y. Lipid droplets: a key cellular organelle associated with cancer cell survival under normoxia and hypoxia. Int J Mol Sci. 2016;17(9):1430.
40. Wilfling F, Haas JT, Walther TC, Farese RV Jr. Lipid droplet biogenesis. Curr Opin Cell Biol. 2014;29:39–45.
41. Ahmadian M, Duncan RE, Jaworski K, Sarkadi-Nagy E, Sul HS. Triacylglycerol metabolism in adipose tissue. Future Lipidol. 2007;2(2):229–37.
42. Bozza PT, Viola JP. Lipid droplets in inflammation and cancer. Prostaglandins Leukot Essent Fatty Acids. 2010;82(4–6):243–50.
43. Mycielska ME, Dettmer K, Rümmele P, Schmidt K, Prehn C, Milenkovic VM, Jagla W, Madej GM, Lantow M, Schladt M, Cecil A, Koehl GE, Eggenhofer E, Wachsmuth CJ, Ganapathy V, Schlitt HJ, Kunzelmann K, Ziegler C, Wetzel CH, Gaumann A, Lang SA, Adamski J, Oefner PJ, Geissler EK. Extracellular citrate affects critical elements of cancer cell metabolism and supports cancer development in vivo. Cancer Res. 2018;78(10):2513–23.
44. Catalina-Rodriguez O, Kolukula VK, Tomita Y, Preet A, Palmieri F, Wellstein A, Byers S, Giaccia AJ, Glasgow E, Albanese C, Avantaggiati ML. The mitochondrial citrate transporter, CIC, is essential for mitochondrial homeostasis. Oncotarget. 2012;3(10):1220–35.
45. Bauer DE, Hatzivassiliou G, Zhao F, Andreadis C, Thompson CB. ATP citrate lyase is an important component of cell growth and transformation. Oncogene. 2005;24(41):6314–22.
46. Hatzivassiliou G, Zhao F, Bauer DE, Andreadis C, Shaw AN, Dhanak D, Hingorani SR, Tuveson DA, Thompson CB. ATP citrate lyase inhibition can suppress tumor cell growth. Cancer Cell. 2005;8(4):311–21.
47. Migita T, Narita T, Nomura K, Miyagi E, Inazuka F, Matsuura M, Ushijima M, Mashima T, Seimiya H, Satoh Y, Okumura S, Nakagawa K, Ishikawa Y. ATP citrate lyase: activation and therapeutic implications in non-small cell lung cancer. Cancer Res. 2008;68(20):8547–54.
48. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP- citrate lyase links cellular metabolism to histone acetylation. Science. 2009;324(5930):1076–80.
49. Zaidi N, Swinnen JV, Smans K. ATP-citrate lyase: a key player in cancer metabolism. Cancer Res. 2012;72(15):3709–14.
50. Wakil SJ, Abu-Elheiga LA. Fatty acid metabolism: target for metabolic syndrome. J Lipid Res. 2009;50 Suppl(Suppl):S138–43.
51. Brusselmans K, De Schrijver E, Verhoeven G, Swinnen JV. RNA interference- mediated silencing of the acetyl-CoA-carboxylase-alpha gene induces growth inhibition and apoptosis of prostate cancer cells. Cancer Res. 2005;65(15):6719–25.
52. Chajès V, Cambot M, Moreau K, Lenoir GM, Joulin V. Acetyl-CoA carboxylase alpha is essential to breast cancer cell survival. Cancer Res. 2006;66(10):5287–94.
53. Pizer ES, Thupari J, Han WF, Pinn ML, Chrest FJ, Frehywot GL, Townsend CA, Kuhajda FP. Malonyl-coenzyme-A is a potential mediator of cytotoxicity induced by fatty-acid synthase inhibition in human breast cancer cells and xenografts. Cancer Res. 2000;60(2):213–8.
54. Guo D, Hildebrandt IJ, Prins RM, Soto H, Mazzotta MM, Dang J, Czernin J, Shyy JY, Watson AD, Phelps M, Radu CG, Cloughesy TF, Mischel PS. The AMPK agonist AICAR inhibits the growth of EGFRvIII-expressing glioblastomas by inhibiting lipogenesis. Proc Natl Acad Sci U S A. 2009;106(31):12932–7.
55. Jeon SM, Chandel NS, Hay N. AMPK regulates NADPH homeostasis to promote tumour cell survival during energy stress. Nature. 2012;485(7400):661–5.
56. Carlson CA, Kim KH. Regulation of hepatic acetyl coenzyme A carboxylase by phosphorylation and dephosphorylation. J Biol Chem. 1973;248(1):378–80.
57. Carling D, Zammit VA, Hardie DG. A common bicyclic protein kinase cascade inactivates the regulatory enzymes of fatty acid and cholesterol biosynthesis. FEBS Lett. 1987;223(2):217–22.
58. Fullerton MD, Galic S, Marcinko K, Sikkema S, Pulinilkunnil T, Chen ZP, O'Neill HM, Ford RJ, Palanivel R, O'Brien M, Hardie DG, Macaulay SL,

Schertzer JD, Dyck JR, van Denderen BJ, Kemp BE, Steinberg GR. Single phosphorylation sites in Acc1 and Acc2 regulate lipid homeostasis and the insulin-sensitizing effects of metformin. Nat Med. 2013;19(12):1649–54.
59. Pollak MN. Investigating metformin for cancer prevention and treatment: the end of the beginning. Cancer Discov. 2012;2(9):778–90.
60. De Schrijver E, Brusselmans K, Heyns W, Verhoeven G, Swinnen JV. RNA interference-mediated silencing of the fatty acid synthase gene attenuates growth and induces morphological changes and apoptosis of LNCaP prostate cancer cells. Cancer Res. 2003;63(13):3799–804.
61. Lupu R, Menendez JA. Pharmacological inhibitors of Fatty Acid Synthase (FASN)—catalyzed endogenous fatty acid biogenesis: a new family of anti-cancer agents? Curr Pharm Biotechnol. 2006;7(6):483–93.
62. Resh MD. Targeting protein lipidation in disease. Trends Mol Med. 2012;18(4):206–14.
63. Cao Y, Pearman AT, Zimmerman GA, McIntyre TM, Prescott SM. Intracellular unesterified arachidonic acid signals apoptosis. Proc Natl Acad Sci U S A. 2000;97(21):11280–5.
64. Yamashita Y, Kumabe T, Cho YY, Watanabe M, Kawagishi J, Yoshimoto T, Fujino T, Kang MJ, Yamamoto TT. Fatty acid induced glioma cell growth is mediated by the acyl-CoA synthetase 5 gene located on chromosome 10q25.1-q25.2, a region frequently deleted in malignant gliomas. Oncogene. 2000;19(51):5919–25.
65. Van Horn CG, Caviglia JM, Li LO, Wang S, Granger DA, Coleman RA. Characterization of recombinant long-chain rat acyl-CoA synthetase isoforms 3 and 6: identification of a novel variant of isoform 6. Biochemistry. 2005;44(5):1635–42. https://doi.org/10.1021/bi0477211.
66. Kim JH, Lewin TM, Coleman RA. Expression and characterization of recombinant rat Acyl-CoA synthetases 1, 4, and 5. Selective inhibition by triacsin C and thiazolidinediones. J Biol Chem. 2001;276(27):24667–73.
67. Mashima T, Oh-hara T, Sato S, Mochizuki M, Sugimoto Y, Yamazaki K, Hamada J, Tada M, Moriuchi T, Ishikawa Y, Kato Y, Tomoda H, Yamori T, Tsuruo T. p53-defective tumors with a functional apoptosome-mediated pathway: a new therapeutic target. J Natl Cancer Inst. 2005;97(10):765–77.
68. Weng JR, Chen CY, Pinzone JJ, Ringel MD, Chen CS. Beyond peroxisome proliferator-activated receptor gamma signaling: the multi-facets of the anti-tumor effect of thiazolidinediones. Endocr Relat Cancer. 2006;13(2):401–13.
69. Mashek DG, Li LO, Coleman RA. Rat long-chain acyl-CoA synthetase mRNA, protein, and activity vary in tissue distribution and in response to diet. J Lipid Res. 2006;47(9):2004–10.
70. Paton CM, Ntambi JM. Biochemical and physiological function of stearoyl-CoA desaturase. Am J Physiol Endocrinol Metab. 2009;297(1):E28–37.
71. Igal RA. Stearoyl-CoA desaturase-1: a novel key player in the mechanisms of cell proliferation, programmed cell death and transformation to cancer. Carcinogenesis. 2010;31(9):1509–15.
72. Ariyama H, Kono N, Matsuda S, Inoue T, Arai H. Decrease in membrane phospholipid unsaturation induces unfolded protein response. J Biol Chem. 2010;285(29):22027–35.
73. Fritz V, Benfodda Z, Rodier G, Henriquet C, Iborra F, Avancès C, Allory Y, de la Taille A, Culine S, Blancou H, Cristol JP, Michel F, Sardet C, Fajas L. Abrogation of de novo lipogenesis by stearoyl-CoA desaturase 1 inhibition interferes with oncogenic signaling and blocks prostate cancer progression in mice. Mol Cancer Ther. 2010;9(6):1740–54.
74. Roongta UV, Pabalan JG, Wang X, Ryseck RP, Fargnoli J, Henley BJ, Yang WP, Zhu J, Madireddi MT, Lawrence RM, Wong TW, Rupnow BA. Cancer cell dependence on unsaturated fatty acids implicates stearoyl-CoA desaturase as a target for cancer therapy. Mol Cancer Res. 2011;9(11):1551–61.
75. Ogretmen B, Hannun YA. Biologically active sphingolipids in cancer pathogenesis and treatment. Nat Rev Cancer. 2004;4(8):604–16.
76. Li G, Liu D, Kimchi ET, Kaifi JT, Qi X, Manjunath Y, Liu X, Deering T, Avella DM, Fox T, Rockey DC, Schell TD, Kester M, Staveley-O'Carroll KF. Nanoliposome C6-ceramide increases the anti-tumor immune response and slows growth of liver tumors in mice. Gastroenterology. 2018;154(4):1024–1036.e9.
77. Ordoñez R, Fernández A, Prieto-Domínguez N, Martínez L, García-Ruiz C, Fernández-Checa JC, Mauriz JL, González-Gallego J. Ceramide metabolism regulates autophagy and apoptotic cell death induced by melatonin in liver cancer cells. J Pineal Res. 2015;59(2):178–89.
78. Tagaram HR, Divittore NA, Barth BM, Kaiser JM, Avella D, Kimchi ET, Jiang Y, Isom HC, Kester M, Staveley-O'Carroll KF. Nanoliposomal ceramide prevents in vivo growth of hepatocellular carcinoma. Gut. 2011;60(5):695–701.
79. Griffiths B, Lewis CA, Bensaad K, Ros S, Zhang Q, Ferber EC, Konisti S, Peck B, Miess H, East P, Wakelam M, Harris AL, Schulze A. Sterol regulatory element binding protein-dependent regulation of lipid synthesis supports cell survival and tumor growth. Cancer Metab. 2013;1(1):3.
80. Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, Leevers S, Griffiths JR, Chung YL, Schulze A. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab. 2008;8(3):224–36.
81. Bengoechea-Alonso MT, Ericsson J. Cdk1/cyclin B-mediated phosphorylation stabilizes SREBP1 during mitosis. Cell Cycle. 2006;5(15):1708–18.

82. Scaglia N, Caviglia JM, Igal RA. High stearoyl-CoA desaturase protein and activity levels in simian virus 40 transformed-human lung fibroblasts. Biochim Biophys Acta. 2005;1687(1–3):141–51.
83. Scaglia N, Igal RA. Stearoyl-CoA desaturase is involved in the control of proliferation, anchorage-independent growth, and survival in human transformed cells. J Biol Chem. 2005;280(27):25339–49.
84. Li J, Ding SF, Habib NA, Fermor BF, Wood CB, Gilmour RS. Partial characterization of a cDNA for human stearoyl-CoA desaturase and changes in its mRNA expression in some normal and malignant tissues. Int J Cancer. 1994;57(3):348–52.
85. Falvella FS, Pascale RM, Gariboldi M, Manenti G, De Miglio MR, Simile MM, Dragani TA, Feo F. Stearoyl-CoA desaturase 1 (Scd1) gene overexpression is associated with genetic predisposition to hepatocarcinogenesis in mice and rats. Carcinogenesis. 2002;23(11):1933–6.
86. Scaglia N, Chisholm JW, Igal RA. Inhibition of stearoylCoA desaturase-1 inactivates acetyl-CoA carboxylase and impairs proliferation in cancer cells: role of AMPK. PLoS One. 2009;4(8):e6812.
87. Dobrzyn P, Dobrzyn A, Miyazaki M, Cohen P, Asilmaz E, Hardie DG, Friedman JM, Ntambi JM. Stearoyl-CoA desaturase 1 deficiency increases fatty acid oxidation by activating AMP-activated protein kinase in liver. Proc Natl Acad Sci U S A. 2004;101(17):6409–14.
88. Hess D, Chisholm JW, Igal RA. Inhibition of stearoylCoA desaturase activity blocks cell cycle progression and induces programmed cell death in lung cancer cells. PLoS One. 2010;5(6):e11394.
89. Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell. 2008;13(6):472–82.
90. Parks SK, Chiche J, Pouyssegur J. pH control mechanisms of tumor survival and growth. J Cell Physiol. 2011;226(2):299–308.
91. Hochachka PW, Rupert JL, Goldenberg L, Gleave M, Kozlowski P. Going malignant: the hypoxia-cancer connection in the prostate. Bioessays. 2002;24(8):749–57.
92. Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. Cancer Cell. 2012;21(3):297–308.
93. Wise DR, Ward PS, Shay JE, Cross JR, Gruber JJ, Sachdeva UM, Platt JM, DeMatteo RG, Simon MC, Thompson CB. Hypoxia promotes isocitrate dehydrogenase-dependent carboxylation of α-ketoglutarate to citrate to support cell growth and viability. Proc Natl Acad Sci U S A. 2011;108(49):19611–6.
94. Rydström J. Mitochondrial NADPH, transhydrogenase and disease. Biochim Biophys Acta. 2006;1757(5–6):721–6.
95. Rysman E, Brusselmans K, Scheys K, Timmermans L, Derua R, Munck S, Van Veldhoven PP, Waltregny D, Daniëls VW, Machiels J, Vanderhoydonc F, Smans K, Waelkens E, Verhoeven G, Swinnen JV. De novo lipogenesis protects cancer cells from free radicals and chemotherapeutics by promoting membrane lipid saturation. Cancer Res. 2010;70(20):8117–26.
96. Rodrigues C, Milkovic L, Bujak IT, Tomljanovic M, Soveral G, Cipak GA. Lipid profile and aquaporin expression under oxidative stress in breast cancer cells of different malignancies. Oxidative Med Cell Longev. 2019;2019:2061830.
97. Riahi H, Brekelmans C, Foriel S, Merkling SH, Lyons TA, Itskov PM, Kleefstra T, Ribeiro C, van Rij RP, Kramer JM, Schenck A. The histone methyltransferase G9a regulates tolerance to oxidative stress-induced energy consumption. PLoS Biol. 2019;17(3):e2006146.
98. Schlaepfer IR, Rider L, Rodrigues LU, Gijón MA, Pac CT, Romero L, Cimic A, Sirintrapun SJ, Glodé LM, Eckel RH, Cramer SD. Lipid catabolism via CPT1 as a therapeutic target for prostate cancer. Mol Cancer Ther. 2014;13(10):2361–71.
99. Kim S, Yang X, Yin A, Zha J, Beharry Z, Bai A, Bielawska A, Bartlett MG, Yin H, Cai H. Dietary palmitate cooperates with Src kinase to promote prostate tumor progression. Prostate. 2019;79(8):896–908.
100. Deberardinis RJ, Lum JJ, Thompson CB. Phosphatidylinositol 3-kinase-dependent modulation of carnitine palmitoyltransferase 1A expression regulates lipid metabolism during hematopoietic cell growth. J Biol Chem. 2006;281(49):37372–80.
101. Buzzai M, Bauer DE, Jones RG, Deberardinis RJ, Hatzivassiliou G, Elstrom RL, Thompson CB. The glucose dependence of Akt-transformed cells can be reversed by pharmacologic activation of fatty acid beta-oxidation. Oncogene. 2005;24(26):4165–73.
102. Pike LS, Smift AL, Croteau NJ, Ferrick DA, Wu M. Inhibition of fatty acid oxidation by etomoxir impairs NADPH production and increases reactive oxygen species resulting in ATP depletion and cell death in human glioblastoma cells. Biochim Biophys Acta. 2011;1807(6):726–34.
103. Eberlé D, Hegarty B, Bossard P, Ferré P, Foufelle F. SREBP transcription factors: master regulators of lipid homeostasis. Biochimie. 2004;86(11):839–48.
104. Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J Clin Invest. 1997;99(5):838–45.
105. Bengoechea-Alonso MT, Ericsson J. SREBP in signal transduction: cholesterol metabolism and beyond. Curr Opin Cell Biol. 2007;19(2):215–22.
106. Espenshade PJ, Li WP, Yabe D. Sterols block binding of COPII proteins to SCAP, thereby controlling SCAP sorting in ER. Proc Natl Acad Sci U S A. 2002;99(18):11694–9.
107. Rawson RB. The SREBP pathway—insights from Insigs and insects. Nat Rev Mol Cell Biol. 2003;4(8):631–40.
108. Dobrosotskaya IY, Seegmiller AC, Brown MS, Goldstein JL, Rawson RB. Regulation of SREBP processing and membrane lipid produc-

tion by phospholipids in Drosophila. Science. 2002;296(5569):879–83.
109. Walker AK, Jacobs RL, Watts JL, Rottiers V, Jiang K, Finnegan DM, Shioda T, Hansen M, Yang F, Niebergall LJ, Vance DE, Tzoneva M, Hart AC, Näär AM. A conserved SREBP-1/phosphatidylcholine feedback circuit regulates lipogenesis in metazoans. Cell. 2011;147(4):840–52.
110. Oliner JD, Andresen JM, Hansen SK, Zhou S, Tjian R. SREBP transcriptional activity is mediated through an interaction with the CREB-binding protein. Genes Dev. 1996;10(22):2903–11. https://doi.org/10.1101/gad.10.22.2903.
111. Yang F, Vought BW, Satterlee JS, Walker AK, Jim Sun ZY, Watts JL, DeBeaumont R, Saito RM, Hyberts SG, Yang S, Macol C, Iyer L, Tjian R, van den Heuvel S, Hart AC, Wagner G, Näär AM. An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. Nature. 2006;442(7103):700–4.
112. Sundqvist A, Bengoechea-Alonso MT, Ye X, Lukiyanchuk V, Jin J, Harper JW, Ericsson J. Control of lipid metabolism by phosphorylation-dependent degradation of the SREBP family of transcription factors by SCF(Fbw7). Cell Metab. 2005;1(6):379–91.
113. Bengoechea-Alonso MT, Ericsson J. A phosphorylation cascade controls the degradation of active SREBP1. J Biol Chem. 2009;284(9):5885–95.
114. Berwick DC, Hers I, Heesom KJ, Moule SK, Tavare JM. The identification of ATP-citrate lyase as a protein kinase B (Akt) substrate in primary adipocytes. J Biol Chem. 2002;277(37):33895–900.
115. Porstmann T, Griffiths B, Chung YL, Delpuech O, Griffiths JR, Downward J, Schulze A. PKB / Akt induces transcription of enzymes involved in cholesterol and fatty acid biosynthesis via activation of SREBP. Oncogene. 2005;24:6465–81.
116. Li S, Brown MS, Goldstein JL. Bifurcation of insulin signaling pathway in rat liver: mTORC1 required for stimulation of lipogenesis, but not inhibition of gluconeogenesis. Proc Natl Acad Sci U S A. 2010;107(8):3441–6.
117. Li Y, Xu S, Mihaylova MM, Zheng B, Hou X, Jiang B, Park O, Luo Z, Lefai E, Shyy JY, Gao B, Wierzbicki M, Verbeuren TJ, Shaw RJ, Cohen RA, Zang M. AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. Cell Metab. 2011;13(4):376–88.
118. Freed-Pastor William A, Mizuno H, Zhao X, Langerød A, Moon S-H, Rodriguez-Barrueco R, Barsotti A, Chicas A, Li W, Polotskaia A, et al. Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. Cell. 2012;148:244–58.
119. Guo D, Prins RM, Dang J, Kuga D, Iwanami A, Soto H, Lin KY, Huang TT, Akhavan D, Hock MB, Zhu S, Kofman AA, Bensinger SJ, Yong WH, Vinters HV, Horvath S, Watson AD, Kuhn JG, Robins HI, Mehta MP, Wen PY, DeAngelis LM, Prados MD, Mellinghoff IK, Cloughesy TF, Mischel PS. EGFR signaling through an Akt- SREBP-1-dependent, rapamycin-resistant pathway sensitizes glioblastomas to antilipogenic therapy. Sci Signal. 2009;2(101):ra82.
120. Guo D, Reinitz F, Youssef M, Hong C, Nathanson D, Akhavan D, Kuga D, Amzajerdi AN, Soto H, Zhu S, Babic I, Tanaka K, Dang J, Iwanami A, Gini B, Dejesus J, Lisiero DD, Huang TT, Prins RM, Wen PY, Robins HI, Prados MD, Deangelis LM, Mellinghoff IK, Mehta MP, James CD, Chakravarti A, Cloughesy TF, Tontonoz P, Mischel PS. An LXR agonist promotes glioblastoma cell death through inhibition of an EGFR/AKT/SREBP-1/LDLR-dependent pathway. Cancer Discov. 2011;1(5):442–56.
121. Kaelin WG Jr, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell. 2008;30(4):393–402.
122. Linehan WM, Srinivasan R, Schmidt LS. The genetic basis of kidney cancer: a metabolic disease. Nat Rev Urol. 2010;7(5):277–85.
123. Laughner E, Taghavi P, Chiles K, Mahon PC, Semenza GL. HER2 (neu) signaling increases the rate of hypoxia-inducible factor 1alpha (HIF-1alpha) synthesis: novel mechanism for HIF-1-mediated vascular endothelial growth factor expression. Mol Cell Biol. 2001;21(12):3995–4004.
124. Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell. 2005;7(1):77–85.
125. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, Semenza GL. Activation of vascular endothelial growth factor gene transcription by hypoxia- inducible factor 1. Mol Cell Biol. 1996;16(9):4604–13.
126. Ravi R, Mookerjee B, Bhujwalla ZM, Sutter CH, Artemov D, Zeng Q, Dillehay LE, Madan A, Semenza GL, Bedi A. Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. Genes Dev. 2000;14(1):34–44.
127. Ebert BL, Firth JD, Ratcliffe PJ. Hypoxia and mitochondrial inhibitors regulate expression of glucose transporter-1 via distinct Cis-acting sequences. J Biol Chem. 1995;270(49):29083–9.
128. Denko NC. Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat Rev Cancer. 2008;8(9):705–13.
129. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. 2006;3(3):177–85.
130. Furuta E, Pai SK, Zhan R, Bandyopadhyay S, Watabe M, Mo YY, Hirota S, Hosobe S, Tsukada T, Miura K, Kamada S, Saito K, Iiizumi M, Liu W, Ericsson J, Watabe K. Fatty acid synthase gene is upregulated by hypoxia via activation of Akt and

sterol regulatory element binding protein-1. Cancer Res. 2008;68(4):1003–11.
131. Yoshii Y, Furukawa T, Yoshii H, Mori T, Kiyono Y, Waki A, Kobayashi M, Tsujikawa T, Kudo T, Okazawa H, Yonekura Y, Fujibayashi Y. Cytosolic acetyl-CoA synthetase affected tumor cell survival under hypoxia: the possible function in tumor acetyl-CoA/acetate metabolism. Cancer Sci. 2009;100(5):821–7.
132. Whitmer JT, Idell-Wenger JA, Rovetto MJ, Neely JR. Control of fatty acid metabolism in ischemic and hypoxic hearts. J Biol Chem. 1978;253(12):4305–9.
133. Boström P, Magnusson B, Svensson PA, Wiklund O, Borén J, Carlsson LM, Ståhlman M, Olofsson SO, Hultén LM. Hypoxia converts human macrophages into triglyceride-loaded foam cells. Arterioscler Thromb Vasc Biol. 2006;26(8):1871–6.
134. Gimm T, Wiese M, Teschemacher B, Deggerich A, Schödel J, Knaup KX, Hackenbeck T, Hellerbrand C, Amann K, Wiesener MS, Höning S, Eckardt KU, Warnecke C. Hypoxia-inducible protein 2 is a novel lipid droplet protein and a specific target gene of hypoxia-inducible factor-1. FASEB J. 2010;24(11):4443–58.
135. Rankin EB, Rha J, Selak MA, Unger TL, Keith B, Liu Q, Haase VH. Hypoxia- inducible factor 2 regulates hepatic lipid metabolism. Mol Cell Biol. 2009;29(16):4527–38.
136. Du W, Zhang L, Brett-Morris A, Aguila B, Kerner J, Hoppel CL, Puchowicz M, Serra D, Herrero L, Rini BI, Campbell S, Welford SM. HIF drives lipid deposition and cancer in ccRCC via repression of fatty acid metabolism. Nat Commun. 2017;8(1):1769.
137. Samudio I, Harmancey R, Fiegl M, Kantarjian H, Konopleva M, Korchin B, Kaluarachchi K, Bornmann W, Duvvuri S, Taegtmeyer H, Andreeff M. Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction. J Clin Invest. 2010;120(1):142–56.
138. Zaugg K, Yao Y, Reilly PT, Kannan K, Kiarash R, Mason J, Huang P, Sawyer SK, Fuerth B, Faubert B, Kalliomäki T, Elia A, Luo X, Nadeem V, Bungard D, Yalavarthi S, Growney JD, Wakeham A, Moolani Y, Silvester J, Ten AY, Bakker W, Tsuchihara K, Berger SL, Hill RP, Jones RG, Tsao M, Robinson MO, Thompson CB, Pan G, Mak TW. Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. Genes Dev. 2011;25(10):1041–51.
139. Michalik L, Desvergne B, Wahli W. Peroxisome-proliferator-activated receptors and cancers: complex stories. Nat Rev Cancer. 2004;4(1):61–70.
140. Yokoyama Y, Mizunuma H. Peroxisome proliferator-activated receptor and epithelial ovarian cancer. Eur J Gynaecol Oncol. 2010;31(6):612–5.
141. Farese RV Jr, Walther TC. Lipid droplets finally get a little R-E-S-P-E-C-T. Cell. 2009;139(5):855–60.
142. Takeuchi K, Reue K. Biochemistry, physiology, and genetics of GPAT, AGPAT, and lipin enzymes in triglyceride synthesis. Am J Physiol Endocrinol Metab. 2009;296(6):E1195–209.
143. Agarwal AK, Garg A. Enzymatic activity of the human 1-acylglycerol-3-phosphate-O--acyltransferase isoform 11: upregulated in breast and cervical cancers. J Lipid Res. 2010;51(8):2143–52.
144. Ishimoto K, Nakamura H, Tachibana K, Yamasaki D, Ota A, Hirano K, Tanaka T, Hamakubo T, Sakai J, Kodama T, Doi T. Sterol-mediated regulation of human lipin 1 gene expression in hepatoblastoma cells. J Biol Chem. 2009;284(33):22195–205.
145. Peterson TR, Sengupta SS, Harris TE, Carmack AE, Kang SA, Balderas E, Guertin DA, Madden KL, Carpenter AE, Finck BN, Sabatini DM. mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. Cell. 2011;146(3):408–20.
146. Bagnato C, Igal RA. Overexpression of diacylglycerol acyltransferase-1 reduces phospholipid synthesis, proliferation, and invasiveness in simian virus 40-transformed human lung fibroblasts. J Biol Chem. 2003;278(52):52203–11.
147. Cheng X, Geng F, Pan M, Wu X, Zhong Y, Wang C, Tian Z, Cheng C, Zhang R, Puduvalli V, Horbinski C, Mo X, Han X, Chakravarti A, Guo D. Targeting DGAT1 ameliorates glioblastoma by increasing fat catabolism and oxidative stress. Cell Metab. 2020;32(2):229–242.e8.
148. Wang J, Li Y. CD36 tango in cancer: signaling pathways and functions. Theranostics. 2019;9(17):4893–908.
149. McKillop IH, Girardi CA, Thompson KJ. Role of fatty acid binding proteins (FABPs) in cancer development and progression. Cell Signal. 2019;62:109336.
150. Bensaad K, Favaro E, Lewis CA, Peck B, Lord S, Collins JM, Pinnick KE, Wigfield S, Buffa FM, Li JL, Zhang Q, Wakelam MJO, Karpe F, Schulze A, Harris AL. Fatty acid uptake and lipid storage induced by HIF-1α contribute to cell growth and survival after hypoxia-reoxygenation. Cell Rep. 2014;9(1):349–65.
151. Forootan FS, Forootan SS, Gou X, Yang J, Liu B, Chen D, Al Fayi MS, Al-Jameel W, Rudland PS, Hussain SA, Ke Y. Fatty acid activated PPARγ promotes tumorigenicity of prostate cancer cells by up regulating VEGF via PPAR responsive elements of the promoter. Oncotarget. 2016;7(8):9322–39.
152. Wymann MP, Schneiter R. Lipid signalling in disease. Nat Rev Mol Cell Biol. 2008;9(2):162–76.
153. Togashi A, Katagiri T, Ashida S, Fujioka T, Maruyama O, Wakumoto Y, Sakamoto Y, Fujime M, Kawachi Y, Shuin T, Nakamura Y. Hypoxia-inducible protein 2 (HIG2), a novel diagnostic marker for renal cell carcinoma and potential target for molecular therapy. Cancer Res. 2005;65(11):4817–26.
154. Qi W, Fitchev PS, Cornwell ML, Greenberg J, Cabe M, Weber CR, Roy HK, Crawford SE, Savkovic SD. FOXO3 growth inhibition of colonic cells is dependent on intraepithelial lipid droplet density. J Biol Chem. 2013;288(23):16274–81.

155. Qiu B, Ackerman D, Sanchez DJ, Li B, Ochocki JD, Grazioli A, Bobrovnikova-Marjon E, Diehl JA, Keith B, Simon MC. HIF2α-dependent lipid storage promotes endoplasmic reticulum homeostasis in clear-cell renal cell carcinoma. Cancer Discov. 2015;5(6):652–67.
156. Penrose H, Heller S, Cable C, Makboul R, Chadalawada G, Chen Y, Crawford SE, Savkovic SD. Epidermal growth factor receptor mediated proliferation depends on increased lipid droplet density regulated via a negative regulatory loop with FOXO3/Sirtuin6. Biochem Biophys Res Commun. 2016;469(3):370–6.
157. Falasca M, Corda D. Elevated levels and mitogenic activity of lysophosphatidylinositol in k-ras-transformed epithelial cells. Eur J Biochem. 1994;221(1):383–9.
158. Ha JH, Radhakrishnan R, Jayaraman M, Yan M, Ward JD, Fung KM, Moxley K, Sood AK, Isidoro C, Mukherjee P, Song YS, Dhanasekaran DN. LPA induces metabolic reprogramming in ovarian cancer via a pseudohypoxic response. Cancer Res. 2018;78(8):1923–34.
159. Morgan-Lappe SE, Tucker LA, Huang X, Zhang Q, Sarthy AV, Zakula D, Vernetti L, Schurdak M, Wang J, Fesik SW. Identification of Ras-related nuclear protein, targeting protein for xenopus kinesin-like protein 2, and stearoyl-CoA desaturase 1 as promising cancer targets from an RNAi-based screen. Cancer Res. 2007;67(9):4390–8.
160. Young RM, Ackerman D, Quinn ZL, Mancuso A, Gruber M, Liu L, Giannoukos DN, Bobrovnikova-Marjon E, Diehl JA, Keith B, Simon MC. Dysregulated mTORC1 renders cells critically dependent on desaturated lipids for survival under tumor-like stress. Genes Dev. 2013;27(10):1115–31.
161. Caro P, Kishan AU, Norberg E, Stanley IA, Chapuy B, Ficarro SB, Polak K, Tondera D, Gounarides J, Yin H, Zhou F, Green MR, Chen L, Monti S, Marto JA, Shipp MA, Danial NN. Metabolic signatures uncover distinct targets in molecular subsets of diffuse large B cell lymphoma. Cancer Cell. 2012;22(4):547–60.
162. Bhatt V, Khayati K, Hu ZS, Lee A, Kamran W, Su X, Guo JY. Autophagy modulates lipid metabolism to maintain metabolic flexibility for LKB1-deficient Kras-driven lung tumorigenesis. Genes Dev. 2019;33(3–4):150–65.
163. Patra KC, Kato Y, Mizukami Y, Widholz S, Boukhali M, Revenco I, Grossman EA, Ji F, Sadreyev RI, Liss AS, Screaton RA, Sakamoto K, Ryan DP, Mino-Kenudson M, Castillo CF, Nomura DK, Haas W, Bardeesy N. Mutant GNAS drives pancreatic tumourigenesis by inducing PKA-mediated SIK suppression and reprogramming lipid metabolism. Nat Cell Biol. 2018;20(7):811–22.
164. Krishnan J, Suter M, Windak R, Krebs T, Felley A, Montessuit C, Tokarska-Schlattner M, Aasum E, Bogdanova A, Perriard E, Perriard JC, Larsen T, Pedrazzini T, Krek W. Activation of a HIF1alpha-PPARgamma axis underlies the integration of glycolytic and lipid anabolic pathways in pathologic cardiac hypertrophy. Cell Metab. 2009;9(6):512–24.

Lipid Metabolism in Tumor-Associated Natural Killer Cells

5

Yu Chen and Meihua Sui

Abstract

Accumulative data demonstrate that during the initiation and progression of tumors, several types of cellular components in tumor microenvironment, including tumor cells and immune cells, exhibit malfunctions in cellular energy metabolism. For instance, lipid metabolism impairments in immune cells are crucial in coordinating immunosuppression and tumor immune escape. In particular, excessive lipids have been shown to exhibit negative effects on innate immunity. Previous studies on lipid metabolism in immune cells are mainly focused on macrophages and T lymphocytes. Although natural killer (NK) cells are major players in the innate elimination of virus, bacteria, and tumor cells, available literature reports related with lipid metabolism in NK cells and tumor-associated NK (TANK) cells are very sparse. Despite these, the importance and clinical relevance of the crosstalk among lipid metabolism, NK/TANK cells, and tumors have been clearly indicated. In this chapter, following a general description of NK and TANK cells, our knowledge on the regulation of lipid metabolism in NK cells is reviewed, with an emphasis on the roles of mTOR and SREBP signaling. Then the interactions between lipid metabolism and NK/TANK cells under pathological conditions, e.g., obesity and cancer, were carefully introduced. As there is an urgent need to reveal more regulators and to clarify detailed molecular mechanisms by which lipid metabolism interacts with NK/TANK cells, several categories of potential regulators/pathways, as well as the challenges and perspectives in this emerging field, are discussed.

Keywords

Natural killer cells · Lipid metabolism · Cancer
Mammalian target of rapamycin · Sterol regulatory element-binding proteins
Scavenger receptors

5.1 Introduction

Cancer is a disease with impairments in immunity, as cancer cells escape from being recognized and removed by the immune system [1, 2]. Many studies demonstrate that the immune system is tightly intertwined with cancer throughout the whole "journey" of cancer, from carcinogenesis to prognosis. Meanwhile, accumulative data have shown that malfunction in cellular energy

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metabolism is a typical characteristic of practically all kinds of cancers. Importantly, recent reports indicate that during initiation and progression of tumors, the metabolism of immune cells is abnormal and is responsible for functional disorders.

In 2002, Frauwirth et al. reported that costimulatory receptor CD28 is involved in T cell activation through glycolysis, which has provided the first piece of direct evidence linking immune cell metabolism with immune activation [3]. Four years later, Doughty et al. found that glycolysis is required for differentiation and activation of B cells [4]. Afterward, research on immune metabolism has achieved an explosive growth, leading to increasing recognition on the importance of cell metabolism for the function and differentiation of immune cells [5–7]. As glucose remains the key fuel for all immune cells, a majority of the studies focus on glucose metabolism. However, other nutrient substances, such as amino acids and lipids, also play critical roles in cell metabolism and are closely related with a number of metabolism-associated disorders [8–10]. For instance, glutamine metabolism provides carbon and nitrogen for the synthesis of amino acids, nucleic acids, and lipids [11].

Lipids, mainly including triglycerides, cholesterol, phosphatidic acid, polyunsaturated fatty acids, and their metabolites, are important energy storage substances in the human body [12]. They usually enter the mitochondrial tricarboxylic acid (TCA) cycle and eventually power oxidative phosphorylation (OXPHOS) [13–15]. The metabolism of lipids is regulated by complex but precise factors and mechanisms, e.g., insulin, glucagon, diet, nutrition, and biochemical enzyme activity in the body [16, 17]. It has been indicated that abnormal lipid metabolism may negatively affect immune cells and even the whole immune system, leading to various pathological conditions including carcinogenesis [18, 19]. For example, tumor cells usually have increased rate of fatty acid synthesis in order to meet the increased needs of producing plasma membrane phospholipids and signaling molecules [20]. Moreover, a large amount of evidences show that lipid metabolism in many cellular components in tumor microenvironment (TME), including malignant cells and immune cells, is crucial in coordinating immunosuppression and tumor immune escape [21–23]. Although most literature on lipid metabolism in immune cells is focused on macrophages and T lymphocytes [9, 24–27], there are a few valuable studies related to lipid metabolism in natural killer (NK) cells and the tumor-associated NK (TANK) cells, which are carefully reviewed and discussed in this chapter.

5.2 Definition of NK Cells and TANK Cells

NK cells, historically designated as "large granular lymphocytes," are prototype innate lymphoid cells [28, 29]. Functionally, NK cells possess potent anti-infection and antitumor activities [30–32], which are mainly monitored by the expression of activating and inhibitory receptors on the surface of NK cells [33]. Several activating receptors including natural killer group 2 member D (NKG2D), natural cytotoxicity receptors natural killer cell p30-related protein (NKp30; also known as NCR3), and natural killer cell p46-related protein (NKp46; also known as NCR1) recognize molecules that are upregulated on cells during stress. Studies have indicated that tumor cells overexpress several classes of glycoproteins, including peptide related sequences MICA and MICB of MHC-I, to activate their ligand NKG2D on the surface of NK cells and thereby induce release of cytotoxic particles such as perforin (PFN) and granzyme (GZM) from NK cells and initiate apoptosis signal pathways in target cells [32, 34–36]. Killer cell immunoglobulin-like receptors (KIRs) are representatives of inhibitory receptors that can recognize human leukocyte antigen (HLA) class I molecules and subsequently promote the targeting capability of NK cells to cells lacking self-HLA class I expression and ensure self-tolerance [35, 37, 38].

The phenotype of NK cells varies slightly among different species [34, 39–42]. In humans, NK cells are classified according to the expression status of surface T cell receptor (TCR), CD3 molecules, and neural cell adhesion molecular 1 (also known as CD56) [43]. The main phenotype

of NK cells is $CD3^-CD56^+$ lymphocyte in humans, while in mice it is $CD3^-NK1.1^+$ lymphocyte [36, 44, 45]. Moreover, the phenotype and function of human NK cells are diverse and different in peripheral blood and other tissues [33, 41]. For example, there are $CD3^-CD56^{bright}$ and $CD3^-CD56^{dim}$ NK cells in human peripheral blood, of which $CD3^-CD56^{dim}$ NK cells are the dominant (nearly 90%) and show strong cytotoxicity [44, 46, 47]. On the contrary, $CD3^-CD56^{bright}$ NK cells have weak cytotoxicity and mainly contain high levels of cytokine receptors, e.g., IL-2R and IL-12R [48, 49]. NK cells widely exist in many tissues, particularly in the spleen, liver, lung, and uterus [33, 50–52]. Similar to the phenotypes detected in human peripheral blood, there are $CD3^-CD56^{bright}$ and $CD3^-CD56^{dim}$ NK cells in the liver. Although $CD3^-CD56^{dim}$ NK cells in the liver possess similar phenotype and function as those in peripheral blood, including CD16 expression and toxic molecules, they specifically express high levels of CD69 [50, 53]. Interestingly, the presence of CD69 is associated with tissue residence of NK cells in mice and humans [33, 54–57]. Detailed introduction on the characteristics of NK cells residing in various tissues could be found in recent literature [33].

TANK cells include circulating NK cells, peritumor NK cells, and NK cells infiltrated into tumors in cancer patients [58]. Interestingly, malignant cells could affect the characteristics of NK cells through regulating the abovementioned activating and/or inhibitory receptors. In patients with non-small cell lung carcinoma, Platonova and co-workers demonstrated a significantly reduced expression of activating NK receptors in intra-tumoral TANK cells by 50% compared with that of circulating NK cells from the same patients or healthy donors. Meanwhile, CD69 and NKp44 activating receptors, which are almost undetectable in circulating NK cells, were found on intra-tumoral TANK cells with a median positivity of 36% and 12%, respectively. In addition, IFN-γ secretion was undetectable in intra-tumoral TANK cells stimulated with either IL-2 or K562 cells, indicating that intra-tumoral TANK cells have functional deficiencies [59]. Similar findings were discovered in TANK cells in breast cancer patients at different stages of disease [60]. Particularly, upon disease progression, the expression of activating NK cell receptors was significantly decreased, which was accompanied with an increase in inhibitory NK cell receptors in patients with invasive breast tumors [60]. Moreover, evaluation on cytotoxicity-related molecule GZM B suggested that TANK cells in breast cancer patients had a reduced and poor cytotoxic function. A significant decrease of activating NK cell receptors in TANK cells was also observed in colorectal cancer (CRC) patients, which was accompanied with significantly lower degranulation activity of circulating NK cells than those from normal donors (27.3 vs. 45.2%; $P < 0.01$) [61]. In addition, many different cytokines produced in the complex TME have significant impacts on NK cells. For instance, TGF-β in TME could induce downregulation of NKG2D in NK cells, leading to reduced cytotoxic activity of TANK cells [62]. Meanwhile, it affects the production, metabolism, and mitochondrial function of NK in TME [63, 64]. These findings demonstrate the complex interactions between TANK cells, tumors, and TME.

Importantly, several studies have indicated that the activity and tumor infiltration of TANK cells are closely related with prognosis of cancer patients. Ishigami et al. firstly investigated the impact of intra-tumor infiltrating NK cells on prognosis of gastric cancer patients and found that the 5-year survival rate of patients with a high rate of NK infiltration ($n = 39$) was significantly improved compared with that of patients with a low level of NK infiltration ($n = 107$) (cutoff value, 25 NK cells/25 high-power fields, $P < 0.01$) [65]. Later, Villegas et al. determined the correlation between the presence of tumor-infiltrating NK cell subset CD57 and survival in 50 patients with primary squamous cell lung cancer after surgery. Their data demonstrate that patient survival was significantly improved when the amount of above NK cell subset was more than five in each microscopic field. Moreover, the mortality risk of patients with less NK cells increased to 3.50 folds of that in patients with more NK cells (cutoff value, 5 NK cells/field) [66]. In a most recent clinical study with 52 renal

cell carcinoma patients with lung metastases enrolled, the overall survival rate of patients was positively associated with the density of infiltrating NKp46⁺ NK cells [67].

5.3 Current Knowledge on Lipid Metabolism in NK Cells

As the characteristics of immune cells are closely related with their metabolic status, normal lipid metabolism is crucial for maintaining optimal immune functions of NK cells and even the whole immune system [68–70]. O'Brien and Finlay recently published an excellent review describing mouse and human NK cell metabolism (Fig. 5.1) [71]. However, as a newly emerging research area, there are very few studies investigating the signaling pathways mediating lipid metabolism in NK cells, which mainly focus on mammalian target of rapamycin (mTOR) and sterol regulatory element-binding proteins (SREBPs).

5.3.1 mTOR Signaling in Lipid Metabolism in NK Cells

mTOR, a ubiquitous serine/threonine kinase, is a central player integrating various metabolic, antigenic, and inflammatory cues and thus has been termed the metabolic checkpoint kinase [72, 73]. Taking part in two distinct complexes termed mTORC1 and mTORC2, mTOR could promote metabolic reactions through stimulating glycolysis and synthesis of proteins and lipids, and thereby affect the differentiation and proliferation of immune cells [72]. Indeed, the expression of lipogenesis enzymes in NIH3T3 cells is monitored by SREBP1, a transcription factor tightly regulated by mTORC1 [74]. Moreover, mTOR is essential for the development and activation of NK cells stimulated by interleukin-15 (IL-15) [75].

Cytokine stimulation with interleukin can affect the metabolism of NK cells originating from mice spleen and human peripheral blood mononuclear cells (PBMCs) through mTOR sig-

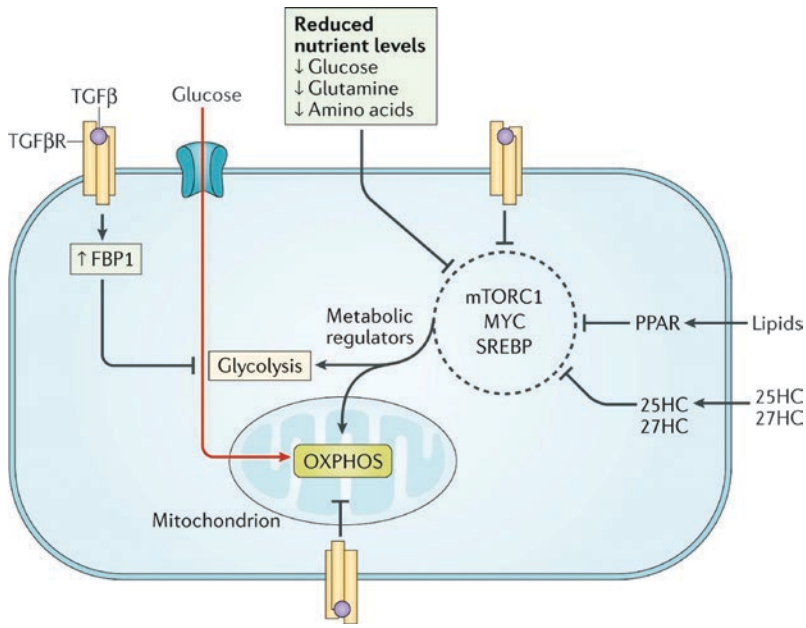

Fig. 5.1 Mechanisms disrupting human/mouse natural killer (NK) cell metabolism in cancer (characterized by reduced nutrient levels) and obesity (characterized by excessive lipids). *mTORC1* mammalian target of rapamycin complex 1, *TGFβ* transforming growth factor-β, *FBP1* fructose bisphosphatase 1, *25HC* 25-hydroxycholesterol, *27HC* 27-hydroxycholesterol, *SREBP* sterol regulatory element-binding protein, *TGFβR* TGFβ receptor, *PPAR* peroxisome proliferator-activated receptor. Reprinted with permission from O'Brien et al. Nat Rev. Immunol. 19(2019)282–290. Published by Springer Nature

naling [76]. It is known that 10–15% of all the human circulating lymphocytes are considered to be NK cells. Interestingly, Xie et al. found that overexpressed DEP domain-containing mTOR-interacting protein (DEPTOR) decreased mTORC1 but increased mTORC2 activity in lymphocytes of human PBMCs. However, the expression of lipid metabolism genes, in particular ATP citrate-lyase (Acl), acetyl-coenzyme A carboxylase 1 (Acc1), fatty acid synthase (Fasn), and stearoyl-coenzyme A desaturase 1 (Scd1), was not significantly reduced by DEPTOR overexpression, indicating that DEPTOR-mediated inhibition on mTORC1 may not suppress lipogenesis in lymphocytes of PBMCs [77]. On the contrary, AKT inhibitors decreased the activity of mTORC1/2 and AKT and downregulated lipogenesis genes in DEPTOR overexpressing lymphocytes of PBMCs. Moreover, knockdown of DEPTOR upregulated mTORC1 activity and enhanced the expression of adipogenic genes (Acl, Acc1, Fasn, etc.) and adipogenesis in lymphocytes of PBMCs [77].

The importance of mTOR signaling in lipid metabolism in NK cells is further demonstrated by a recent report from Miller's group, in which the biochemical properties of human NK cells after continuous or intermittent exposure to IL-15 were determined and compared [78]. Their data showed that continuous treatment of IL-15 led to inhibitory effects on human NK cells, e.g., decreased viability, a cell cycle arrest gene expression pattern, and reduced antitumor capability. In particular, the fatty acid oxidation (FAO)-dependent spare respiratory capacity was decreased in NK cells continuously exposed to IL-15 when compared with those exposed to intermittent treatment, by the inhibitory effect of mTOR on FAO of NK cells via inhibiting carnitine palmitoyltransferase 1a (CPT1a). However, on the contrary, intermittent treatment of IL-15 induced strong antitumor activity of NK cells in mice. Impressively, mTOR inhibitor rapamycin effectively recovered the functions of NK cells continuously exposed to IL-15. This work demonstrated that continuous administration of IL-15 induced NK cell exhaustion through a metabolic defect, including a decrease in FAO via mTOR signaling, which subsequently suppressed the activation and function of NK cells.

5.3.2 SREBP Signaling in Lipid Metabolism in NK Cells

SREBPs are transcription factors that regulate the expression of genes encoding molecules involved in lipid biosynthesis and transport, e.g., almost all lipogenic enzymes including Fasn, Scd1, 3-hydroxy-3-methylglutaryl-CoA synthase 1 (Hmgcs1), and acetyl-CoA acetyltransferase 2 (Acat2), and thus possess well-defined roles in cellular lipid homeostasis. Three SREBPs including SREBP1a, SREBP1c, and SREBP2 are expressed in mammalian cells, which transactivate genes targeted by both SREBP1c and SREBP2. They preferentially drive the expression of genes encoding molecules involved in the biosynthesis of fatty acids, and predominately transactivate genes encoding molecules involved in cholesterol biosynthesis, intracellular lipid movement, and lipoprotein import, respectively [79, 80]. Moreover, it has been indicated that the function of SREBPs in control of de novo lipogenesis and the synthesis/uptake of cholesterol is regulated by mTORC1 [71].

Previous reports have shown that lipid metabolism in immune cells, including human and murine macrophages, murine T cells, and dendritic cells, is also highly dependent on SREBPs [81–83]. Bensinger and colleagues demonstrated that SREBP signaling was essential for meeting the increased lipid demands of membrane synthesis during blastogenesis of mouse $CD8^+$ T cells, which is a required biosynthetic process for clonal expansion. Consequently, SREBP deficiency led to failure of blastogenesis and attenuation of clonal expansion in mouse $CD8^+$ T cells [84]. Moreover, Assmann and co-workers showed that in mouse NK cells, the expression of Srebp target genes encoding key molecules for de novo fatty acid synthesis (Fasn and Scd1) and those encoding molecules involved in mevalonate pathway for cholesterol synthesis (Hmgcs1 and Acat2) was steadily induced after cytokine stimulation with IL-2 and IL-12 for 18 h. However, although there

are studies demonstrating that SREBP can affect the expression of IFN and GZMB in human NK cells, the impact of SREBP in lipid metabolism in human NK cells has not yet been investigated and remains poorly defined [85].

5.4 Altered Lipid Metabolism, Altered Characteristics of NK/TANK Cells

Lipids are not only an important component of cellular membrane and a major energy source but also involved in the complex inter- and intracellular signal transduction network. Previous studies in animal and human have shown that dietary lipids are important mediators of immune cell function and possess a generally negative effect on innate immune cell function [86, 87]. Although to date there is a dearth of information on abnormal lipid metabolism in NK cells, its significant impact on characteristics of NK cells and TANK cells has been clearly indicated.

5.4.1 Altered Lipid Metabolism in NK Cells

Obesity is a disorder of lipid metabolism. With the emerging obesity pandemic, obesity-induced biological changes have attracted increasing attention. In a clinical study conducted by Lynch et al., 52 patients with severe obesity and 11 lean healthy controls were enrolled. Patients were classified into two groups, including 26 metabolically "healthy obese" and 26 "unhealthy obese" patients, respectively, based on standard cutoff points for blood pressure, lipid profile, and fasting glucose. Their data demonstrated that obese patients had remarkably fewer NK cells and cytotoxic T lymphocytes (CTLs) in peripheral blood in comparison with lean controls. Moreover, the percentages of NK cells (11.7 vs. 6.5%, $P < 0.0001$) and CTLs in the healthy obese group were significantly higher than those in the unhealthy obese group, regardless of age and BMI. Moreover, these NK cells were less activated in "healthy obese" in comparison with "unhealthy obese" [88]. This is the first report describing quantitative differences in the circulating immune system of obese patients with similar BMI but varied metabolic status and suggests that the increase of circulating NK cells and CTLs may have protective effect against obesity-related diseases including cancer.

Afterward, O'Shea et al. investigated whether the characteristics of NK cells would be influenced differentially by cigarette smoke in obese and lean population. They collected the blood samples of 40 subjects with severe obesity and 20 lean healthy subjects, together with their clinical data. In particular, the influence of cigarette smoke on the killing capability of NK cells against K562 tumor cells was assessed with or without the co-treatment of adipokines leptin and adiponectin, two crucial regulators of lipid metabolism [89]. The obtained data indicate that the amount, phenotype, and function of NK cells were remarkably changed in obese subjects. For example, the percentage of NK cells was significantly decreased from 16.6% in lean controls to 7.6% in obese subjects ($P = 0.0008$), with a significant compromise in NK cell functions in obese patients. Moreover, NK cells from obese subjects were significantly more sensitive to the inhibitory effects of cigarette smoke on their tumor-killing ability in comparison with lean subjects (33 vs. 28%, $P = 0.01$). Interestingly, co-treatment of adiponectin effectively reversed the influence of cigarette smoke on NK cells in both obese subjects ($P = 0.002$) and lean controls ($P = 0.01$), which was not observed in NK cells co-treated with leptin. These findings suggest that the impaired NK cell activity and higher sensitivity to the toxic effects of cigarette smoke may contribute to the increased risks of cancers and infections seen in obese population. As adiponectin was able to restore the activity of NK cells, it may have therapeutic implication in obese people and smokers.

Most recently, the influence of obesity on NK cell immunosurveillance and the underlying mechanisms was studied deeply and systemically by Michelet et al. in both mice and human models [90]. Transcriptional assay was conducted, respectively, in NK cells from mice fed a short-

term (1 week) high-fat diet (HFD), fed a long-term (8 weeks) HFD, and fed a standard-fat diet. They found that after even just a week of HFD, most of the genes related to lipid metabolism and lipid synthesis, including lipid-droplet formation and lipases (Lipe and Plin2), lipid and glycerol uptake (Cd36, Lpl and Lrp4), and lipid metabolism (Abca1, Scarb2 and Gyk), all of which are peroxisome proliferator-activated receptor (PPAR) target genes, were significantly upregulated in NK cells from mice. Meanwhile, treatment with fatty acid, or PPARα and PPARδ (PPARα/δ) agonists, mimicked obesity and inhibited mTOR-mediated glycolysis. These excessive lipid-induced metabolic defects were accompanied with downregulation of the killing function of NK cells. To confirm these findings in humans, they studied human NK cells from obese individuals and age-matched lean controls, and found that both the amount and functional activity of NK cells were impaired in obese individuals. For instance, there were significantly fewer NK cells ($CD3^{-}CD56^{+}$) in peripheral blood in obese subjects than in lean ones. Meanwhile, both the cytotoxic activity against cancer cells and IFN-γ production were significantly decreased in NK cells from obese individuals in comparison with those from lean ones. Alterations in signaling pathways revealed in mice with HFD were also observed in NK cells in human obesity, which further confirmed that PPAR targeting metabolic pathways could be turned on in NK cells in a lipid-rich environment. Further studies demonstrate that excessive lipids in obesity impaired NK cell-mediated immunosurveillance, leading to deficient antitumor activity in both cultured NK cells and a tumor-bearing mice model. These data have provided additional evidence that altered lipid metabolism in NK cells is closely associated with increased risks of cancers and infections in obesity and suggest that metabolic reprogramming of NK cells may improve the clinical outcomes in cancer patients with obesity. It is worthy to note that interestingly, unlike excessive lipid-induced NK cell incompetence, other features of obesity, e.g., high glucose or high insulin concentrations, did not interfere with NK cell-mediated killing effect on target cells in this study.

5.4.2 Altered Lipid Metabolism in TANK Cells

Studies introduced in Sect. 5.4.1 demonstrate that abnormal lipid metabolism would impair NK cell-mediated immunosurveillance, which may subsequently lead to carcinogenesis and cancer progression. In fact, the presence of tumors can in turn affect the metabolism of immune cells or induce metabolic reprogramming. Previous studies have shown that in mice bearing tumors and in cancer patients, conventional dendritic cells (cDC) had increased level of lipid content and decreased activity in stimulating allogeneic T cells or presenting tumor-associated antigens [68, 91]. Mechanistically, upregulation of scavenger receptors (SR) was found to play a key role in lipid accumulation in cDC [68, 91]. Moreover, increases in lipid accumulation and FAO metabolism were also found in tumor-infiltrating myeloid-derived suppressor cells (MDSCs) [92]. When it comes to NK cells, it has been shown that TANK cells could transform the balance of the core procedure of metabolism from OXPHOS to glycolysis, to fulfill the requirement of increased energy demands to fight against tumor cells [93, 94]. However, there is currently only one published report investigating the alterations of lipid metabolism in TANK cells.

In recent years, perioperative immunology, which aims to reveal the mechanisms mediating cancer surgery-induced immunological effects and develop immunotherapeutic strategies to prevent cancer recurrence after surgical removal of tumors, has become an emerging field with the accumulative literature [95–98]. In a study investigating the influence of surgery on the characteristics of NK cells, Niavarani et al. evaluated the alterations and potential role of lipid metabolism in TANK cells in both tumor-bearing murine models and colorectal cancer patients [70]. In this study, a significant increase of lipid content was observed in splenic NK cells isolated from B16F10lacZ-tumor-bearing C57Bl/6 (B6-B16) mice with surgical treatment in comparison with NK cells from control mice without surgery. This postoperative lipid accumulation after tumor resection in NK cells was additionally confirmed

in surgery-treated BALB/c-CT26 mice bearing colorectal cancer. Meanwhile, expressions of Ly49A, Ly49E/F, Ly49G2, and NKG2D on the surface of NK cells from surgery-treated B6-B16 mice were significantly decreased. Further analysis revealed that gene expressions of scavenger receptors (SR) including MSR1, CD36, and CD68, all of which are crucial in intracellular lipid transport, were remarkably upregulated in postoperative NK cells. Moreover, postoperative NK cells with high lipid content showed defective cytotoxic function against tumors ex vivo and in vivo. Interestingly, differences neither in lipid level nor in surface expression level of SR were observed in macrophages or cDC in the postoperative stage, indicating distinctive effects on NK cells on this matter. Importantly, to determine whether there are the same effects on human NK cells after surgery, they further characterized the NK cells in five colorectal cancer patients after tumor resection. Consistent with the findings in mice, postoperative TANK cells in colorectal cancer patients exhibited enhanced accumulation of lipids, increased CD36 expression, and reduced production of granzyme B and perforin in addition to decreased cell-killing activity. Novel strategies for lipid metabolism reprogramming in NK cells may have implications for improving surgical outcomes in cancer patients.

5.5 Other Potential Regulators Coordinating the Interaction Between Lipid Metabolism and NK/TANK Cells

While the association between NK/TANK cells with lipid metabolism has become an emerging field, very few studies have been conducted to address these important issues, particularly in TANK cells (Fig. 5.2). The underlying mechanisms mediating the interaction between lipid metabolic reprogramming and NK/TANK cells are complicated and still poorly understood. As there is an urgent need to reveal detailed regulators and related signal pathways by which lipid metabolism interacts with NK/TANK cells, we herein briefly introduce several example regulators potentially involved in the lipid metabolism of NK and TANK cells, which deserve further investigation.

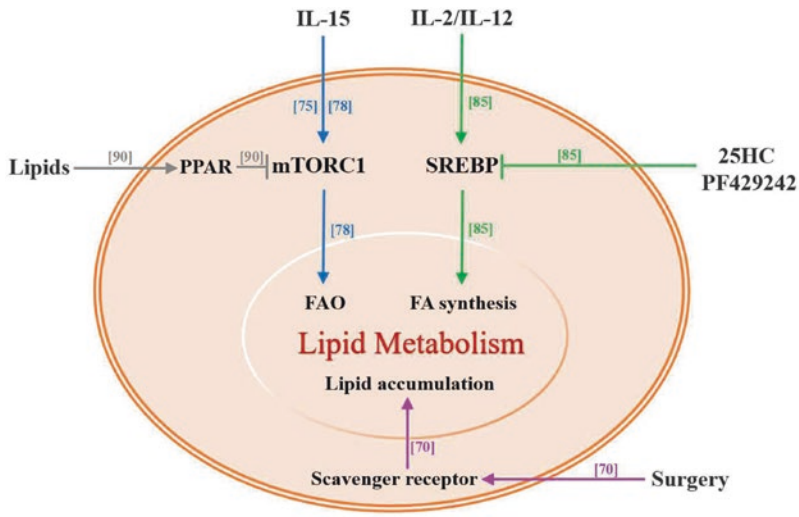

Fig. 5.2 A schematic diagram of current understanding and representative reports on lipid metabolism of human natural killer (NK) and tumor-associated nature killer (TANK) cells. Numbers in frames correspond to the numbers in Reference section. *PPAR* peroxisome proliferator-activated receptor, *mTORC1* mammalian target of rapamycin complex 1, *25HC* 25-hydroxycholesterol, *SREBP* sterol regulatory element-binding protein, *IL-15* interleukin-15, *IL-2* interleukin-2, *IL-12* interleukin-12, *FA* fatty acid

5.5.1 Lipid Molecules

In the abovementioned study conducted by Michelet et al., palmitate and oleate, two representatives of lipids, were used to treat primary NK cells isolated from blood samples and YT-INDY immortalized NK cells, in order to mimic obesity in vitro [90]. Another lipid commonly released into the TME is sphingosine-1-phosphate (S1P), which interacts with five independent high-affinity AG-coupled protein receptors (S1P receptor S1PR1–5) and plays an important extracellular role [99, 100]. NK cells have an overabundance of S1P receptor (S1PR) that contributes to the regulation of NK cell migration. Moreover, S1PR5 expression is required for NK cell localization during homeostasis and rapid secretion of IFN-γ by NK cells [99]. In primary rodent hepatocytes, conjugated bile acids activate S1PR2, leading to subsequent activation of the downstream ERK1/2 and AKT signaling pathways, which is crucial for regulating hepatic lipid metabolism [101]. Whether S1P could affect the lipid metabolism of NK cells and TANK needs to be elucidated. Moreover, considering the essential role of SREBP in lipid metabolism of NK cells, lipid molecules oxysterol 25-hydroxycholesterol and oxysterol 27-hydroxycholesterol may potentially affect the lipid metabolism of NK cells and TANK through targeting SREBP. Particularly, in inflammatory macrophages and specific tumors (e.g., glioblastoma), cholesterol 25-hydroxylase is highly expressed and can be converted to oxysterol 25-hydroxycholesterol by enzymes [85, 102, 103].

5.5.2 Hormones

Many studies have demonstrated the complex regulatory effects of hormones on many different types of cells and tissues, including the regulation on lipid metabolism [104–107]. Particularly, some hormones are directly associated with the functional regulation of NK cells. For instance, prostaglandin E2 (PGE_2) is a well-known tumor-associated mediator that contributes to immunosuppression and carcinogenesis by multiple mechanisms [108]. Multiple lines of in vitro and in vivo evidences indicate that PGE_2 not only interferes with the functions of NK cells (e.g., reducing NK cell production of IFN-γ) but also represses their proliferation and survival via multiple mechanisms (e.g., inducing apoptosis of NK cells) [109–112]. Adiponectin, which is secreted by adipose tissue and participates in the metabolism of fatty acids, has inhibitory effect on NK cell function. Increase of adiponectin could reduce the production of IFN-γ, whereas adiponectin deficiency promoted IFN-γ production in mouse NK cells [113]. Leptin is another hormone derived from adipose tissues, with its receptors (leptin receptors) expressed in 5% of human blood NK cells. Interestingly, leptin shows different regulatory effects on mice NK cells, NK-92 cells, and primary human NK cells [114]. For instance, two recent studies indicated that after stimulation with leptin, both the cytotoxicity toward cancer cells and IFN-γ production were reduced in humans NK cells [114, 115]. However, in contrast, another report showed that stimulation of leptin increased granzyme B expression and in vitro cell-killing activity of NK-92 cells [116]. Moreover, stress hormones could activate lipolysis by adipose tissue and thereby increase the levels of circulating fatty acids. A quick raise in catecholamines and glucocorticoids induced by 3,4-methylenedioxymethamphetamine and methylphenidate could stimulate the migration of NK cells into circulation, which was positively associated with the proportion of $CD56^{dim}$ NK cell subsets. Interestingly, there was a downregulation of NKG2D expression in NK cells during this process [117].

5.5.3 Cytokines

As described above, a recent report from Miller's group demonstrated that human NK cells constantly exposed to IL-15 exhibited a reduction in FAO [78]. In fact, several other cytokines have been shown to affect lipid metabolism in immune cells. For instance, Salvatore et al. characterized human monocyte-derived foamy DCs obtained with exposure to interleukin 17A (IL-17A) [118]. At day 6 of IL-17A treatment, DC-17s (DCs

obtained with exposure to IL-17A) exhibited a mixed DC/macrophage phenotype as they simultaneously expressed DC markers (CD1a) and macrophage markers (CD14, CD68 and CD163). Interestingly, there were significant accumulations of all lipid species in DC-17s in comparison with DCs from three healthy donors, with the levels of phospholipid, cholesterol, triglyceride, and cholesteryl ester increased 2–5 times, 2–4 times, 5–12 times, and 3–9 times, respectively, after IL-17A treatment. However, there was no significant difference in the immunogenic properties between DC-17s and DCs [118]. In another recent report, Autumn G et al. found that treatment of mouse bone marrow-derived macrophages (BMDMs) with either IFN-β or Poly:IC (interferon inducer) remarkably reduced the synthesis of saturated long chain fatty acids, unsaturated long chain fatty acids, and cholesterol. Unexpectedly, isotope tracer analysis demonstrated an increase in total amount of fatty acids and cholesterol on a per cell basis, suggesting an increase of lipid import in these cells. In addition, exposure to type I IFN consistently inhibited de novo synthesis of cholesterol while promoted its import to a similar extent [119].

5.5.4 Regulators Implicated in Lipid Metabolism in iNKTs

Natural killer T (NKT) cells are special T cell subsets with both T cell receptors and NK cell receptors on the cell surface. Fu and co-workers recently demonstrated that co-administration of anti-CD3 with anti-CD28 remarkably increased lipid biosynthesis and IFN-γ production in activated invariant natural killer (iNKT) cells [120]. Further exploration demonstrated that these procedures were mediated by PPARγ, a crucial regulator of adipocyte differentiation and lipid metabolism, and promyelocytic leukemia zinc finger (PLZF) synergistically through enhancing transcription of Srebf1. By using a mouse model bearing subcutaneously inoculated B16F10 melanoma, they demonstrated that lactic acid in TME reduced the expression of PPARγ in iNKT cells infiltrating into tumors and subsequently diminished their lipid synthesis and IFN-γ secretion. Furthermore, they found that in patients with hepatocellular carcinoma, expressions of PPAR-γ, SREBP1, and cholesterol were all reduced in intra-tumoral iNKT cells compared with cells in para-carcinoma tissues, and PPAR-γ expression was positively correlated with SREBP1 and cholesterol in both cancer and paracancerous tissues. These findings indicate that reduction of PPAR-γ leads to deficient cholesterol synthesis and dysfunction of intra-tumoral iNKT cells in humans and mice. Importantly, this study proposed a new strategy to increase the anticancer activity of immunotherapies based on iNKT cells trough enhancing lipid biosynthesis with PPARγ agonist [120].

5.6 Conclusion and Perspective

Interactions between "internal" (intracellular) and "external" (extracellular/environmental) lipid metabolism and the immune cells have become an important research area receiving increased attention. In general, excessive lipids have been shown to exhibit negative effects on innate immunity in vitro and in vivo and are responsible for a number of disorders, including impaired immunosurveillance and tumor immune escape. On the other hand, pathological conditions such as cancer, obesity, and inflammation could alter the metabolism, number, and functional abilities of various types of immune cells. NK cells play critical roles in the innate elimination of virus, bacteria, and malignant cells, including cancer stem cells. Unfortunately, our current understanding on the association between lipid metabolism and NK/TANK cells remains very poor, as available literature reports are very sparse. Despite all this, the importance and clinical relevance of the crosstalk among lipid metabolism, NK/TANK cells, and tumors have already been indicated.

As the limited literature mainly supports the role and involvement of several signal molecules already known to be crucial for general procedures of lipid synthesis and metabolism, e.g., mTOR and SREBP, identification of NK/TANK

cell metabolism-specific molecular mechanisms is an important yet challenging task, considering the diversity of energy metabolism, lipids, NK/TANK cells, and tumors. In addition to the molecules or pathways introduced in this chapter, there are certainly other regulators, and even subcellular fraction carrying bioactive components such as extracellular vesicles, which may mediate the interaction between lipid metabolism and NK/TANK cells. It is important to pay attention that the progression stage of disorders, such as stage of cancer and severity of obesity, may interfere with the characteristics of lipid metabolism in NK/TANK cells and alter the responses to metabolic reprogramming. We also need to keep in mind that NK/TANK cells exist in many different tissues and organs, in which they may not only have different phenotypes and functions but also exhibit varied responses to metabolic stimulation. Moreover, a number of approaches such as chemotherapy and biotherapy have been used for the treatment of cancers, and elucidation of their regulatory effects on lipid metabolism of NK/TANK cells will have great clinical impact. Future research in this field will be a multidiscipline collaboration and will lay foundations for the development of NK/TANK cell-based and metabolism-associated therapeutics.

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References

1. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991.
2. Vesely MD, Schreiber RD. Cancer immunoediting: antigens, mechanisms, and implications to cancer immunotherapy. Ann N Y Acad Sci. 2013;1284:1.
3. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, Elstrom RL, June CH, Thompson CB. The CD28 signaling pathway regulates glucose metabolism. Immunity. 2002;16:769.
4. Doughty CA, Bleiman BF, Wagner DJ, Dufort FJ, Mataraza JM, Roberts MF, Chiles TC. Antigen receptor-mediated changes in glucose metabolism in B lymphocytes: role of phosphatidylinositol 3-kinase signaling in the glycolytic control of growth. Blood. 2006;107:4458.
5. Teng X, Li W, Cornaby C, Morel L. Immune cell metabolism in autoimmunity. Clin Exp Immunol. 2019;197:181.
6. Buck MD, Sowell RT, Kaech SM, Pearce EL. Metabolic instruction of immunity. Cell. 2017;169:570.
7. Vander HM, DeBerardinis RJ. Understanding the intersections between metabolism and cancer biology. Cell. 2017;168:657.
8. Li X, Wenes M, Romero P, Huang SC, Fendt SM, Ho PC. Navigating metabolic pathways to enhance antitumour immunity and immunotherapy. Nat Rev Clin Oncol. 2019;16:425.
9. Remmerie A, Scott CL. Macrophages and lipid metabolism. Cell Immunol. 2018;330:27.
10. Kelly B, Pearce EL. Amino assets: how amino acids support immunity. Cell Metab. 2020;32(2):154–75.
11. Altman BJ, Stine ZE, Dang CV. From Krebs to clinic: glutamine metabolism to cancer therapy. Nat Rev Cancer. 2016;16:619.
12. Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CR, Shimizu T, Spener F, van Meer G, Wakelam MJ, Dennis EA. Update of the LIPID MAPS comprehensive classification system for lipids. J Lipid Res. 2009;50(Suppl):S9.
13. Wu D, Sanin DE, Everts B, Chen Q, Qiu J, Buck MD, Patterson A, Smith AM, Chang CH, Liu Z, Artyomov MN, Pearce EL, Cella M, Pearce EJ. Type 1 interferons induce changes in core metabolism that are critical for immune function. Immunity. 2016;44:1325.
14. Waters LR, Ahsan FM, Wolf DM, Shirihai O, Teitell MA. Initial B cell activation induces metabolic reprogramming and mitochondrial remodeling. iScience. 2018;5:99.
15. Gardiner CM. NK cell metabolism. J Leukoc Biol. 2019;105:1235.
16. Lee S, Dong HH. FoxO integration of insulin signaling with glucose and lipid metabolism. J Endocrinol. 2017;233:R67.
17. Perry RJ, Zhang D, Guerra MT, Brill AL, Goedeke L, Nasiri AR, Rabin-Court A, Wang Y, Peng L, Dufour S, Zhang Y, Zhang XM, Butrico GM, Toussaint K, Nozaki Y, Cline GW, Petersen KF, Nathanson MH, Ehrlich BE, Shulman GI. Glucagon stimulates gluconeogenesis by INSP3R1-mediated hepatic lipolysis. Nature. 2020;579:279.
18. McDonald G, Deepak S, Miguel L, Hall CJ, Isenberg DA, Magee AI, Butters T, Jury EC. Normalizing glycosphingolipids restores function in CD4+ T cells from lupus patients. J Clin Invest. 2014;124:712.
19. Bettencourt IA, Powell JD. Targeting metabolism as a novel therapeutic approach to autoimmunity, inflammation, and transplantation. J Immunol. 2017;198:999.
20. Currie E, Schulze A, Zechner R, Walther TC, Farese RJ. Cellular fatty acid metabolism and cancer. Cell Metab. 2013;18:153.

21. Bougarne N, Weyers B, Desmet SJ, Deckers J, Ray DW, Staels B, De Bosscher K. Molecular actions of PPARalpha in lipid metabolism and inflammation. Endocr Rev. 2018;39:760.
22. Merino SM, Gomez DCM, Moreno RJ, Falagan MS, Sanchez MR, Casado E, Ramirez DMA, Sereno M. Lipid metabolism and lung cancer. Crit Rev Oncol Hematol. 2017;112:31.
23. Wang G, Xu J, Zhao J, Yin W, Liu D, Chen W, Hou SX. Arf1-mediated lipid metabolism sustains cancer cells and its ablation induces anti-tumor immune responses in mice. Nat Commun. 2020;11:1.
24. Raud B, McGuire PJ, Jones RG, Sparwasser T, Berod L. Fatty acid metabolism in CD8(+) T cell memory: challenging current concepts. Immunol Rev. 2018;283:213.
25. Mao K, Baptista AP, Tamoutounour S, Zhuang L, Bouladoux N, Martins AJ, Huang Y, Gerner MY, Belkaid Y, Germain RN. Innate and adaptive lymphocytes sequentially shape the gut microbiota and lipid metabolism. Nature. 2018;554:255.
26. Kannan Y, Perez-Lloret J, Li Y, Entwistle LJ, Khoury H, Papoutsopoulou S, Mahmood R, Mansour NR, Ching-Cheng HS, Pearce EJ, Pedro SDCL, Ley SC, Wilson MS. TPL-2 regulates macrophage lipid metabolism and M2 differentiation to control TH2-mediated immunopathology. PLoS Pathog. 2016;12:e1005783.
27. Yang W, Bai Y, Xiong Y, Zhang J, Chen S, Zheng X, Meng X, Li L, Wang J, Xu C, Yan C, Wang L, Chang CC, Chang TY, Zhang T, Zhou P, Song BL, Liu W, Sun SC, Liu X, Li BL, Xu C. Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. Nature. 2016;531:651.
28. Shimasaki N, Jain A, Campana D. NK cells for cancer immunotherapy. Nat Rev Drug Discov. 2020;19:200.
29. Timonen T, Ortaldo JR, Herberman RB. Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. J Exp Med. 1981;153:569.
30. Lanier LL. Evolutionary struggles between NK cells and viruses. Nat Rev Immunol. 2008;8:259.
31. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. Nat Rev Cancer. 2016;16:7.
32. Vivier E, Tomasello E, Baratin M, Walzer T, Ugolini S. Functions of natural killer cells. Nat Immunol. 2008;9:503.
33. Bjorkstrom NK, Ljunggren HG, Michaelsson J. Emerging insights into natural killer cells in human peripheral tissues. Nat Rev Immunol. 2016;16:310.
34. Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. Nat Rev Immunol. 2018;18:671.
35. Bauer S. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. Science. 1999;285:727.
36. Hu W, Wang G, Huang D, Sui M, Xu Y. Cancer immunotherapy based on natural killer cells: current progress and new opportunities. Front Immunol. 2019;10:1205.
37. Anfossi N, Andre P, Guia S, Falk CS, Roetynck S, Stewart CA, Breso V, Frassati C, Reviron D, Middleton D, Romagne F, Ugolini S, Vivier E. Human NK cell education by inhibitory receptors for MHC class I. Immunity. 2006;25:331.
38. Kim S, Poursine-Laurent J, Truscott SM, Lybarger L, Song YJ, Yang L, French AR, Sunwoo JB, Lemieux S, Hansen TH, Yokoyama WM. Licensing of natural killer cells by host major histocompatibility complex class I molecules. Nature. 2005;436:709.
39. Chiossone L, Chaix J, Fuseri N, Roth C, Vivier E, Walzer T. Maturation of mouse NK cells is a 4-stage developmental program. Blood. 2009;113:5488.
40. Hayakawa Y, Smyth MJ. CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity. J Immunol. 2006;176:1517.
41. Bald T, Krummel MF, Smyth MJ, Barry KC. The NK cell-cancer cycle: advances and new challenges in NK cell-based immunotherapies. Nat Immunol. 2020;21:835.
42. Wu C, Li B, Lu R, Koelle SJ, Yang Y, Jares A, Krouse AE, Metzger M, Liang F, Lore K, Wu CO, Donahue RE, Chen I, Weissman I, Dunbar CE. Clonal tracking of rhesus macaque hematopoiesis highlights a distinct lineage origin for natural killer cells. Cell Stem Cell. 2014;14:486.
43. Lanier LL, Testi R, Bindl J, Phillips JH. Identity of Leu-19 (CD56) leukocyte differentiation antigen and neural cell adhesion molecule. J Exp Med. 1989;169:2233.
44. Sedlmayr P, Schallhammer L, Hammer A, Wilders-Truschnig M, Wintersteiger R, Dohr G. Differential phenotypic properties of human peripheral blood CD56dim+ and CD56bright+ natural killer cell subpopulations. Int Arch Allergy Immunol. 1996;110:308.
45. Hayakawa Y, Huntington ND, Nutt SL, Smyth MJ. Functional subsets of mouse natural killer cells. Immunol Rev. 2006;214:47.
46. Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaheri BA, Ghayur T, Carson WE, Caligiuri MA. Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset. Blood. 2001;97:3146.
47. Fauriat C, Long EO, Ljunggren HG, Bryceson YT. Regulation of human NK-cell cytokine and chemokine production by target cell recognition. Blood. 2010;115:2167.
48. Kunikata T, Torigoe K, Ushio S, Okura T, Ushio C, Yamauchi H, Ikeda M, Ikegami H, Kurimoto M. Constitutive and induced IL-18 receptor expression by various peripheral blood cell subsets as determined by anti-hIL-18R monoclonal antibody. Cell Immunol. 1998;189:135.
49. Caligiuri MA, Zmuidzinas A, Manley TJ, Levine H, Smith KA, Ritz J. Functional consequences of interleukin 2 receptor expression on resting human lymphocytes. Identification of a novel natural killer

cell subset with high affinity receptors. J Exp Med. 1990;171:1509.
50. Burt BM, Plitas G, Zhao Z, Bamboat ZM, Nguyen HM, Dupont B, DeMatteo RP. The lytic potential of human liver NK cells is restricted by their limited expression of inhibitory killer Ig-like receptors. J Immunol. 2009;183:1789.
51. Schleypen JS, Baur N, Kammerer R, Nelson PJ, Rohrmann K, Grone EF, Hohenfellner M, Haferkamp A, Pohla H, Schendel DJ, Falk CS, Noessner E. Cytotoxic markers and frequency predict functional capacity of natural killer cells infiltrating renal cell carcinoma. Clin Cancer Res. 2006;12:718.
52. Verma S, King A, Loke YW. Expression of killer cell inhibitory receptors on human uterine natural killer cells. Eur J Immunol. 1997;27:979.
53. Marquardt N, Beziat V, Nystrom S, Hengst J, Ivarsson MA, Kekalainen E, Johansson H, Mjosberg J, Westgren M, Lankisch TO, Wedemeyer H, Ellis EC, Ljunggren HG, Michaelsson J, Bjorkstrom NK. Cutting edge: identification and characterization of human intrahepatic CD49a+ NK cells. J Immunol. 2015;194:2467.
54. Cortez VS, Fuchs A, Cella M, Gilfillan S, Colonna M. Cutting edge: salivary gland NK cells develop independently of Nfil3 in steady-state. J Immunol. 2014;192:4487.
55. Ottaviani C, Nasorri F, Bedini C, de Pita O, Girolomoni G, Cavani A. CD56brightCD16(−) NK cells accumulate in psoriatic skin in response to CXCL10 and CCL5 and exacerbate skin inflammation. Eur J Immunol. 2006;36:118.
56. Ali TH, Pisanti S, Ciaglia E, Mortarini R, Anichini A, Garofalo C, Tallerico R, Santinami M, Gulletta E, Ietto C, Galgani M, Matarese G, Bifulco M, Ferrone S, Colucci F, Moretta A, Karre K, Carbone E. Enrichment of CD56(dim)KIR + CD57 + highly cytotoxic NK cells in tumour-infiltrated lymph nodes of melanoma patients. Nat Commun. 2014;5:5639.
57. Takayama T, Kamada N, Chinen H, Okamoto S, Kitazume MT, Chang J, Matuzaki Y, Suzuki S, Sugita A, Koganei K, Hisamatsu T, Kanai T, Hibi T. Imbalance of NKp44(+)NKp46(−) and NKp44(−)NKp46(+) natural killer cells in the intestinal mucosa of patients with Crohn's disease. Gastroenterology. 2010;139(882):891.
58. Bruno A, Ferlazzo G, Albini A, Noonan DM. A think tank of TINK/TANKs: tumor-infiltrating/tumor-associated natural killer cells in tumor progression and angiogenesis. J Natl Cancer Inst. 2014;106:u200.
59. Platonova S, Cherfils-Vicini J, Damotte D, Crozet L, Vieillard V, Validire P, Andre P, Dieu-Nosjean MC, Alifano M, Regnard JF, Fridman WH, Sautes-Fridman C, Cremer I. Profound coordinated alterations of intratumoral NK cell phenotype and function in lung carcinoma. Cancer Res. 2011;71:5412.
60. Mamessier E, Sylvain A, Thibult ML, Houvenaeghel G, Jacquemier J, Castellano R, Goncalves A, Andre P, Romagne F, Thibault G, Viens P, Birnbaum D, Bertucci F, Moretta A, Olive D. Human breast cancer cells enhance self tolerance by promoting evasion from NK cell antitumor immunity. J Clin Invest. 2011;121:3609.
61. Rocca YS, Roberti MP, Arriaga JM, Amat M, Bruno L, Pampena MB, Huertas E, Loria FS, Pairola A, Bianchini M, Mordoh J, Levy EM. Altered phenotype in peripheral blood and tumor-associated NK cells from colorectal cancer patients. Innate Immun. 2013;19:76.
62. Hodgins JJ, Khan ST, Park MM, Auer RC, Ardolino M. Killers 2.0: NK cell therapies at the forefront of cancer control. J Clin Invest. 2019;129:3499.
63. Zaiatz-Bittencourt V, Finlay DK, Gardiner CM. Canonical TGF-beta signaling pathway represses human NK cell metabolism. J Immunol. 2018;200:3934.
64. Viel S, Marcais A, Guimaraes FS, Loftus R, Rabilloud J, Grau M, Degouve S, Djebali S, Sanlaville A, Charrier E, Bienvenu J, Marie JC, Caux C, Marvel J, Town L, Huntington ND, Bartholin L, Finlay D, Smyth MJ, Walzer T. TGF-beta inhibits the activation and functions of NK cells by repressing the mTOR pathway. Sci Signal. 2016;9:a19.
65. Ishigami S, Natsugoe S, Tokuda K, Nakajo A, Che X, Iwashige H, Aridome K, Hokita S, Aikou T. Prognostic value of intratumoral natural killer cells in gastric carcinoma. Cancer. 2000;88:577.
66. Villegas FR, Coca S, Villarrubia VG, Jimenez R, Chillon MJ, Jareno J, Zuil M, Callol L. Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer. Lung Cancer. 2002;35:23.
67. Remark R, Alifano M, Cremer I, Lupo A, Dieu-Nosjean MC, Riquet M, Crozet L, Ouakrim H, Goc J, Cazes A, Flejou JF, Gibault L, Verkarre V, Regnard JF, Pages ON, Oudard S, Mlecnik B, Sautes-Fridman C, Fridman WH, Damotte D. Characteristics and clinical impacts of the immune environments in colorectal and renal cell carcinoma lung metastases: influence of tumor origin. Clin Cancer Res. 2013;19:4079.
68. Herber DL, Cao W, Nefedova Y, Novitskiy SV, Nagaraj S, Tyurin VA, Corzo A, Cho HI, Celis E, Lennox B, Knight SC, Padhya T, McCaffrey TV, McCaffrey JC, Antonia S, Fishman M, Ferris RL, Kagan VE, Gabrilovich DI. Lipid accumulation and dendritic cell dysfunction in cancer. Nat Med. 2010;16:880.
69. Al-Khami AA, Zheng L, Del VL, Hossain F, Wyczechowska D, Zabaleta J, Sanchez MD, Dean MJ, Rodriguez PC, Ochoa AC. Exogenous lipid uptake induces metabolic and functional reprogramming of tumor-associated myeloid-derived suppressor cells. Oncoimmunology. 2017;6:e1344804.
70. Niavarani SR, Lawson C, Bakos O, Boudaud M, Batenchuk C, Rouleau S, Tai LH. Lipid accumulation impairs natural killer cell cytotoxicity and tumor control in the postoperative period. BMC Cancer. 2019;19:823.

71. O'Brien KL, Finlay DK. Immunometabolism and natural killer cell responses. Nat Rev Immunol. 2019;19:282.
72. Linke M, Fritsch SD, Sukhbaatar N, Hengstschlager M, Weichhart T. mTORC1 and mTORC2 as regulators of cell metabolism in immunity. FEBS Lett. 2017;591:3089.
73. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. Cell. 2012;149:274.
74. Peterson TR, Sengupta SS, Harris TE, Carmack AE, Kang SA, Balderas E, Guertin DA, Madden KL, Carpenter AE, Finck BN, Sabatini DM. mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. Cell. 2011;146:408.
75. Marcais A, Cherfils-Vicini J, Viant C, Degouve S, Viel S, Fenis A, Rabilloud J, Mayol K, Tavares A, Bienvenu J, Gangloff YG, Gilson E, Vivier E, Walzer T. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. Nat Immunol. 2014;15:749.
76. Keating SE, Zaiatz-Bittencourt V, Loftus RM, Keane C, Brennan K, Finlay DK, Gardiner CM. Metabolic reprogramming supports IFN-gamma production by CD56bright NK cells. J Immunol. 2016;196:2552.
77. Xie QB, Liang Y, Yang M, Yang Y, Cen XM, Yin G. DEPTOR-mTOR signaling is critical for lipid metabolism and inflammation homeostasis of lymphocytes in human PBMC culture. J Immunol Res. 2017;2017:5252840.
78. Felices M, Lenvik AJ, McElmurry R, Chu S, Hinderlie P, Bendzick L, Geller MA, Tolar J, Blazar BR, Miller JS. Continuous treatment with IL-15 exhausts human NK cells via a metabolic defect. JCI Insight. 2018;3:e96219.
79. Kobayashi T, Mattarollo SR. Natural killer cell metabolism. Mol Immunol. 2019;115:3.
80. Shimano H, Sato R. SREBP-regulated lipid metabolism: convergent physiology—divergent pathophysiology. Nat Rev Endocrinol. 2017;13:710.
81. Xu Y, Tertilt C, Krause A, Quadri LE, Crystal RG, Worgall S. Influence of the cystic fibrosis transmembrane conductance regulator on expression of lipid metabolism-related genes in dendritic cells. Respir Res. 2009;10:26.
82. Lee JH, Phelan P, Shin M, Oh BC, Han X, Im SS, Osborne TF. SREBP-1a-stimulated lipid synthesis is required for macrophage phagocytosis downstream of TLR4-directed mTORC1. Proc Natl Acad Sci U S A. 2018;115:E12228.
83. Im SS, Yousef L, Blaschitz C, Liu JZ, Edwards RA, Young SG, Raffatellu M, Osborne TF. Linking lipid metabolism to the innate immune response in macrophages through sterol regulatory element binding protein-1a. Cell Metab. 2011;13:540.
84. Kidani Y, Elsaesser H, Hock MB, Vergnes L, Williams KJ, Argus JP, Marbois BN, Komisopoulou E, Wilson EB, Osborne TF, Graeber TG, Reue K, Brooks DG, Bensinger SJ. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. Nat Immunol. 2013;14:489.
85. Assmann N, O'Brien KL, Donnelly RP, Dyck L, Zaiatz-Bittencourt V, Loftus RM, Heinrich P, Oefner PJ, Lynch L, Gardiner CM, Dettmer K, Finlay DK. Srebp-controlled glucose metabolism is essential for NK cell functional responses. Nat Immunol. 2017;18:1197.
86. de Pablo MA, Angeles PM, Alvarez DCG. Immune cell functions, lipids and host natural resistance. FEMS Immunol Med Microbiol. 2000;29:323.
87. Yaqoob P, Newsholme EA, Calder PC. Inhibition of natural killer cell activity by dietary lipids. Immunol Lett. 1994;41:241.
88. Lynch LA, O'Connell JM, Kwasnik AK, Cawood TJ, O'Farrelly C, O'Shea DB. Are natural killer cells protecting the metabolically healthy obese patient? Obesity (Silver Spring). 2009;17:601.
89. O'Shea D, Cawood TJ, O'Farrelly C, Lynch L. Natural killer cells in obesity: impaired function and increased susceptibility to the effects of cigarette smoke. PLoS One. 2010;5:e8660.
90. Michelet X, Dyck L, Hogan A, Loftus RM, Duquette D, Wei K, Beyaz S, Tavakkoli A, Foley C, Donnelly R, O'Farrelly C, Raverdeau M, Vernon A, Pettee W, O'Shea D, Nikolajczyk BS, Mills KHG, Brenner MB, Finlay D, Lynch L. Metabolic reprogramming of natural killer cells in obesity limits antitumor responses. Nat Immunol. 2018;19:1330.
91. Ramakrishnan R, Tyurin VA, Veglia F, Condamine T, Amoscato A, Mohammadyani D, Johnson JJ, Zhang LM, Klein-Seetharaman J, Celis E, Kagan VE, Gabrilovich DI. Oxidized lipids block antigen cross-presentation by dendritic cells in cancer. J Immunol. 2014;192:2920.
92. Hossain F, Al-Khami AA, Wyczechowska D, Hernandez C, Zheng L, Reiss K, Valle LD, Trillo-Tinoco J, Maj T, Zou W, Rodriguez PC, Ochoa AC. Inhibition of fatty acid oxidation modulates immunosuppressive functions of myeloid-derived suppressor cells and enhances cancer therapies. Cancer Immunol Res. 2015;3:1236.
93. Yang K, Neale G, Green DR, He W, Chi H. The tumor suppressor Tsc1 enforces quiescence of naive T cells to promote immune homeostasis and function. Nat Immunol. 2011;12:888.
94. Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS, Goldstein JL. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J Clin Invest. 1996;98:1575.
95. Wang J, Su X, Yang L, Qiao F, Fang Y, Yu L, Yang Q, Wang Y, Yin Y, Chen R, Hong Z. The influence of myeloid-derived suppressor cells on angiogenesis and tumor growth after cancer surgery. Int J Cancer. 2016;138:2688.
96. Zhu X, Pribis JP, Rodriguez PC, Morris SJ, Vodovotz Y, Billiar TR, Ochoa JB. The central role of arginine catabolism in T-cell dysfunction and increased

susceptibility to infection after physical injury. Ann Surg. 2014;259:171.
97. Tai LH, Alkayyal AA, Leslie AL, Sahi S, Bennett S, Tanese DSC, Baxter K, Angka L, Xu R, Kennedy MA, Auer RC. Phosphodiesterase-5 inhibition reduces postoperative metastatic disease by targeting surgery-induced myeloid derived suppressor cell-dependent inhibition of Natural Killer cell cytotoxicity. Oncoimmunology. 2018;7:e1431082.
98. Shaashua L, Shabat-Simon M, Haldar R, Matzner P, Zmora O, Shabtai M, Sharon E, Allweis T, Barshack I, Hayman L, Arevalo J, Ma J, Horowitz M, Cole S, Ben-Eliyahu S. Perioperative COX-2 and beta-Adrenergic Blockade Improves Metastatic Biomarkers in Breast Cancer Patients in a Phase-II Randomized Trial. Clin Cancer Res. 2017;23:4651.
99. Fang V, Chaluvadi VS, Ramos-Perez WD, Mendoza A, Baeyens A, Rivera R, Chun J, Cammer M, Schwab SR. Gradients of the signaling lipid S1P in lymph nodes position natural killer cells and regulate their interferon-gamma response. Nat Immunol. 2017;18:15.
100. Molino S, Tate E, McKillop WM, Medin JA. Sphingolipid pathway enzymes modulate cell fate and immune responses. Immunotherapy. 2017;9:1185.
101. Kwong E, Li Y, Hylemon PB, Zhou H. Bile acids and sphingosine-1-phosphate receptor 2 in hepatic lipid metabolism. Acta Pharm Sin B. 2015;5:151.
102. Li D, Long W, Huang R, Chen Y, Xia M. 27-hydroxycholesterol inhibits sterol regulatory element-binding protein 1 activation and hepatic lipid accumulation in mice. Obesity (Silver Spring). 2018;26:713.
103. Adams CM, Reitz J, De Brabander JK, Feramisco JD, Li L, Brown MS, Goldstein JL. Cholesterol and 25-hydroxycholesterol inhibit activation of SREBPs by different mechanisms, both involving SCAP and Insigs. J Biol Chem. 2004;279:52772.
104. Titov VN. [Leptin and adiponectin in pathogenesis of metabolic syndrome]. Klin Med (Mosk). 2014;92:20.
105. Li R, Lau WB, Ma XL. Adiponectin resistance and vascular dysfunction in the hyperlipidemic state. Acta Pharmacol Sin. 2010;31:1258.
106. Dyck DJ, Heigenhauser GJ, Bruce CR. The role of adipokines as regulators of skeletal muscle fatty acid metabolism and insulin sensitivity. Acta Physiol (Oxf). 2006;186:5.
107. Dyck DJ. Adipokines as regulators of muscle metabolism and insulin sensitivity. Appl Physiol Nutr Metab. 2009;34:396.
108. Wang D, DuBois RN. Role of prostanoids in gastrointestinal cancer. J Clin Invest. 2018;128:2732.
109. Wang D, DuBois RN. Role of prostanoids in gastrointestinal cancer. J Clin Investig. 2018;128:2732.
110. Martinet L, Jean C, Dietrich G, Fournie JJ, Poupot R. PGE2 inhibits natural killer and gamma delta T cell cytotoxicity triggered by NKR and TCR through a cAMP-mediated PKA type I-dependent signaling. Biochem Pharmacol. 2010;80:838.
111. Walker W, Rotondo D. Prostaglandin E2 is a potent regulator of interleukin-12- and interleukin-18-induced natural killer cell interferon-gamma synthesis. Immunology. 2004;111:298.
112. Mailliard RB, Alber SM, Shen H, Watkins SC, Kirkwood JM, Herberman RB, Kalinski P. IL-18-induced CD83+CCR7+ NK helper cells. J Exp Med. 2005;202:941.
113. Wilk S, Jenke A, Stehr J, Yang CA, Bauer S, Goldner K, Kotsch K, Volk HD, Poller W, Schultheiss HP, Skurk C, Scheibenbogen C. Adiponectin modulates NK-cell function. Eur J Immunol. 2013;43:1024.
114. Bahr I, Goritz V, Doberstein H, Hiller GG, Rosenstock P, Jahn J, Portner O, Berreis T, Mueller T, Spielmann J, Kielstein H. Diet-induced obesity is associated with an impaired NK cell function and an increased colon cancer incidence. J Nutr Metab. 2017;2017:4297025.
115. Jahn J, Spielau M, Brandsch C, Stangl GI, Delank KS, Bahr I, Berreis T, Wrann CD, Kielstein H. Decreased NK cell functions in obesity can be reactivated by fat mass reduction. Obesity (Silver Spring). 2015;23:2233.
116. Lamas B, Goncalves-Mendes N, Nachat-Kappes R, Rossary A, Caldefie-Chezet F, Vasson MP, Farges MC. Leptin modulates dose-dependently the metabolic and cytolytic activities of NK-92 cells. J Cell Physiol. 2013;228:1202.
117. Bigler MB, Egli SB, Hysek CM, Hoenger G, Schmied L, Baldin FS, Marquardsen FA, Recher M, Liechti ME, Hess C, Berger CT. Stress-induced in vivo recruitment of human cytotoxic natural killer cells favors subsets with distinct receptor profiles and associates with increased epinephrine levels. PLoS One. 2015;10:e145635.
118. Salvatore G, Bernoud-Hubac N, Bissay N, Debard C, Daira P, Meugnier E, Proamer F, Hanau D, Vidal H, Arico M, Delprat C, Mahtouk K. Human monocyte-derived dendritic cells turn into foamy dendritic cells with IL-17A. J Lipid Res. 2015;56:1110.
119. York AG, Williams KJ, Argus JP, Zhou QD, Brar G, Vergnes L, Gray EE, Zhen A, Wu NC, Yamada DH, Cunningham CR, Tarling EJ, Wilks MQ, Casero D, Gray DH, Yu AK, Wang ES, Brooks DG, Sun R, Kitchen SG, Wu TT, Reue K, Stetson DB, Bensinger SJ. Limiting cholesterol biosynthetic flux spontaneously engages type I IFN signaling. Cell. 2015;163:1716.
120. Fu S, He K, Tian C, Sun H, Zhu C, Bai S, Liu J, Wu Q, Xie D, Yue T, Shen Z, Dai Q, Yu X, Zhu S, Liu G, Zhou R, Duan S, Tian Z, Xu T, Wang H, Bai L. Impaired lipid biosynthesis hinders anti-tumor efficacy of intratumoral iNKT cells. Nat Commun. 2020;11:438.

6 Lipid Metabolism in Tumor-Associated Macrophages

Yuancai Xiang and Hongming Miao

Abstract

Macrophages are essential components of the immune system in tumors. It can be recruited and educated to two mainly polarized subpopulations (M1-like and M2-like) of tumor-associated macrophages (TAMs) to display anti-tumor or protumor function during the tumor occurrence and progression. Reprogramming of metabolism, especially lipid metabolism, is a typical characteristic of TAMs polarization, which was confirmed recently as a vital target for tumor therapy. However, the relationship between TAMs and lipid metabolism is still obscure in the past decade. In this review, we will first introduce the historical aspects of TAMs, and then discuss the correlation of main lipids (triglycerides, cholesterol, and phospholipids) to TAMs activation and summarize the mechanisms by which lipid metabolism mediated tumor escape the immunological surveillance as well as currently available drugs targeting these mechanisms. We hope that this chapter will give a better understanding of lipid metabolism in TAMs for those who are interested in this field, and lay a foundation to develop novel strategies for tumor therapy by targeting lipid metabolism.

Keywords

Tumor-associated macrophages · Lipid metabolism · Macrophage polarization Tumor therapy

6.1 Introduction

Macrophages, an important member of the mononuclear phagocyte immune system, exist widely in almost all tissues of multicellular organisms. In human adults, they were estimated to total of 10^{10} cells, which are essential for mediating innate immunity (non-specific defense) and helping initiate adaptive immunity (specific defense) [1]. Macrophages are a kind of leukocytes and play vital roles in the maintenance of tissue homeostasis, microbial killing, inflammation regulation, immune suppression, antigen processing, and tissue remodeling [2–4]. Also, macrophages can activate the complement system by secreting various cytokines and are involved in the process of immune regulation [5].

A mounting number of observations have demonstrated that macrophages display impressive plasticity in adapting to the local microenvironment, which contains chemokines, lipids, growth factors, and other molecules secreted by tumor stromal cells [6–8]. During these processes, macrophages are generally differenti-

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ated into two polarized states, *i.e.*, classically activated macrophages (M1) and alternatively activated macrophages (M2), to perform distinct functions. Interestingly, macrophages also infiltrate into solid tumors (Tumor-associated macrophage, TAM) and develop into M1-like or M2-like cells, which interact with tumor cells to eliminate or promote tumor progression, respectively. Moreover, TAMs can also directly kill tumor cells, clear cellular debris, or eliminate apoptotic cells through phagocytosis, although it shows distinct phagocytic ability in different TAM phenotypes [5, 9]. Thus, understanding the mechanisms of TAM activation and/or differentiation is essential for developing strategies for tumor therapy.

6.2 Historical Aspects of TAMs

Host leukocytes were first discovered in and/or at the edge of tumor tissue by Virchow in the 1860s [10], which linked the chronic inflammation to tumor origin. Then the phagocytosis and macrophages were identified by Ilya Mechnikov in 1887 [11]. However, the classification of macrophages in the mononuclear phagocyte system was determined by van Furth and Cohn till in the 1970s [12, 13]. In mononuclear phagocyte theory, the tissue-resident macrophages depend upon the replenishment from bone marrow-derived blood monocytes [13]. Although this concept has been debated in several aspects, it is generally accepted that adult-derived tissue-resident macrophages, for instance, microglia, Kupffer cells, and alveolar macrophages resided in the brain, liver, and lungs, respectively, and embryonic-derived tissue-specific macrophages could coexist in the given tissues [14]. Over the next two decades, macrophages were demonstrated to exert distinct even opposite functions under different circumstances, especially in tumor progression [15, 16]. In 1992, Mantovani and colleges proposed a "macrophage balance" hypothesis to interpret the dual functions of TAMs (i.e., cytotoxicity and growth promotion) in their interaction with tumor cells through secreted cytokines by careful analysis of these seemingly contradictory results [10]. When determining the different metabolism of arginine in distinct mouse model-derived macrophages stimulated by IFN-γ, lipopolysaccharides (LPS) or TGF-β, Mills and colleges correlated with these differences between T helper 1 (Th1) and Th2 cell responses and proposed M1–M2 dichotomy to distinguish the two types of polarized macrophages [17]. This classification was commonly used in later research, although there are several other nomenclatures [18].

Currently, it is well established that macrophages are stimulated to M1 by IFN-γ, LPS, or granulocyte-macrophage colony-stimulating factors (GM-CSF). M1 macrophages produce high levels of pro-inflammatory cytokines such as TNFα, IL-1β, IL-6, IL-12, IL-8, and IL-23 to facilitate the clearance of invading pathogens. In contrast, when primed with cytokines like IL-4, IL-13, and IL-10, macrophages are prone to M2 phenotype and secret IL-10, arginase 1 (Arg1), and other type 2 cytokines to participate in the process of anti-inflammation, allergic response, tissue repair, and immunoregulation (Fig. 6.1) [4, 19, 20].

Due to high heterogeneity, TAMs also possess M1-like and M2-like subpopulations, which can transform into each other with the change of microenvironment. It was evidenced that, in addition to regulating the inflammatory response, M1-like and M2-like TAMs can exert anti-tumor and protumor functions, respectively. In the course of tumor progression, TAMs are the main recruited immune cell population (up to 50%) and are finally educated by tumors to a M2-like phenotype, which promotes tumor immune escape, tumor growth, angiogenesis, and metastasis [21]. Similarly, the CC chemokines like CCL17, CCL22, and CCL24 produced by M2-like macrophages can also inhibit the effector functions of $CD4^+$ and $CD8^+$ T cells and recruit regulatory T cells (Tregs) to regulate the tumor microenvironment [22–24]. Although TAMs were reported to be present in all stages of tumor progression, M1-like macrophages with a strong anti-tumor activity are mostly activated in the early stage, while M2-like macrophages with a protumor ability are mainly activated in

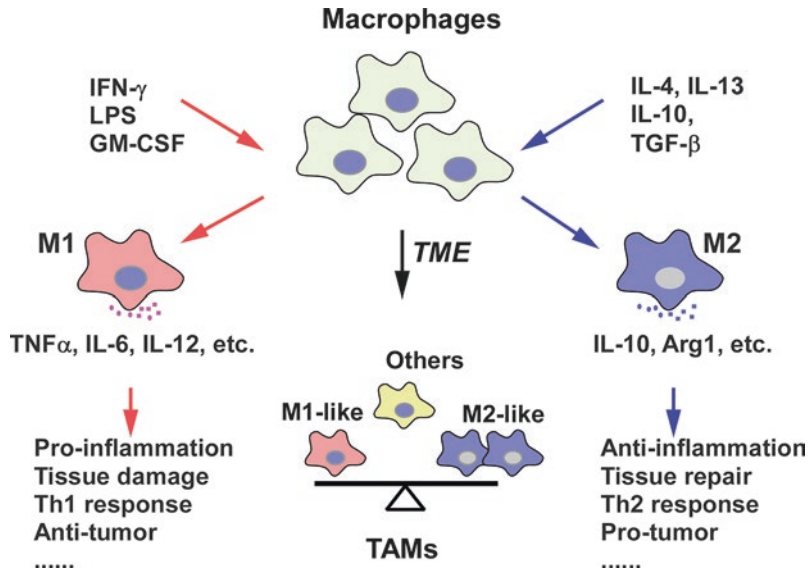

Fig. 6.1 The characteristic of macrophages and TAMs. Macrophages mainly differentiated into two subpopulations (M1 and M2) with distinct stimulations in the local microenvironment. Generally, M1 macrophages, stimulated by IFN-γ, LPS, or GM-CSF in inflammatory state, produced some pro-inflammatory cytokines (TNFα, IL-6, IL-12, etc.) to facilitate pro-inflammation, tissue damage, Th1 response, and tumor suppression. M2 macrophages, activated by IL-4, IL-13, IL-10, or TGF-β, generated a series of cytokines (IL-10, Arg1, etc.) to participate in the process of anti-inflammation, tissue repair, Th2 response, and tumor promotion. Once recruited into the tumor microenvironment (TME), macrophages were polarized to M1-like, M2-like tumor-associated macrophages (TAMs), and/or other subpopulations

the late stage. It should be noted that M1-like and M2-like macrophages are the two typical phenotypes of TAMs rather than the only phenotypes in various stages, which was confirmed by a series of research groups [25–28].

6.3 Overview of Metabolic Reprogramming in TAMs

It is well known that energy is one of the most important factors for tumor cell proliferation. In order to meet the demand, cancer cell was metabolically reprogrammed to quickly obtain adequate energy. The most famous metabolic reprogramming in cancer cells is the Warburg effect, a glucose metabolic shift from oxidative phosphorylation to aerobic glycolysis [29, 30]. Similarly, once macrophages are recruited into the tumor microenvironment, they have to be reprogrammed in the metabolic pathways in order to adapt to the special environment [1]. For example, TAMs differentiate into M2-like macrophages while stimulated with Th2 cytokines (IL-4 and IL-13). The M2-like macrophages have increased L-arginine metabolism and produce polyamines by increasing Arg1 expression, thereby suppressing the immune response and promoting tumor growth [31, 32]. Moreover, the reprogramming of glucose and lipid metabolisms were confirmed in murine or human TAMs. For instance, several lines of evidence have shown that TAMs utilize glycolysis in the early stages of cancer progression, but oxidative phosphorylation is preferred in the late stages with the accumulation of lactic acid [33–35]. The fatty acid uptake and oxidation are suppressed in M1 macrophages, which is totally opposite in M2 macrophages [36]. Furthermore, in addition to the variation of these nutrient metabolisms, some other metabolism patterns like heme oxygenase 1 mediated iron metabolic activation also contributed to TAM polarization [37]. Collectively, metabolic reprogramming can regulate TAM differentiation, polarization, and anti-tumor responses.

6.3.1 The Role of Lipid Metabolism for TAMs

6.3.1.1 General Process of Cellular Lipid Metabolism

Among the metabolic alterations in TAMs, lipid metabolism has been associated with the tumoricidal capability. Lipids are essential components of cell membrane structure and are composed of different types of molecules, including fatty acids, triglycerides, cholesterol, phospholipids, sphingolipids, etc. [38, 39]. Besides their roles as structural components, lipids can transduce signals within cells as second messengers, and provide energy sources when nutrients are limited [40–42]. In normal conditions, the cellular lipid sustains a homeostasis by regulating its biosynthesis and lipolysis. It has been well demonstrated that the digested fatty acids can be uptaken directly or de novo synthesized with the help of several key fatty acid synthases including sterol regulatory element-binding proteins (SREBPs), acetyl-CoA carboxylase (ACC), and stearoyl-CoA desaturase 1 (SCD1) [43]. When cellular lipids are excessive, these fatty acids will further generate triglycerides (TGs) with the aid of monoacylglycerol O-acyltransferases (MGATs) and diacylglycerol O-acyltransferases (DGATs) (Fig. 6.2). Fatty acids can also be converted to cholesteryl esters and those lipids are further stored in the cytosol as lipid droplets [44–46]. TG, cholesterol, and phospholipids are the main components within lipid droplets. To maintain the normal physiological state of cells, they are catalyzed by a series of enzymes to produce distinct derivatives for fulfilling diverse functions. However, the lipid metabolism was reprogrammed during TAM differentiation. Abnormal accumulation or decrease of metabolites not only directly affected TAM polarization, angiogenesis, lymphangiogenesis, or fatty acid oxidation but also indirectly changed these functions via transcriptional regulation mediated by nuclear factor kappa B (NF-κB), peroxisome proliferator-activated receptor (PPAR), CCAAT/enhancer-binding protein δ (C/EBPδ), signal transducer and activator of transcription 3 (STAT3) or SREBP1, which will be discussed as follows (Fig. 6.2).

6.3.1.2 Triglyceride Metabolism in TAMs

During the early comprehensive analysis of the gene expression profiles between human mono-

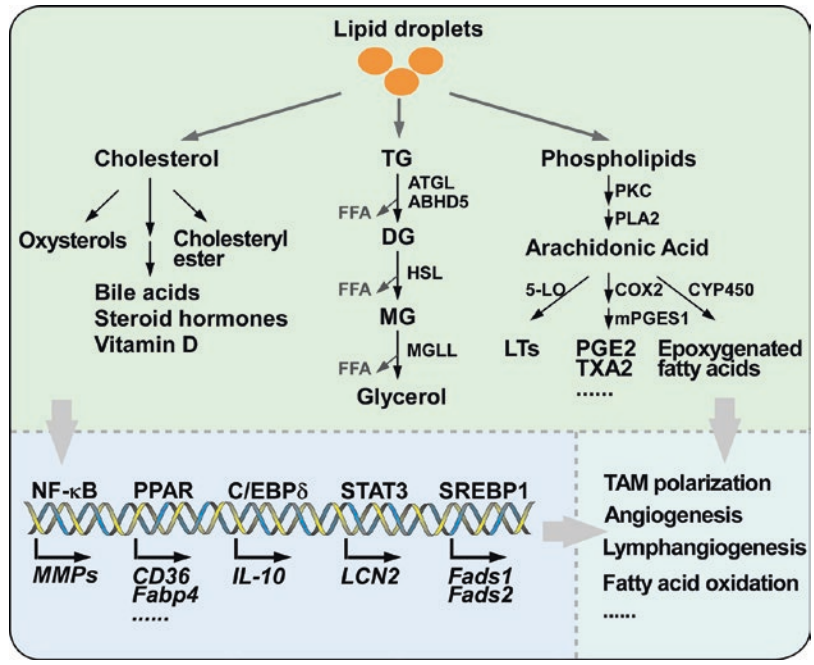

Fig. 6.2 Regulation of lipid catabolism or transformation in TAMs. *TG* triglycerides, *DG* diacylglycerol, *MG* monoacylglycerol, *ATGL* adipose triglyceride lipase, *ABHD5* abhydrolase domain-containing 5, *HSL* hormone sensitive lipase, *MGLL* monoacylglycerol lipase, *PKC* protein kinase C, *PLA2* phospholipase A2, *5-LO* 5-lipoxygenases, *COX2* cyclooxygenases 2, *PGE2* prostaglandin E2, *mPGES1* microsomal PGE2 synthase 1, *CYP450* cytochrome p450, *LTs* leukotrienes

cytes and differentiated macrophages (M1 and M2), lipid metabolism was found in the mainly altered category of modulated transcripts [47]. Even so, the relative mechanisms were largely unknown. Triglycerides, also known as neutral fats, are the main form of lipids. When cells need, TGs can be catalyzed by adipose triglyceride lipase (ATGL) to generate diacylglycerols (DGs), and then hydrolyzed via hormone sensitive lipase (HSL) to form monoacylglycerols (MGs), which can further produce free fatty acids and glycerols by monoacylglycerol lipase (MAGL/MGLL) [43, 48]. Recently, several studies from a same research group correlated the reprogrammed TG catabolism to macrophage activation. Xiang et al. demonstrated that MGLL was deficient and contributed to lipid accumulation in TAMs from colorectal cancer. MGLL deficiency induced an accumulation of MGs, DGs, and TGs. 2-Arachidonoylglycerol (2-AG), a subtype of DGs, functioned as an endogenous ligand of cannabinoid receptor-2 (CB2). Xiang et al. claimed that MGLL deficiency-induced 2-AG could activate CB2, inhibit the signal transduction of TLR4, and finally polarized TAMs to an M2-like phenotype, which maintained an immunosuppressive tumor microenvironment [49]. Another work screened a small TAM subpopulation with low expression of abhydrolase domain-containing 5 (ABHD5), a coactivator of ATGL to catalyze the hydrolysis of cellular triglycerides, in colorectal cancer tissues. The TAMs with ABHD5 deficiency had higher levels of ROS and matrix metalloproteinases (MMPs), which facilitated the migration and invasiveness of colorectal cancer cells [28]. Interestingly, the TG catabolism associated factor ABHD5 could also regulate polyamine metabolism in TAMs and cell growth of colorectal cancer [50]. Moreover, melanoma and colon cancer cells-educated macrophages can accumulate lipids through enhanced lipid uptake mediated by surface scavenger receptor CD36. These TAMs, in turn, generate some tumor proliferation- and progression-promoting molecules, such as *Arg1*, *Vegf*, and *Hif1a*. Noteworthily increased lipid accumulation in TAMs was positively and significantly associated with the development of multiple myeloma [51]. Taken together, these findings suggest that reprogramming of TG metabolism is crucial for TAM activation and tumor progression.

6.3.1.3 Cholesterol Metabolism in TAMs

Cholesterol homeostasis, which is modulated by the process of de novo synthesis, uptake, storage, and efflux, is important for the maintaining of cellular structure and function [52, 53]. Cholesterol de novo synthesis from acetyl-CoA is a complex process that involved in a series of enzymes to catalyze approximately 30 steps in cells [54]. It is well documented that cholesterols can be further processed to generate vitamin D, bile acids, steroid hormones, and cholesterol derivatives (cholesteryl esters, oxysterols) [54]. Accordingly, the metabolism of cholesterol theoretically might affect macrophage activation, although an early study showed that cholesterol-enriched peritoneal macrophages induced by casein did not demonstrate any different changes relative to the controls in tumoricidal activity [55]. This notion was gradually confirmed in the following studies. For instance, disruption of cholesterol synthesis by the inhibition of ATP citrate lyase can regulate membrane fluidity and decrease the anti-tumor activity in CpG-induced macrophages [56]. More convincingly, Goossens et al. reported that ovarian cancer cells promoted the efflux of membrane cholesterols, which made TAMs shift to M2-like phenotype and promote tumor progression [57].

In addition to cholesterols, the cholesterol-associated metabolites also regulate the activation of TAMs. For example, 27-Hydroxycholesterol (27HC), a kind of oxysterol, is a primary metabolite of cholesterols catalyzed by cytochrome p450 oxidase (CYP27A1) and degraded by oxysterol 7α-hydroxylase (CYP7B1). It was reported that CYP27A1 was highly expressed in TAMs and regulated TAM activation-associated tumor progression in breast cancer. The loss of the protumoral function of these CYP27A1 deficient TAMs was rescued by 27HC supplementation [58]. Moreover, when cocultured with breast cancer cells, THP-1 monocytes were differentiated into M2-like macrophages with high levels of CYP27A1 and 27HC. Accumulated 27HC was

not only secreted to promote tumor cell proliferation but also recruited monocytes and stimulated them to produce tumor favoring chemokines such as CCL2. Consistently, higher expression of CYP27A1 was also observed in IL-4-induced M2 macrophages [59]. These results indicated that 27HC accumulation stimulated TAMs to an M2-like phenotype and modified the microenvironment for cancer progression. In addition, vitamin D3 is a converted product of cholesterols. It is reported that tumor-produced versican V1 promoted LL-37 expression in macrophages through activation of toll-like receptor 2 (TLR2) and vitamin D3 signaling to facilitate ovarian cancer development in vitro [60]. Based on these studies, it seems that reducing cholesterols in TAMs is a benefit for tumor survival. However, recent research on lowering cholesterol with a chemical inhibitor showed an anti-tumoral function. Simvastatin, a cholesterol-lowering drug by disrupting lipid rafts, was able to re-polarize TAMs, promoting M2-to-M1 phenotype switch via cholesterol-associated LXR/ABCA1 regulation [61]. Collectively, the functions and mechanisms of cholesterol metabolism in TAMs is extremely complex owning to various components, which warrants further research in the future.

6.3.1.4 Phospholipid Metabolism in TAMs

Phospholipids are another group of lipids that contain phosphoric acids, mainly including glycerophospholipids and sphingomyelin, and are widely distributed in the cellular membrane system and influence cellular behavior in a variety of ways [62]. One of the most focused subgroups is arachidonic acid (AA) related phospholipids because they are key regulators of inflammation and cancers. AA will be released from the sn-2 position of membrane phospholipids through enzymatic reaction mediated by protein kinase C (PKC) and phospholipase A2 (PLA2) [63]. Free AA can be further transformed via three major pathways, cyclooxygenases (COX), cytochrome p450, and lipoxygenases (LO), into prostaglandins (PGs), epoxygenated fatty acids, and leukotrienes (LTs), respectively [64]. Among these mediators, it is not difficult to conclude that phospholipid metabolism must change in TAMs after infiltrating into the tumor microenvironment because COX1 and COX2 often changed in different stages of macrophages or in response to IFN-γ and LPS treatment [47]. For example, the most abundant prostaglandin E2 (PGE2), a COX2 mediated AA metabolic product, was markedly secreted by granulocyte-type $CD15^+$ myeloid cells from cancer patients compared to these in healthy donors [65]. Moreover, autocrine PGE2 from osteopontin (OPN)-stimulated TAMs could improve tumor microenvironment by affecting angiogenesis for tumor growth [66]. Interestingly, increased PGE2 could also, in turn, prevent myeloid differentiation into mature APCs and turn to a phenotype of myeloid-derived suppressor cells [65]. However, Ringleb et al. revealed another AA catalyzed enzyme, 5-LO, was decreased by apoptotic breast cancer cells, which attenuated T cell recruitment to benefit cancer progression [67]. In addition, high levels of 5-LO metabolites from hypoxic ovarian cancer cells promoted TAMs infiltration [68]. Of note, the role of 5-LO in the tumor microenvironment (TME) remains controversial [69–72].

6.3.1.5 Fatty Acid Oxidation in TAM

It is well documented that the way of energy production is a clear difference between M1 and M2 macrophages. Related to glycolysis dominantly used in M1, M2 macrophages seem to prefer oxidative phosphorylation via enhancing fatty acid oxidation (FAO) [73]. Moreover, it is reported that FAO inhibitor can markedly suppress IL-4-induced M2 macrophage activation [74]. However, this notion was challenged by Namgaladze and Brune, who demonstrated no apparent changes in human M2 macrophages treated by the FAO inhibitor [75]. Although the role of FAO in macrophage polarization was still ambiguous, recent studies uncovered that FAO indeed participated in the activation of TAMs. Among them, Zhang et al. demonstrated that the protumoral effects of conditioned medium from M2 polarized human monocyte-derived macrophages (M2-like TAMs) on hepatocellular carcinoma (HCC) were blocked by the FAO

inhibitor. Furthermore, a more direct method using different inhibitors against glutamine and glucose oxidation and fatty acid synthesis demonstrated that the effect of M2-like TAMs on HCC depends on the modulation of FAO activity [76]. Similarly, etomoxir could impede oleate-induced colon cancer-associated macrophages acquiring the M2-like phenotype [77]. These parallel results were also shown in PPAR activation-stimulated M2-like polarization in HCC-associated macrophages [78]. Of note, the opposite role of FAO was also reported by Niu et al., who demonstrated that truncated PPARγ inhibited fatty acid oxidation, contributed to tumor-associated macrophage differentiation, and promoted cancer progression [79]. After all, these findings suggest that FAO is an important factor in TAM activation.

6.3.2 Lipid Metabolites in TME Affects TAMs Polarization

Lipids are widely distributed and can affect cell functions in many ways. Importantly, cellular lipids can also be secreted and influence cell function in an autocrine or paracrine manner. For instance, the metabolites in TME derive from tumor cells, interstitial cells, and the vasculature systems. All those metabolites might have a communication with TAMs. As mentioned above, 27HC and PGE2 are secreted into TME to recruit monocytes and stimulate TAMs to produce tumor beneficial chemokines or affect angiogenesis [58, 59, 65]. Beyond these, there are a few lipid metabolites secreted by TAMs or tumors that have been identified to participate in TAMs polarization (Table 6.1).

Table 6.1 The role of secreted lipid metabolites on TAMs

Lipid metabolites	Origin	TAMs	Cancer type	Function	References
PGE2	Autocrine	RAW264.7	Melanoma	Angiogenesis	[66]
	Tumor cells	Tumor tissue-infiltrated CD11b + myeloid cells	Bladder tumors	Differentiation into mature APCs; acquiring phenotype of the myeloid-derived suppressor cells or inflammatory macrophages	[65]
	Tumor cells	RAW264.7 macrophages	Lung cancer	M2 macrophage differentiation	[80]
5-LO metabolite	Tumor cells	THP-1	Ovarian cancer	Macrophages migration and invasion	[68]
27HC	Autocrine	Bone marrow-derived macrophages	Breast Cancer	Cancer cell proliferation	[58]
	Autocrine	THP-1; murine bone marrow cells		ER+ cancer cell proliferation; recruitment of CCR2- and CCR5-expressing monocytes	[59]
25HC	Tumor cells	THP-1 monoblastic leukemia cell line; primary human peripheral blood monocytes	Glioblastoma multiforme	Macrophage chemotactic migration	[81]
S1P	Tumor cells	Human primary macrophages; isolated murine macrophages;	Breast Cancer	Macrophages differentiation into M2-like TAMs	[82, 83]
		Primary human macrophages	Breast Cancer	Lymphangiogenesis and tumor metastasis	[84]
LPA	Tumor cells	Primary macrophages	Colorectal cancer	M1-like polarization	[85]
OPN	Autocrine	RAW264.7	Melanoma	Angiogenesis; cancer cells migration; macrophage recruitment	[66]

6.4 Lipid Metabolic Reprogramming-Related Signaling Pathways in TAMs

TME is a special microenvironment with oxygen and nutrition deficiency. After entering into TME, macrophages will reprogram their metabolisms in an active or passive manner. The reprogrammed lipid metabolisms will determine the secreta of TAMs and further influence the immune status of TME and the progression of tumors. Knowing lipid metabolic reprogramming-associated signaling pathways in TAMs are beneficial for developing new targets for cancer treatment.

6.4.1 COX2/PGE2 Axis Mediates Phospholipid Metabolism Signal Network

COX2 is one of the most important inflammatory mediators and is abnormally expressed in many cancers. Multiple lines of evidence revealed that COX2 and its catalyzed product PGE2 converted from AA not only stimulated inflammation but also enhanced tumor cell proliferation and survival [86]. Likewise, the roles of COX2/PGE2 axis in TAMs and tumors have also been widely studied in recent years. For instance, TAM-derived OPN could bind to α9β1 integrin to promote AP1 signaling-mediated COX2 expression, which further increased PGE2 and MMP9 expression, resulting in angiogenesis and melanoma growth [66]. Besides, in nasopharyngeal carcinoma-associated macrophages, PGE2 stimulated the production of IL-10 and pentraxin 3 by adjusting RNA binding protein Hu antigen R (HuR)-mediated C/EBPδ expression (Fig. 6.2). According to this mechanism, PGE2 inhibited the tumor-phagocytic activity of TAMs [87]. More interestingly, PGE2 was demonstrated to trigger the expression of programmed cell death protein ligand 1 (PD-L1) in TAMs which is a chief culprit molecule helping tumors to escape from immunological surveillance [88]. Of note, a study in colon cancer showed that receptor-interacting protein kinase 3 (RIPK3) deficiency elicited NFκB-transcribed COX2 to elevate PEG2 levels. Moreover, elevated PEG2 in TME could suppress RIPK3. This RIPK3-PGE2 circuit further enlarged the protumoral function of TAMs [89]. Certainly, PGE2 could also alter TAMs functions via other signaling pathways such as PGE2/STAT3 [90]. Taken together, those studies indicated that the COX2/PGE2 axis might be a promising target for tumor treatment.

6.4.2 Caspase 1/PPAR Axis Regulates Fatty Acid Metabolism

In addition to regulating the abundance of PGE2, RIPK3 involving in fatty acid oxidation was reported by Wu et al. [78]. They found that RIPK3 deficiency in HCC-associated macrophages reduced ROS-mediated caspase 1 activation. Caspase 1 inactivation potentiated PPAR signaling to promote CD36 and Fabp4 mediated-FAO pathways and finally induce M2-like polarization (Fig. 6.2). Disruption of this pathway can alleviate the immunosuppressive activity of TAMs and suppress tumorigenesis of liver cancer [78]. However, caspase 1/PPARγ axis-induced FAO inhibition also promotes TAMs differentiation for breast cancer progression [79]. The seemingly contradicted findings in those two studies indicated that the TAMs in two different tumor tissues might have different secreta downstream of the FAO pathway. Another report pointed out that FAO activation could stimulate STAT6-mediated gene expression which was related to TAM generation and function [51]. Apart from FAO, SREBP1-regulated fatty acid desaturase 1 (*Fads1*) and *Fads2* (Fig. 6.2), i.e., fatty acid synthesis, was indirectly activated by regulatory T cells in the tumor microenvironment, which contributed to M2-like TAM polarization [91]. These results suggest that the mechanisms of fatty acid metabolism in TAMs are varied in the different tumor microenvironments, which shed light on personalized treatment in the future.

6.4.3 MGLL and ABHD5-Mediated Cross-Talk Between Triglyceride Metabolism and Other Signaling Pathways

Xiang et al. reported that TAMs in colorectal cancer tissues are rich in lipid droplets, which were mainly neutral fats. According to the high throughput analysis, they mapped the genes involved in TG metabolism. They claimed that MGLL was deficient and contributed to lipid accumulation in TAMs. MGLL deficiency resulted in 2-AG accumulation and CB2 activation, which blocked TLR4 signaling and polarized TAMs to an M2-like phenotype. In contrast, forced expression of MGLL in TAMs promoted the release of free fatty acid and reduced the levels of 2-AG. It has been reported that saturated fatty acid can activate TLR4-dependent M1 activation. Therefore, overexpression of MGLL in TAMs potentiated M1 polarization by potentiating TLR4 signaling and suppressing CB2 pathways and subsequently activated $CD8^+$ T cells to kill cancer cells [49]. Unexpectedly, another TG lipolytic activator ABHD5 was aberrantly overexpressed in colorectal cancer-associated macrophages. Miao et al. claimed that ABHD5 could inhibit C/EBPε-dependent expression of spermidine synthase and reduce the spermidine production, which finally promote colorectal cancer growth [50]. Interestingly, the same research group later reported a small population of TAMs with lower expression of ABHD5 in mouse colorectal cancer tissues were correlated to cancer metastasis. Mechanistically, they found that ABHD5 deficiency resulted in the overproduction of ROS and IL-1β, which activated NF-κB-dependent matrix metalloproteinase production and cancer cell metastasis (Fig. 6.2) [28]. These studies indicated that TG-associated enzymes were heterogeneously expressed in TAMs and might affect cancer cell biology in different aspects. Therefore, the heterogeneity of TG metabolism in TAMs should not be neglected when designing the strategies for cancer therapy.

6.4.4 Apoptosis Signals of Cancer Cells Affect TAM Activity

Survival tumor cells dynamically educated the tumor microenvironment by secreting a variety of protein and metabolic molecules. However, recent reports showed that apoptotic tumor cells could also alter the lipid metabolism of TAMs, especially during the early stages of tumor formation. Weigert et al. revealed that the activation of M2-like TAMs required tumor cell death [82]. This activation depends on sphingosine kinase 2 (Sphk2)-catalyzed production of Sphingosine-1-Phosphate (S1P) [82, 83]. Once secreted into the tumor microenvironment, S1P bound to its receptor S1PR1 before inducing LCN2 expression via activating STAT3 (Fig. 6.2), promoting lymphangiogenesis and enabling breast cancer cell metastasis [84]. Moreover, apoptotic cancer cells also could suppress 5-LO through Mer tyrosine kinase-mediated upregulation of transcription repression factor c-Myb, so that the loss of 5-LO expression provided a tumor-favored microenvironment [67].

6.4.5 "Do-Not-Eat" Signal Affects the Phagocytosis of TAMs

Phagocytosis is an essential role of macrophage in the elimination of pathogen, cellular debris, apoptotic cells, and neoplastic cells to maintain tissue homoeostasis. Under normal condition, macrophages express PD-1, signal regulatory protein α (SIRPα), and leukocyte immunoglobulin-like receptor subfamily B member 1 (LILRB1) to pair corresponding ligands PD-L1, CD47, major histocompatibility complex class I (MHC I) expressing in target cells, respectively, which help to distinguish self and non-self-cells [5]. However, neoplastic cells were evolutionarily expressed these "do-not-eat" signaling molecules in their surface to avoid macrophage engulfment and promote tumor immune evasion. It has been proved that lipid metabolism contributes to mac-

rophage phagocytosis via providing energy and regulating membrane fluidity. Thus, similar to macrophage, lipid metabolism, and its metabolite should theoretically modulate the phagocytic ability of TAMs by regulating these "do-not-eat" signaling molecules. Virtually this notion was reflected in several recent studies. Gordon et al. revealed that PD-1 was also expressed in the surface of TAMs and served as a suppression molecule of TAMs phagocytosis in mouse and human samples [92]. Meanwhile, Alvey et al. demonstrated that CD47 knockdown tumors were shrunk by TAM phagocytosis [93], which was further consolidated in cholangiocarcinoma [94]. Moreover, as described before, lipid metabolite PGE2 can induce PD-L1 expression in cancer and assist to escape immune surveillance [88], and directly affect the tumor-phagocytic activity of TAMs [87]. It is worth to note that the ex vivo phagocytosis assay performed by Gordon et al. uncovered the PD-1^{+} TAMs, with low phagocytosis, and PD-1^{-} TAMs, with high phagocytosis, were expressed an M2-like and M1-like surface profile, respectively [92], suggesting M1-like and M2-like TAMs displayed different phagocytic ability which contributes to their distinct function. Taken together, given that TAMs are less phagocytic than macrophages in other tissues [9], it is important to reactivate the phagocytic activity of TAMs in cancer treatment.

6.4.6 Other Identified Lipid Metabolism-Related Signaling Pathways

There are several other identified signaling pathways in TAMs for cancer cell growth and proliferation, although less research revealed the mechanisms of lipid metabolism. Li et al. revealed that ovarian cancer cell-derived versican V1 induced HCAP18/LL-37 expression by TLR-mediated vitamin D3 generation and further promoted tumor cell growth and invasion [60]. Besides, Wen et al. reported that the 5-LO produced by ovarian cancer cells within hypoxic conditions could promote macrophages migration and invasion through p38-mediated MMP-7 upregulation [68]. Interestingly, a positive effect of p38 signaling in TAMs was recently demonstrated by Zhang et al., who found that colon cancer cell-produced lysophosphatidic acid (LPA) could signal to its receptor to elevate p38/p65 signaling pathways in TAMs so as to acquire an M1-like phenotype and suppress cancer progression [85].

Altogether, the mechanisms of lipid metabolic reprogramming in TAMs are complicated, which makes TAMs shift their phenotypes actively or passively to adapt to the microenvironment. This nature not only easily can be used by tumor cells for survival, but also offers us a chance for tumor therapy.

6.5 Strategies for Tumor Therapy by Targeting TAM Lipid Metabolism

Given the potent regulatory roles in the occurrence and development of various cancers, TAMs, the main group of immune cells in TME, are considered to be a promising target for cancer treatment [95]. Currently, many drugs based on lipid metabolism-associated pathways have been applied to tumor therapies (Table 6.2). There are several lipid metabolism targeted strategies that have been utilized and briefly described as follows. (1) Depleting TAMs directly. As most TAMs demonstrate M2-like phenotype, destroying TAMs to remove tumor favoring microenvironment could be an effective way for cancer treatment, which was confirmed by treatment of clodronate in myeloma bearing mice [96]. (2) Promoting TAM differentiated into M1-like phenotype. This strategy was utilized by Zhang, et al. who augment M1-like TAMs through LPA mediated p38/p65 signaling pathway to enhance the suppression of colon cancer progression [85]. (3) Preventing TAMs from polarizing to the M2-like phenotype. For example, Mira et al. used lovastatin, an inhibitor of cholesterol biosynthesis, and revealed the inhibition of M2-like polarization of TAM which render it anti-tumor immunity in spontaneous mouse mammary tumors [97].

Table 6.2 Chemicals against lipid metabolism in TAMs for cancer therapy

Target protein	Chemicals	Cancer type	Preclinical model	References
COX2/PGE2	Aspirin; AH6809	Colon cancer	Xenografts	[89]
5-lipoxygenase	Zileuton	Ovarian cancer	Xenografts	[68]
Cholesterol	Simvastatin (combined with paclitaxel)	Lung cancer	Xenografts	[61]
Carnitine palmitoyl transferase 1α/PPARγ	Etomoxir; GW9662	Multiple myeloma; monoclonal gammopathy of undetermined significance; HCC	Xenografts	[51, 78]
Caspase-1	YVAD	Breast cancer	MMTV-PyMT mice	[79]
SREBP	Fatostatin (combination with anti-PD-1)	Melanoma; colon adenocarcinoma tumor	Xenografts	[91]
Cannabinoid receptor 2	AM-630; JTE-907	Colorectal cancer; breast cancer	Xenografts; MMTV-PyMT mice	[49]
Agpat4/LPA/p38/p65 axis	LPA; SB203580; BAY 11-7082	Colorectal cancer	Xenografts	[28, 85]

(4) Reeducation of M2-like TAMs. Regarding this, a study from Simões et al. showed that the stable synthetic analog of aspirin-triggered lipoxin—15-epi-LXA4 (ATL-1) could selectively promote M2-like TAMs to transform into M1-like profile, thereby triggering apoptosis of melanoma cells and suppressing its progression (Lipoxin A4 selectively programs) [98]. (5) Improving TAM phagocytosis. Phagocytosis of TAMs was enhanced by inhibition of "do-not-eat" signals like PD-1/PD-L1 axis [99].

6.6 Challenges and Perspective

As discussed above, TAMs are the key link connecting tumor immunosuppression to tumor occurrence and progression. Reprogramming of lipid metabolism plays a crucial role in regulating TAM activity. With the development of detection technology, more than 10,000 lipid species have been identified, indicating that lipid metabolism-based therapy possesses a great potential for cancer treatment. However, limited studies have been performed on the lipid metabolic reprogramming in TAMs. Therefore, there are still many problems that need to be resolved urgently. First, identifying the key target molecules or signaling pathways that display a decisive role to sustain the steady state of TAMs. Second, developing or optimizing the delivery methods of the targeted drug for precise treatment. Third, discovering new chemicals for changing the function of TAMs. Thus, by exploring these intrinsic mechanisms, we can deepen our understanding of the significance of lipid metabolism in TAMs and make more potential targeted strategies for cancer therapy.

References

1. Ley K. M1 means kill; M2 means heal. J Immunol. 2017;199:2191–3.
2. Haniffa M, Bigley V, Collin M. Human mononuclear phagocyte system reunited. Semin Cell Dev Biol. 2015;41:59–69.
3. Yona S, Gordon S. From the reticuloendothelial to mononuclear phagocyte system—the unaccounted years. Front Immunol. 2015;6:328.
4. Seyedizade SS, Afshari K, Bayat S, Rahmani F, Momtaz S, Rezaei N, et al. Current status of M1 and M2 macrophages pathway as drug targets for inflammatory bowel disease. Arch Immunol Ther Exp. 2020;68:10.
5. Zhou J, Tang Z, Gao S, Li C, Feng Y, Zhou X. Tumor-associated macrophages: recent insights and therapies. Front Oncol. 2020;10:188.
6. Laskin DL, Sunil VR, Gardner CR, Laskin JD. Macrophages and tissue injury: agents of defense or destruction? Annu Rev Pharmacol Toxicol. 2011;51:267–88.

7. Snyder RJ, Lantis J, Kirsner RS, Shah V, Molyneaux M, Carter MJ. Macrophages: a review of their role in wound healing and their therapeutic use. Wound Repair Regen. 2016;24:613–29.
8. Shi R, Tang Q, Miao H. Metabolism in tumor microenvironment: implications for cancer immunotherapy. MedComm. 2020;1:1–22.
9. Rodriguez D, Silvera R, Carrio R, Nadji M, Caso R, Rodriguez G, et al. Tumor microenvironment profoundly modifies functional status of macrophages: peritoneal and tumor-associated macrophages are two very different subpopulations. Cell Immunol. 2013;283:51–60.
10. Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L. The origin and function of tumor-associated macrophages. Immunol Today. 1992;13:265–70.
11. Mildner A, Yona S, Jung S. A close encounter of the third kind: monocyte-derived cells. Adv Immunol. 2013;120:69–103.
12. van Furth R, Cohn ZA. The origin and kinetics of mononuclear phagocytes. J Exp Med. 1968;128:415–35.
13. van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. Bull World Health Organ. 1972;46:845–52.
14. Varol C, Mildner A, Jung S. Macrophages: development and tissue specialization. Annu Rev Immunol. 2015;33:643–75.
15. Adams DO, Snyderman R. Do macrophages destroy nascent tumors? J Natl Cancer Inst. 1979;62:1341–5.
16. Mantovani A, Ming WJ, Balotta C, Abdeljalil B, Bottazzi B. Origin and regulation of tumor-associated macrophages: the role of tumor-derived chemotactic factor. Biochim Biophys Acta. 1986;865:59–67.
17. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol. 2000;164:6166–73.
18. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity. 2014;41:14–20.
19. Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, et al. Macrophage polarization in tumour progression. Semin Cancer Biol. 2008;18:349–55.
20. Jenkins SJ, Ruckerl D, Thomas GD, Hewitson JP, Duncan S, Brombacher F, et al. IL-4 directly signals tissue-resident macrophages to proliferate beyond homeostatic levels controlled by CSF-1. J Exp Med. 2013;210:2477–91.
21. Yunna C, Mengru H, Lei W, Weidong C. Macrophage M1/M2 polarization. Eur J Pharmacol. 2020;877:173090.
22. Zheng X, Turkowski K, Mora J, Brune B, Seeger W, Weigert A, et al. Redirecting tumor-associated macrophages to become tumoricidal effectors as a novel strategy for cancer therapy. Oncotarget. 2017;8:48436–52.
23. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25:677–86.
24. Franklin RA, Liao W, Sarkar A, Kim MV, Bivona MR, Liu K, et al. The cellular and molecular origin of tumor-associated macrophages. Science. 2014;344:921–5.
25. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. J Clin Invest. 2012;122:787–95.
26. Liao J, Feng W, Wang R, Ma S, Wang L, Yang X, et al. Diverse in vivo effects of soluble and membrane-bound M-CSF on tumor-associated macrophages in lymphoma xenograft model. Oncotarget. 2016;7:1354–66.
27. Yang Y, Zhang R, Xia F, Zou T, Huang A, Xiong S, et al. LPS converts Gr-1(+)CD115(+) myeloid-derived suppressor cells from M2 to M1 via P38 MAPK. Exp Cell Res. 2013;319:1774–83.
28. Shang S, Ji X, Zhang L, Chen J, Li C, Shi R, et al. Macrophage ABHD5 suppresses NFkappaB-dependent matrix metalloproteinase expression and cancer metastasis. Cancer Res. 2019;79:5513–26.
29. Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. Cancer Cell. 2012;21:297–308.
30. Yousefzadeh Y, Hallaj S, Baghi Moornani M, Asghary A, Azizi G, Hojjat-Farsangi M, et al. Tumor associated macrophages in the molecular pathogenesis of ovarian cancer. Int Immunopharmacol. 2020;84:106471.
31. Rath M, Muller I, Kropf P, Closs EI, Munder M. Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages. Front Immunol. 2014;5:532.
32. Chang CI, Liao JC, Kuo L. Macrophage arginase promotes tumor cell growth and suppresses nitric oxide-mediated tumor cytotoxicity. Cancer Res. 2001;61:1100–6.
33. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. Nature. 2014;513:559–63.
34. Dietl K, Renner K, Dettmer K, Timischl B, Eberhart K, Dorn C, et al. Lactic acid and acidification inhibit TNF secretion and glycolysis of human monocytes. J Immunol. 2010;184:1200–9.
35. DeNardo DG, Barreto JB, Andreu P, Vasquez L, Tawfik D, Kolhatkar N, et al. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell. 2009;16:91–102.
36. Odegaard JI, Chawla A. Alternative macrophage activation and metabolism. Annu Rev Pathol. 2011;6:275–97.
37. Zhu L, Zhao Q, Yang T, Ding W, Zhao Y. Cellular metabolism and macrophage functional polarization. Int Rev Immunol. 2015;34:82–100.

38. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol. 2008;9:112–24.
39. Holthuis JC, Menon AK. Lipid landscapes and pipelines in membrane homeostasis. Nature. 2014;510:48–57.
40. Guo D, Bell EH, Chakravarti A. Lipid metabolism emerges as a promising target for malignant glioma therapy. CNS Oncol. 2013;2:289–99.
41. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer. 2007;7:763–77.
42. Efeyan A, Comb WC, Sabatini DM. Nutrient-sensing mechanisms and pathways. Nature. 2015;517:302–10.
43. Cheng C, Geng F, Cheng X, Guo D. Lipid metabolism reprogramming and its potential targets in cancer. Cancer Commun (Lond). 2018;38:27.
44. Walther TC, Farese RV Jr. The life of lipid droplets. Biochim Biophys Acta. 1791;2009:459–66.
45. Walther TC, Farese RV Jr. Lipid droplets and cellular lipid metabolism. Annu Rev Biochem. 2012;81:687–714.
46. Fei W, Shui G, Zhang Y, Krahmer N, Ferguson C, Kapterian TS, et al. A role for phosphatidic acid in the formation of "supersized" lipid droplets. PLoS Genet. 2011;7:e1002201.
47. Martinez FO, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. J Immunol. 2006;177:7303–11.
48. Ou J, Miao H, Ma Y, Guo F, Deng J, Wei X, et al. Loss of abhd5 promotes colorectal tumor development and progression by inducing aerobic glycolysis and epithelial-mesenchymal transition. Cell Rep. 2014;9:1798–811.
49. Xiang W, Shi R, Kang X, Zhang X, Chen P, Zhang L, et al. Monoacylglycerol lipase regulates cannabinoid receptor 2-dependent macrophage activation and cancer progression. Nat Commun. 2018;9:2574.
50. Miao H, Ou J, Peng Y, Zhang X, Chen Y, Hao L, et al. Macrophage ABHD5 promotes colorectal cancer growth by suppressing spermidine production by SRM. Nat Commun. 2016;7:11716.
51. Su P, Wang Q, Bi E, Ma X, Liu L, Yang M, et al. Enhanced lipid accumulation and metabolism are required for the differentiation and activation of tumor-associated macrophages. Cancer Res. 2020;80:1438–50.
52. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. Cell. 2015;161:161–72.
53. Goldstein JL, Brown MS. The LDL receptor. Arterioscler Thromb Vasc Biol. 2009;29:431–8.
54. Huang B, Song B, Xu C. Cholesterol metabolism in cancer: mechanisms and therapeutic opportunities. Nat Metab. 2020;2:132–41.
55. Schlager SI, Madden LD, Meltzer MS, Bara S, Mamula MJ. Role of macrophage lipids in regulating tumoricidal activity. Cell Immunol. 1983;77:52–68.
56. Yang H, Bi Y, Xue L, Wang J, Lu Y, Zhang Z, et al. Multifaceted modulation of SIRT1 in cancer and inflammation. Crit Rev Oncog. 2015;20:49–64.
57. Goossens P, Rodriguez-Vita J, Etzerodt A, Masse M, Rastoin O, Gouirand V, et al. Membrane cholesterol efflux drives tumor-associated macrophage reprogramming and tumor progression. Cell Metab. 2019;29:1376–89.e4.
58. Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, et al. 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. Science. 2013;342:1094–8.
59. Shi SZ, Lee EJ, Lin YJ, Chen L, Zheng HY, He XQ, et al. Recruitment of monocytes and epigenetic silencing of intratumoral CYP7B1 primarily contribute to the accumulation of 27-hydroxycholesterol in breast cancer. Am J Cancer Res. 2019;9:2194–208.
60. Li D, Wang X, Wu JL, Quan WQ, Ma L, Yang F, et al. Tumor-produced versican V1 enhances hCAP18/LL-37 expression in macrophages through activation of TLR2 and vitamin D3 signaling to promote ovarian cancer progression in vitro. PLoS One. 2013;8:e56616.
61. Jin H, He Y, Zhao P, Hu Y, Tao J, Chen J, et al. Targeting lipid metabolism to overcome EMT-associated drug resistance via integrin beta3/FAK pathway and tumor-associated macrophage repolarization using legumain-activatable delivery. Theranostics. 2019;9:265–78.
62. Bohdanowicz M, Grinstein S. Role of phospholipids in endocytosis, phagocytosis, and macropinocytosis. Physiol Rev. 2013;93:69–106.
63. Poczobutt JM, Gijon M, Amin J, Hanson D, Li H, Walker D, et al. Eicosanoid profiling in an orthotopic model of lung cancer progression by mass spectrometry demonstrates selective production of leukotrienes by inflammatory cells of the microenvironment. PLoS One. 2013;8:e79633.
64. Colby JK, Jaoude J, Liu F, Shureiqi I. Oxygenated lipid signaling in tumor-associated macrophages-focus on colon cancer. Cancer Metastasis Rev. 2018;37:289–315.
65. Eruslanov E, Daurkin I, Vieweg J, Daaka Y, Kusmartsev S. Aberrant PGE(2) metabolism in bladder tumor microenvironment promotes immunosuppressive phenotype of tumor-infiltrating myeloid cells. Int Immunopharmacol. 2011;11:848–55.
66. Kale S, Raja R, Thorat D, Soundararajan G, Patil TV, Kundu GC. Osteopontin signaling upregulates cyclooxygenase-2 expression in tumor-associated macrophages leading to enhanced angiogenesis and melanoma growth via alpha9beta1 integrin. Oncogene. 2014;33:2295–306.
67. Ringleb J, Strack E, Angioni C, Geisslinger G, Steinhilber D, Weigert A, et al. Apoptotic cancer cells

suppress 5-lipoxygenase in tumor-associated macrophages. J Immunol. 2018;200:857–68.
68. Wen Z, Liu H, Li M, Li B, Gao W, Shao Q, et al. Increased metabolites of 5-lipoxygenase from hypoxic ovarian cancer cells promote tumor-associated macrophage infiltration. Oncogene. 2015;34:1241–52.
69. Wculek SK, Malanchi I. Neutrophils support lung colonization of metastasis-initiating breast cancer cells. Nature. 2015;528:413–7.
70. Cheon EC, Khazaie K, Khan MW, Strouch MJ, Krantz SB, Phillips J, et al. Mast cell 5-lipoxygenase activity promotes intestinal polyposis in APCDelta468 mice. Cancer Res. 2011;71:1627–36.
71. Poczobutt JM, Nguyen TT, Hanson D, Li H, Sippel TR, Weiser-Evans MC, et al. Deletion of 5-lipoxygenase in the tumor microenvironment promotes lung cancer progression and metastasis through regulating T cell recruitment. J Immunol. 2016;196:891–901.
72. Poczobutt JM, De S, Yadav VK, Nguyen TT, Li H, Sippel TR, et al. Expression profiling of macrophages reveals multiple populations with distinct biological roles in an immunocompetent orthotopic model of lung cancer. J Immunol. 2016;196:2847–59.
73. Kelly B, O'Neill LA. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. Cell Res. 2015;25:771–84.
74. Huang SC-C, Everts B, Ivanova Y, O'Sullivan D, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol. 2014;15:846–55.
75. Namgaladze D, Brüne B. Fatty acid oxidation is dispensable for human macrophage IL-4-induced polarization. Biochim Biophys Acta. 1841;2014:1329–35.
76. Zhang Q, Wang H, Mao C, Sun M, Dominah G, Chen L, et al. Fatty acid oxidation contributes to IL-1beta secretion in M2 macrophages and promotes macrophage-mediated tumor cell migration. Mol Immunol. 2018;94:27–35.
77. Wu H, Han Y, Rodriguez Sillke Y, Deng H, Siddiqui S, Treese C, et al. Lipid droplet-dependent fatty acid metabolism controls the immune suppressive phenotype of tumor-associated macrophages. EMBO Mol Med. 2019;11:e10698.
78. Wu L, Zhang X, Zheng L, Zhao H, Yan G, Zhang Q, et al. RIPK3 orchestrates fatty acid metabolism in tumor-associated macrophages and hepatocarcinogenesis. Cancer Immunol Res. 2020;8:710–21.
79. Niu Z, Shi Q, Zhang W, Shu Y, Yang N, Chen B, et al. Caspase-1 cleaves PPARgamma for potentiating the pro-tumor action of TAMs. Nat Commun. 2017;8:766.
80. Liu L, Ge D, Ma L, Mei J, Liu S, Zhang Q, et al. Interleukin-17 and prostaglandin E2 are involved in formation of an M2 macrophage-dominant microenvironment in lung cancer. J Thorac Oncol. 2012;7:1091–100.
81. Eibinger G, Fauler G, Bernhart E, Frank S, Hammer A, Wintersperger A, et al. On the role of 25-hydroxycholesterol synthesis by glioblastoma cell lines. Implications for chemotactic monocyte recruitment. Exp Cell Res. 2013;319:1828–38.
82. Weigert A, Tzieply N, von Knethen A, Johann AM, Schmidt H, Geisslinger G, et al. Tumor cell apoptosis polarizes macrophages role of sphingosine-1-phosphate. Mol Biol Cell. 2007;18:3810–9.
83. Weigert A, Schiffmann S, Sekar D, Ley S, Menrad H, Werno C, et al. Sphingosine kinase 2 deficient tumor xenografts show impaired growth and fail to polarize macrophages towards an anti-inflammatory phenotype. Int J Cancer. 2009;125:2114–21.
84. Jung M, Oren B, Mora J, Mertens C, Dziumbla S, Popp R, et al. Lipocalin 2 from macrophages stimulated by tumor cell-derived sphingosine 1-phosphate promotes lymphangiogenesis and tumor metastasis. Sci Signal. 2016;9:ra64.
85. Zhang D, Shi R, Xiang W, Kang X, Tang B, Li C, et al. The Agpat4/LPA axis in colorectal cancer cells regulates antitumor responses via p38/p65 signaling in macrophages. Signal Transduct Target Ther. 2020;5:24.
86. Tong D, Liu Q, Wang LA, Xie Q, Pang J, Huang Y, et al. The roles of the COX2/PGE2/EP axis in therapeutic resistance. Cancer Metastasis Rev. 2018;37:355–68.
87. Hsiao YW, Li CF, Chi JY, Tseng JT, Chang Y, Hsu LJ, et al. CCAAT/enhancer binding protein delta in macrophages contributes to immunosuppression and inhibits phagocytosis in nasopharyngeal carcinoma. Sci Signal. 2013;6:ra59.
88. Prima V, Kaliberova LN, Kaliberov S, Curiel DT, Kusmartsev S. COX2/mPGES1/PGE2 pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells. Proc Natl Acad Sci U S A. 2017;114:1117–22.
89. Yan G, Zhao H, Zhang Q, Zhou Y, Wu L, Lei J, et al. A RIPK3-PGE2 circuit mediates myeloid-derived suppressor cell-potentiated colorectal carcinogenesis. Cancer Res. 2018;78:5586–99.
90. Mao Y, Poschke I, Wennerberg E, Pico de Coana Y, Egyhazi Brage S, Schultz I, et al. Melanoma-educated CD14+ cells acquire a myeloid-derived suppressor cell phenotype through COX-2-dependent mechanisms. Cancer Res. 2013;73:3877–87.
91. Liu C, Chikina M, Deshpande R, Menk AV, Wang T, Tabib T, et al. Treg cells promote the SREBP1-dependent metabolic fitness of tumor-promoting macrophages via repression of CD8(+) T cell-derived interferon-gamma. Immunity. 2019;51:381–97.e6.
92. Gordon SR, Maute RL, Dulken BW, Hutter G, George BM, McCracken MN, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. Nature. 2017;545:495–9.
93. Alvey CM, Spinler KR, Irianto J, Pfeifer CR, Hayes B, Xia Y, et al. SIRPA-inhibited, marrow-derived macrophages engorge, accumulate, and differentiate in antibody-targeted regression of solid tumors. Curr Biol. 2017;27:2065–77.e6.
94. Vaeteewoottacharn K, Kariya R, Pothipan P, Fujikawa S, Pairojkul C, Waraasawapati S, et al. Attenuation of CD47-SIRPalpha signal in cholangiocarcinoma potentiates tumor-associated macrophage-mediated

phagocytosis and suppresses intrahepatic metastasis. Transl Oncol. 2019;12:217–25.
95. Mantovani A, Allavena P. The interaction of anticancer therapies with tumor-associated macrophages. J Exp Med. 2015;212:435–45.
96. Cabrales P, Carter C, Oronsky B, Reid T. Rrx-001 is a phase 3 small molecule dual inhibitor of CD47 and Sirpα with activity in multiple myeloma. Blood. 2018;132:5623.
97. Mira E, Carmona-Rodriguez L, Tardaguila M, Azcoitia I, Gonzalez-Martin A, Almonacid L, et al. A lovastatin-elicited genetic program inhibits M2 macrophage polarization and enhances T cell infiltration into spontaneous mouse mammary tumors. Oncotarget. 2013;4:2288–301.
98. Simoes RL, De-Brito NM, Cunha-Costa H, Morandi V, Fierro IM, Roitt IM, et al. Lipoxin A4 selectively programs the profile of M2 tumor-associated macrophages which favour control of tumor progression. Int J Cancer. 2017;140:346–57.
99. Li CW, Lai YJ, Hsu JL, Hung MC. Activation of phagocytosis by immune checkpoint blockade. Front Med. 2018;12:473–80.

7 Lipid Metabolism in Tumor-Associated Myeloid-Derived Suppressor Cells

Wei Liu, Hua Song, Xiaojing Li, Deshan Ren, Shuai Ding, and Yan Li

Abstract

Myeloid-derived suppressor cells (MDSCs) are a heterogenous population of myeloid cells with immature phenotypes and immunosuppressive functions. This population of cells has been extensively studied over the past decade owing to an increasing recognition of their pivotal role in pathological conditions including cancers, infectious diseases, sepsis, and autoimmune diseases. Various treatments targeting MDSCs are currently under development or in clinical trials with the aim to restore functional immunity against tumors or pathogens. Recent advances in immune metabolism demonstrate the role of metabolic pathways, especially lipid metabolism, in the differentiation and function of MDSCs in tumor environments. Therefore, a comprehensive understanding of lipid metabolism in MDSCs would facilitate the development of novel therapies against tumors through metabolic reprograming of MDSCs.

Keywords

Lipid metabolism · MDSC · Fatty acid Tumor microenvironment · Immune metabolism · Humanized mice

7.1 Lipid Metabolism and the Origin of MDSCs

Lipid metabolism involves lipid catabolism and anabolism, providing energy and substrate to sustain cellular activities. Lipid metabolism begins with food digestion to breakdown triglycerides into fatty acids, glycerol, and cholesterol by lipases. Small intestinal epithelial cells absorb fatty acids and monoglycerides to reassemble into triglycerides. Subsequently, triglyceride and cholesterol are packed with lipoproteins to form lipid transport complexes like chylomicrons, which travel into the blood circulation [1]. When lipid transport complexes move across tissues, capillary endothelial cells release and breakdown triglycerides into fatty acids and glycerol for consumption in metabolizing cells [2]. Glycerol participates in glycolysis or gluconeogenesis mostly in the cytosol of liver cells whereas fatty acids are stored mainly in the form of triglycerides or used through fatty acid beta-oxida-

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tion (FAO) for energy [3]. Long-chain fatty acids are transported via carnitine palmitoyltransferase system from cytosol to mitochondrial where the FAO takes place. The sequential removal of two carbon units from a beta position of fatty acyl-CoA molecules produces acetyl-CoA, a key intermediate for carbohydrate, protein, and lipid metabolism. Acetyl-CoA sustains tricarboxylic acid cycle (TCA) and oxidative phosphorylation (OXPHOS) [4]. If fatty acids are completely oxidized to CO_2 and water, they yield the highest ATP on an energy per gram basis compared to carbohydrates and proteins. On the contrary to FAO, fatty acid synthesis commences with carboxylation of acetyl-CoA derived from citrate in cytosol into malonyl CoA by acetyl CoA carboxylase 1 (ACC-1). Malonyl CoA in turn potently inhibits the carnitine palmitoyltransferase 1 (CPT-1) activity, the rate-limiting enzyme in FAO. Condensation of malonyl CoA to acetyl CoA and another seven cycles of reaction to produce a saturated long-chain fatty acid palmitate by fatty acid synthase. De novo synthesized fatty acids and intermediates of other metabolic pathways participate in the generation of other lipids or incorporated into triglycerides for lipid droplet formation.

Tissue-specific metabolic environment has a great influence on the hematopoiesis and peripheral immune homeostasis. Take bone marrow (BM) as an example (Fig. 7.1), adipocytes occupy up to 70% of adult BM cavity and thus make BM the third largest fat depot in the body behind subcutaneous and visceral fat [5]. These cells are not just "space fillers" [6], but rather metabolically active and release fatty acids from triacylglycerol droplet into hematopoietic milieu, therefore creating a lipid rich microenvironment for hematopoietic cells [7]. While long-term hematopoietic stem cell (LT-HSC) relies on anaerobic glycolysis for energy [8], short-term HSC (ST-HSC) and committed progenitors live on FAO for self-renew and differentiation [9]. When LT-HSCs differentiate, the expression of genes related to lipid metabolism became increasingly vigorous. HSC numbers in BM of mice and human increase with age, partially due to loss of quiescence for LT-HSCs and enhanced proliferation of ST-HSCs [10, 11]. A similar observation was reported in diet-induced obesity mice [12]. Moreover, aged and obese mice manifest biased hematopoiesis toward myeloid lineages and reduced lymphoid progenitors [13, 14]. Excessive accumulation of fat content in the marrow with aging and obesity is thought to contribute to this altered hematopoiesis through lipolysis of triglycerides in adipocytes. Indeed, fatty acids released from BM adipocytes are utilized by leukemic blasts via fatty acid-binding protein (FABP)-4, supporting the survival and proliferation of acute myeloid leukemia blast cells [15]. As such, lipid-rich BM microenvironment may favor the hematopoiesis toward myelopoiesis through metabolic reprogramming of HSC for FAO.

Myeloid-derived suppressor cells (MDSCs) (mouse: $CD11b^+$ $Gr1^+$; human: $CD11b^+$ $CD33^+$ HLA−DR−/lo) are immunosuppressive myeloid cell populations first described in tumors, subsequently identified in chronic inflammation, and recently in neonates [16–19]. Inhibition of MDSC mediated suppression of innate and adaptive immunity is crucial for effective immunotherapy against tumor and pathogens. Based on surface markers, MDSCs resemble neutrophils and monocytes with immature phenotypes, thus can be further divided into PMN-MDSCs (mouse: $Ly6G^+Ly6C^{-/lo}$; human: $CD15^+CD14^-$) and M-MDSCs (mouse: $Ly6C^{+/hi}Ly6G^-$; human: $CD14^+CD15^-$) subpopulations, respectively [20]. The origin of MDSCs is still under debate. Initially, MDSCs are proposed as myeloid precursors blocked from differentiation in BM and recruited by chemokines secreted from tumor cells. This hypothesis is supported by in vivo experiments that all-trans retinoic acid and vitamin D3 drive the immature myeloid cells in tumors into functional macrophages, granulocytes, and DCs [21, 22]. However, recent studies challenge this theory by demonstrating that monocytes and neutrophils are plastic and acquire immunosuppressive function within tumor microenvironment (TME) [23]. In any case, both hypotheses acknowledge the role of TME in the development of MDSCs. Although cytokines secreted by tumor cells have been indicated in the manipulation of myelopoiesis in BM or TME [24, 25], accumulating evidence points that metabolic reprogramming of MDSCs from

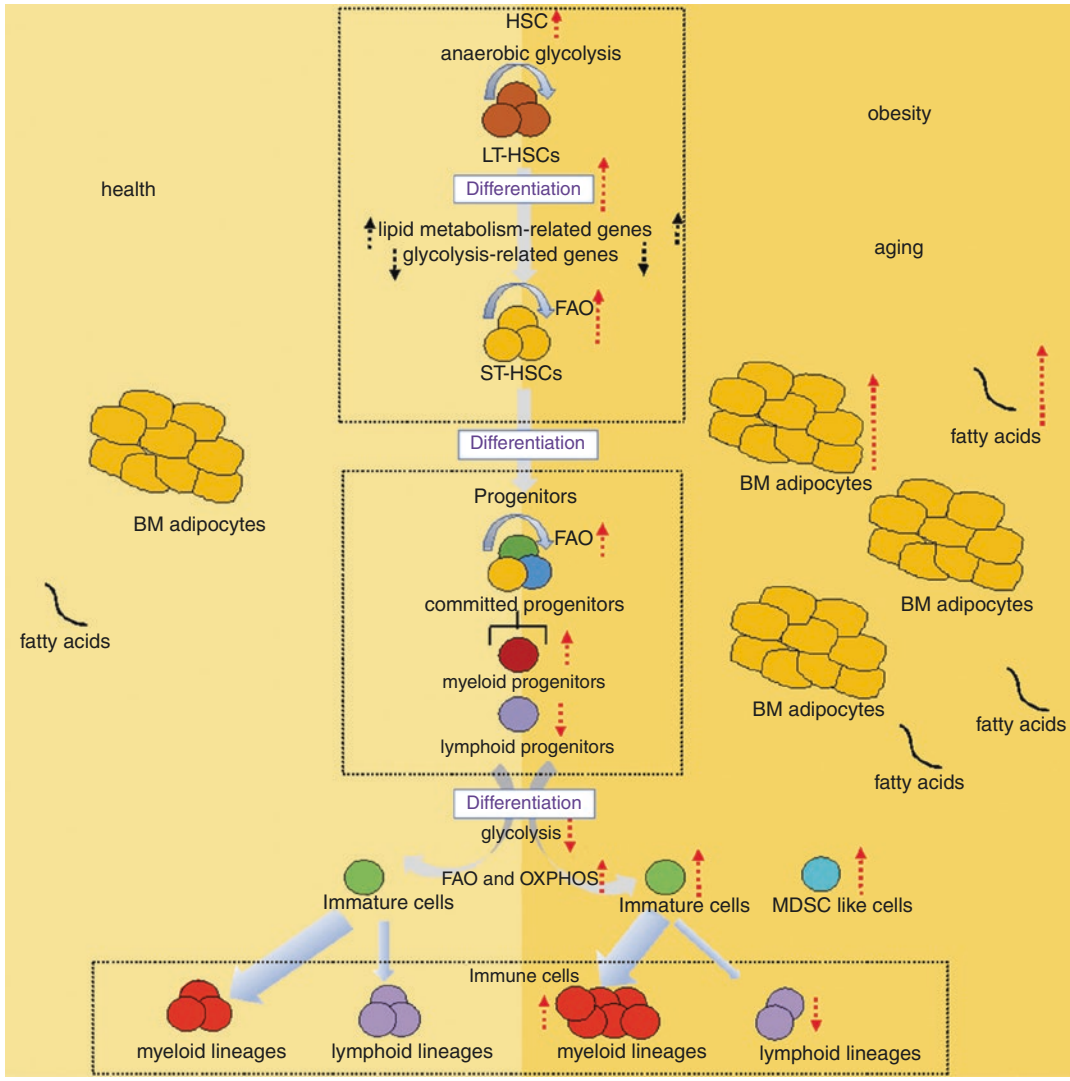

Fig. 7.1 Lipid metabolism and the origin of MDSCs. (i) LT-HSC relies on anaerobic glycolysis for energy, while ST-HSC and committed progenitors live on FAO for energy. (ii) During LT-HSC differentiation, the expression of lipid metabolism-related genes increases, accompanied by a decrease in the expression of glycolysis-related genes. (iii) Aging or obesity would lead to an increase in adipocytes and fatty acids in BM, forming a lipid-rich microenvironment. In the BM of aged and obese human or mice: (iv) the quiescence of LT-HSC is disrupted and ST-HSCs prefer to differentiate into myeloid progenitors, accompanied by a metabolic switch from glycolysis to FAO and OXPHOS for energy supply. (v) Immature myeloid cells accumulate and generate immunosuppressive MDSCs. *MDSC* Myeloid-derived suppressor cells, *LT-HSCs* Long-term hematopoietic stem cells, *ST-HSCs* Short-term hematopoietic stem cells, *FAO* Fatty acid beta oxidation, *BM* Bone marrow, *OXPHOS* Oxidative phosphorylation

glycolysis to FAO and OXPHOS by tumor cells play a pivotal role in the generation and function of MDSCs. Besides tumor tissues, MDSCs also accumulate in adipose tissue and bone marrow, two other lipid-rich environments. It has been reported that MDSCs are elevated in obese individuals, as well as in spleen and adipose tissue in obese mice. The level of MDSCs in adipose tissue of mice on a high-fat diet is 1.5–3 times higher than their lean counterparts [26]. As discussed above, bone marrow of obese and aged individuals also harbors a large reservoir of

immature myeloid cells. These evidence together suggest that tissue-specific lipid microenvironment greatly impacts MDSC generation.

7.2 Influence of TME on MDSC Energy Metabolism

Four distinct mechanisms are employed by MDSCs in TME to promote tumor growth and metastases: (a) suppression of tumor-specific immunity, (b) establishing a TME to benefit tumor growth, (c) facilitating tumor metastasis, (d) induction of tumor stem cell and promotion of epithelial-to-mesenchymal transition [27]. In TME, factors determining MDSC expansion, differentiation, migration, and immunosuppression, include cytokines and growth hormones (e.g., PGE2, TGF-β, GM-CSF, IL-6, and IFN-γ), low PH, hypoxia, and nutrient availability [28]. These triggers participate in shaping MDSC functions by exploiting their plasticity and reprogramming their metabolic fate [29]. Below, we discuss the key metabolic features in TME and its influence on the MDSC energy metabolism (Fig. 7.2).

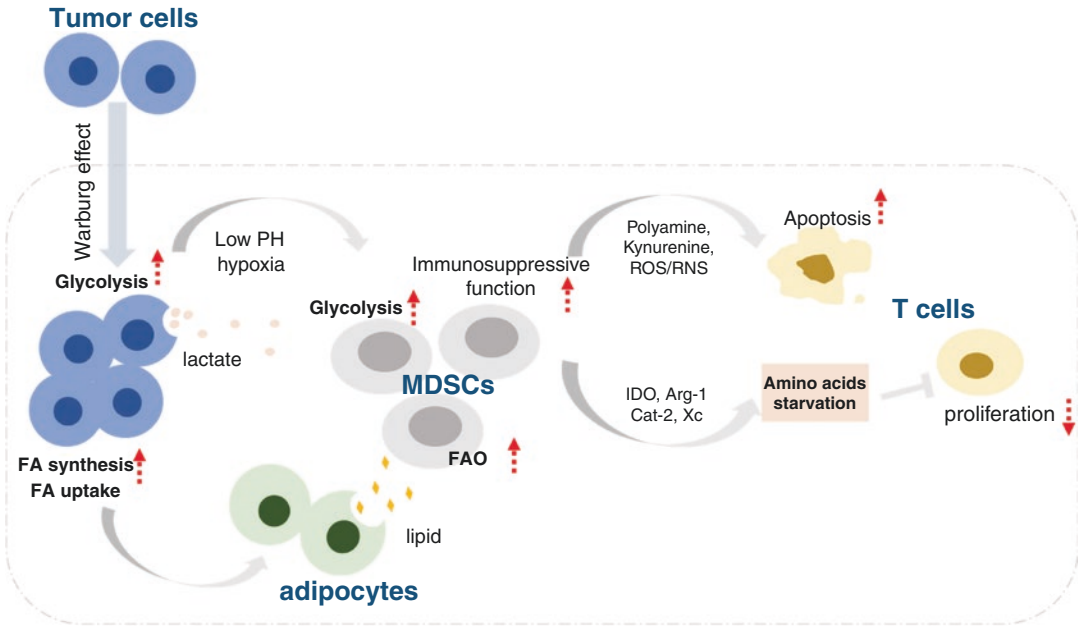

Fig. 7.2 TME shapes the energy metabolism of MDSCs. (i) To meet the demand for rapid proliferation, tumor cells initiate a metabolic shift toward glycolysis, which is known as Warburg Effect. The high glycolytic rate in tumor cells results in massive lactate release, forming an acidulated microenvironment. Besides, uncontrolled proliferation of tumor cells and abnormal blood vessel formation cause hypoxia in TME. (ii) MDSCs reprogram their metabolic pathways toward glycolysis to compete with tumor cells for limited oxygen and glucose. (iii) Tumor cells enhance fatty acid uptake and synthesis and recruit adipocytes in TME to produce excessive exogenous fatty acids, which makes a lipid-rich environment for infiltrating MDSCs. MDSCs are then driven to adopt FAO as their primary energy source. (iv) The acidic and lipid-rich TME promotes the development of MDSCs and enhances their immunosuppressive function. MDSCs consume amino acids in the TME through enzymes (Arg-1, IDO, iNOS, etc.) and transporters like Cat-2 and Xc, contributing to amino acid starvation in the TME, which in turn leads to inhibition of tumor antigen-specific proliferation of T cells. (v) Toxic ROS/RNS and amino acid metabolites (polyamine and kynurenine) released by MDSCs induce apoptosis of T cells and promote the generation of Treg from naïve CD4 T cells. *TME* Tumor microenvironment, *MDSC* Myeloid-derived suppressor cells, *FAO* Fatty acid beta oxidation, *Arg-1* arginase-1, *iNOS* induced nitric oxide synthase, *IDO* indoleamine 2,3 dioxygenase, *Cat-2* amino acid transporter 2B, *Xc* cysteine-glutamine antiporter, *ROS* reactive oxygen species, *RNS* reactive nitrogen species

Rapid proliferation of tumor cells requires large amounts of ATPs and substrates to fulfill energetic and biosynthetic demands. As such, tumor cells rewire their metabolic pathway toward glycolysis even under aerobic condition, also known as Warburg Effect [30]. Although aerobic glycolysis is insufficient to generate ATP per unit of glucose compared to mitochondrial respiration, it generates a comparable amount of ATP within a given period of time [31]. Massive lactate secretion from tumor glycolysis acidifies TME and altered the myelopoiesis to promote the generation of MDSCs [32]. Uncontrolled proliferation of tumor cells and abnormal blood vessel formation cause hypoxia in TME. Hypoxia stimulates the expression of HIF-1α in tumor-infiltrating immune cells, which is the downstream of PI3K-AKT-mTOR pathway [33]. Activation of HIF-1α favors the glycolysis over OXPHOS by upregulation of glucose and lactate transporters, along with glycolytic enzymes [34, 35]. This results in dampened lymphocyte activation and blocked maturation of myeloid subsets, which requires sufficient glucose for effector functions. Dynamic metabolic flux analysis (dMFA) study shows that MDSC maturation in TME correlates with a high glycolytic flux contributing to up to 95% of the global ATP turnover rate, demonstrating that developing MDSCs obtain energy primarily through glycolysis [36]. In addition, glycolysis activation by metformin or providing lactate promotes the proliferation of MDSCs in TME [37]. Hence, MDSCs would compete with tumor cells for limited glucose and oxygen, but eventually acclimate its metabolic pathway to the available nutrients and hypoxia in TME.

Nitrogen metabolism of MDSCs has been extensively studied in the context of immunosuppression of T cell function in TME. Amino acids such as L-arginine, tryptophan, and cysteine are either depleted from TME by MDSCs expressed enzymes like Arginase-1 (Arg-1), iNOS, and indoleamine 2,3 dioxygenase (IDO), or sequestered by transporters like cationic amino acid transporter 2B (Cat-2) or cysteine-glutamine antiporter (Xc) in MDSCs [38–40]. Amino acid starvation leads to inhibition of tumor antigen-specific proliferation of T cells through downregulation of CD3ζ and induction of cell cycle arrest at G0–G1 phase [41–43]. Moreover, products from amino acid metabolism such as polyamine, kynurenine, and toxic ROS/RNS (NO, O^{2-}, H2O2, and PNT) can further induce apoptosis of cytotoxic CD8 T cells and promote conversion of naïve CD4 T cells into Tregs [43–46].

Aerobic glycolysis provides tumor cells with excessive carbon for de novo synthesis of nucleotides, lipids, and proteins to support their uncontrolled proliferation. Of note, enhanced uptake of glucose promotes synthesis of reducing agent NADPH from oxidative branch of pentose phosphate pathway to participate in lipid synthesis [47, 48]. As a result, reactivation of lipid biosynthesis and storage have been frequently reported in cancer tissues, especially when tumor cells outgrew tissue blood supply of nutrients and oxygen. Despite that tumor cells synthesize most fatty acids de novo [49], aggressive tumor cells often intermingle with adipocytes in TME to obtain exogenous fatty acids [50, 51]. Therefore, similar to BM and adipose tissue microenvironment, TME is also a lipid-rich environment for infiltrating immune cells. We will discuss further on how this lipid-rich TME influences immunosuppressive function of MDSCs.

7.3 Lipid Metabolism and Immunosuppressive Function of MDSC

Recent studies on mouse models conclude that tumor-associated MDSCs upregulate FAO as a primary energy source as opposed to glycolysis to exert immunosuppressive functions, in comparison to peripheral myeloid cells and spleen MDSCs. This conclusion is supported by increased mitochondrial mass, fatty acid uptake via CD36, expression of key enzymes in FAO (CPT-1, acyl-CoA dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase), and oxygen consumption rate (OCR)/extracellular acidification rate (ECAR) ratio [52]. Of note, consistent with what has been observed in the mouse study, enhanced fatty acid uptake and increased level of FAO-related

enzymes in MDSCs were also detected in the blood and tumor from cancer patients. Along this line, lipid overload in MDSCs correlates with their suppressive function. Interestingly, most lipids in MDSCs are found to be oxidized via MPO and ROS, and the polyunsaturated fatty acids, which are more susceptible to oxidation, could promote the suppressive function of MDSCs [29]. Importantly, pharmacologic inhibition of FAO in mouse significantly alleviated tumor progression and improved antitumor outcome of adoptive T-cell therapy. Mechanically, this is because pharmacologic inhibition of FAO decreased the production of inhibitory cytokines and prevented the immune inhibitory capability of MDSCs. Moreover, combining chemotherapy and pharmacologic FAO inhibition eliminated the immunosuppressive effects of MDSCs and consequently enhanced the therapeutic outcome [52]. Lysosomal acid lipase (LAL) is required for hydrolysis of cholesteryl and triglycerides in lysosomes to generate fatty acid for FAO. MDSCs in LAL-deficient mice switched from FAO to glucose-dependent oxidative pathway, with enhanced proliferation but compromised immunosuppression [53]. Together, these results indicate that modulating FAO activity may provide an interesting option to inhibit the immunosuppressive function of MDSCs and improve the outcome of clinical therapy [54].

Besides FAO for ATP supply, fatty acid synthesis, lipogenesis, and lipid accumulation also link to suppressive function of MDSCs [55, 56]. Gabrilovich et al. demonstrated that targeting key enzymes of lipid synthesis ACC-1 with 5-tetradecycloxy-2 furoic acid (TOFA) to block fatty acid synthesis in MDSCs revert the suppression of T cell activation. Recently, Veglia, et al. identified MDSCs utilize fatty acid transport protein 2 (FATP-2) for transporting arachidonic acid and synthesizing prostaglandin E2 (PGE-2) to suppress T cell-mediated antitumor immunity. Selective inhibition of FATP2 abolished the suppressive activity of PMN-MDSCs and substantially delayed tumor progression in mice [57]. Interestingly, PGE2, a lipid mediator, generated by COX-2 from fatty acid arachidonic acid [58] was proved to promote MDSC generation in tumor-bearing mice [59]. And further research indicated that inhibiting PEG2 or COX2 could decrease MDSC generation and delay tumor progression [59, 60]. Prevention of lipid droplet formation in MDSC like cell line MSC-2 by diacylglycerol acyltransferases impairs the immunosuppression [61]. Therefore, although fatty acid synthesis and oxidation cannot occur under classical view, it appears that MDSCs adapt to hypoxic and nutrient-deprived TME to activate both metabolic pathways simultaneously to exert its suppressive function (Fig. 7.3).

7.4 Genetic and Epigenetic Pathways Involved in Lipid Metabolisms of MDSC

Although how lipid metabolism in MDSCs influences their immunosuppressive function is not fully understood, several signaling pathways have been implicated to play a role in this process. For example, liver X receptors (LXR) are members of nuclear hormone receptor family that regulate lipid homeostasis. Their physiological activators are oxysterols and intermediates in the cholesterol biosynthetic pathway. Under the administration of LXR agonist, PMN-MDSCs and M-MDSCs were effectively decreased in mouse models and cancer patients [62]. In addition, the LXR target gene ApoE triggers activation of cytotoxic T cells to enhance antitumor immune defenses [62]. Another known "lipid sensing" receptor is peroxisome proliferator-activator receptors (PPARs), which are activated by free fatty acids, prostaglandins, and sterols. Several PPARs subtypes elicit the expression of FAO genes and coordinate cell fate and inflammation. Cardiolipin, the main phospholipid in the inner mitochondrial membrane, promotes IL-10 expression in MDSCs of tumor-bearing mice by activating PPARγ [63, 64], and the effect can be reversed by PPARγ inhibitor [65]. Moreover, PPARα agonist activated CPT and thereby enhanced fatty acid catabolism in melanoma-specific $CD8^+$ TILs, ultimately leading to delayed

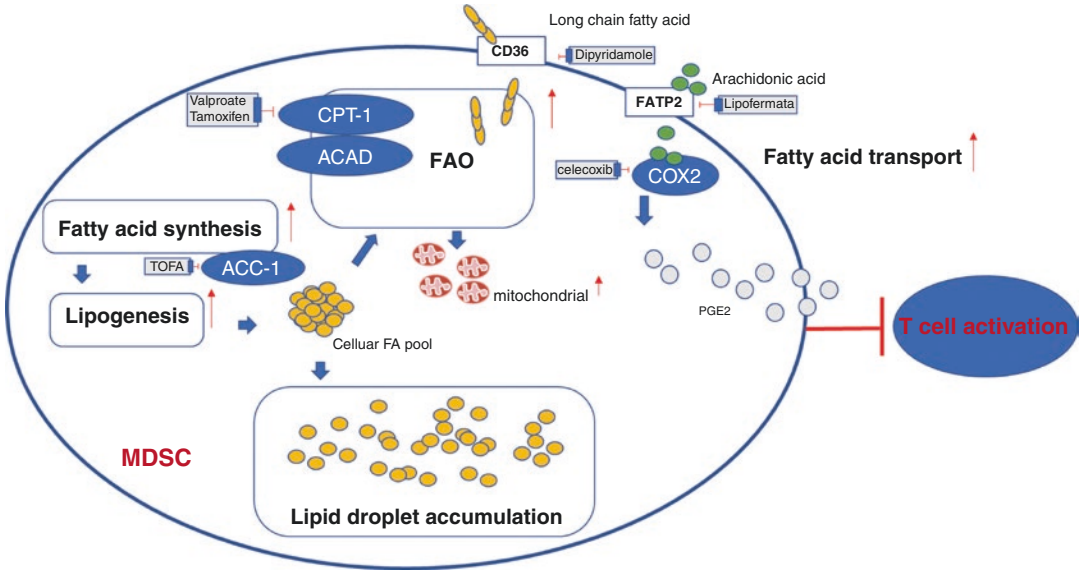

Fig. 7.3 lipid metabolism in MDSCs correlate with their suppressive function. (i) Fatty acid uptake, fatty acid beta oxidation (FAO), fatty acid synthesis, and lipogenesis increase in MDSCs and result in upregulation of immunosuppressive function. (ii) Lipid accumulation in MDSC leads to lipid droplet formation and associate with their immunosuppressive functions. (iii) Pharmacologic inhibition of fatty acid transport, FAO, and fatty acid synthesis in MDSCs blocks immunosuppressive functions. *MDSC* Myeloid-derived suppressor cells, *FAO* Fatty acid beta oxidation, *FATP2* fatty acid transport protein 2, *COX2* cyclooxygenase 2, *PGE2* prostaglandin E2, *CPT-1* Carnitine palmitoyl transferase 1, *ACAD* acyl-CoA dehydrogenase, *ACC-1* Acetyl-CoA carboxylase, *TOFA* 5-tetradecycloxy-2 furoic acid

tumor progression [66]. These studies indicate targeting PPAR pathways as a promising strategy for rebalancing lipid metabolism in MDSCs. Further research supported cross-regulation between PPARs and LXRs pathways to increase atherosclerosis susceptibility and elucidated that activation of this regulatory network has additive effects in controlling ATP binding cassette transporter A1 (ABCA1) expression, thereby reversing cholesterol transport in macrophages [67, 68].

Changes in lipid metabolism can also influence cell differentiation and functions by epigenetic modifications. For example, the phospholipid derivative lysophosphatidic acid (LPA) activates a family of GPCRs named LPAR1–6 and leads to recruitment of HDAC1 coincides with decreased histone acetylation in the TRAIL death receptors promoter, which ultimately promotes survival of cancer cells [69]. Moreover, many DNA/histone-modifying enzymes often require cofactors and substrates that are also critical intermediate metabolites. For example, increased FAO leads to elevation of acetyl-CoA, which served as a carrier of acyl groups by histone acetyltransferases to promote open chromatin state and activate transcription. Compared with other immune cells, evidence to support the direct link between metabolites or cofactors and epigenetic regulation in MDSCs is still lacking [70, 71].

Besides providing most of the acetyl groups on histones, fatty acids are also involved in another histone modification: histone acylation. Up till now, a variety of short and long-chain acyl groups have been identified covalently attached to a histone lysine residue, including propyl, butyryl, crotonyl, myristoyl, and palmitoyl. Each fatty acid modifying group confers distinct biochemical properties that influence subcellular localization, intracellular trafficking, and protein–lipid interactions. A compelling study to link lysine fatty acylation to immune response shows

the role of HDAC11 in the regulation of serine hydroxymethyltransferase 2 (SHMT2) defatty-acylation in type I IFN-mediated signaling [72]. HDAC11, the only class IV HDAC member, has been rediscovered as a highly potent and efficient defatty-acylase of lysine as compared to its deacetylase activity [72]. Of note, HDAC11 acts as a negative regulator of MDSCs expansion and function [73]. A better understanding of the mechanisms and functional consequences of reversible lysine fatty acylation may provide new insights into regulating lipid metabolic homeostasis. In the future, we expect to see the signaling pathway inhibitors, epigenetic inhibitors, and metabolite antagonists that specifically interfere with the above mentioned regulatory process being applied in combination to manipulate the fate of MDSCs and its function.

7.5 Issues of Translating MDSC Knowledge from Bench to Bed

Although several key discoveries made in mouse studies have been validated on human, there are still differences between human and murine MDSCs. For example, LOX-1, a low-density lipoprotein receptor, has been proposed as a candidate marker for human immunosuppressive PMN-MDSCs and numbers of LOX-1$^+$ PMN-MDSCs correlate with cancer progression [74]. On the contrary, PMN-MDSCs from LOX-1 knockout mice did not exhibit differences in migration toward tumor tissue, nor in suppressive function against T cells compared to wild type PMN-MDSCs. Moreover, Arg-1 expression in M-MDSCs and TAMs are well documented in murine studies, whereas Arg-1 is constitutively expressed in human neutrophils and inducible in other monocytic cells under disease setting [75]. Another example of these differences could be the fact that mice lacks cholesteryl ester transfer protein (CETP), which transferring CE from HDL toward ApoB containing lipoproteins [76]. Consequently, mice and human display a substantially different plasma lipoprotein profile. These species barriers might hinder the translation process from mouse studies to effective therapies targeting metabolisms of MDSCs.

Despite the success achieved in past years on studying lipid metabolism and mouse MDSCs, research on human MDSCs is still scarce and key questions regarding human MDSC biology remain largely unexplored. How chronic tumor microenvironment influence the generation process of human MDSCs? Through which mechanism are they recruited to the tumor microenvironments? Would it be possible to manipulate human MDSC generation or migration toward tumor microenvironment through altering lipid metabolism? To address these questions, novel research models and tools are required. For instance, severe immune-deficient mice engrafted with human CD34$^+$ HSC develop multi-lineage human immune cells, which can serve as a unique platform to study human MDSC biology in vivo [77, 78]. These humanized mouse models can be further transplanted with tumor cells or tissues to dissect the factors of TME on both the generation and the recruitment of MDSCs [79, 80]. Moreover, liver humanization protocol has succeeded in addition to human immune system reconstitution to create a double humanized mouse. These complex yet highly clinically relevant models would provide an in vivo platform to investigate the metabolic and immune cross-talk among human MDSCs, tumor, and liver (Fig. 7.4). On average, 95% of new cancer drug candidates failed during clinical tests due to the inconsistency between murine and human immune systems [81] and lack of reliable preclinical animal models, which could accurately recapitulate patient tumor microenvironment [82]. Hence, it is critical to appreciate the differences between human and murine MDSCs in order to accelerate therapeutic development. With the advance of humanized mouse models, screening, and validation of MDSC targeted therapeutics would be easier and faster in the future.

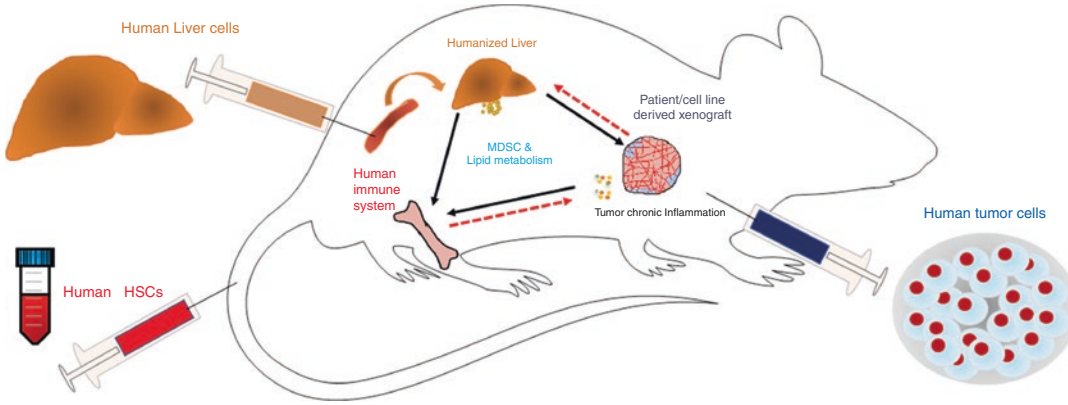

Fig. 7.4 Liver/Tumor/Immune humanized mouse models. To further investigate lipid metabolism in human tumor-associated MDSCs in vivo, we propose a liver, tumor, and immune system triple humanized mouse model. Human MDSC and immune system can be generated from human $CD34^+$ HSC engrafted to severe immune-deficient mice. And the liver can be humanized by injecting human liver cells into spleen of a genetic engineered mouse strain with spontaneous or induced liver damage. This humanized mouse model can be further transplanted with primary tumor cells or tissues from patients or tumor cell lines to create a triple humanized mouse model. With the availability of this triple humanized mouse model, we can decipher the factors involved in the generation and recruitment of MDSCs to tumor microenvironment and calibrate the balance between lipid catabolism and anabolism in MDSC function and tumor progression. *MDSC* Myeloid-derived suppressor cell, *HSC* hematopoietic stem cells

References

1. Jo Y, Okazaki H, Moon YA, Zhao T. Regulation of lipid metabolism and beyond. Int J Endocrinol. 2016;2016:5415767. https://doi.org/10.1155/2016/5415767.
2. Feingold KR, Grunfeld C. Introduction to lipids and lipoproteins. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext. South Dartmouth, MA: MDText.com; 2000.
3. Berg JM, Tymoczko J L, Stryer L. Section 16.3. Glucose can be synthesized from noncarbohydrate precursors. In: Biochemistry. 5th ed. New York: W H Freeman; 2002.
4. Abo Alrob O, Lopaschuk GD. Role of CoA and acetyl-CoA in regulating cardiac fatty acid and glucose oxidation. Biochem Soc Trans. 2014;42(4):1043–51. https://doi.org/10.1042/BST20140094.
5. Fazeli PK, Horowitz MC, MacDougald OA, Scheller EL, Rodeheffer MS, Rosen CJ, Klibanski A. Marrow fat and bone—new perspectives. J Clin Endocrinol Metab. 2013;98(3):935–45. https://doi.org/10.1210/jc.2012-3634.
6. Guerra DAP, Paiva AE, Sena IFG, Azevedo PO, Batista ML Jr, Mintz A, Birbrair A. Adipocytes role in the bone marrow niche. Cytometry A. 2018;93(2):167–71. https://doi.org/10.1002/cyto.a.23301.
7. Robles H, Park S, Joens MS, Fitzpatrick JAJ, Craft CS, Scheller EL. Characterization of the bone marrow adipocyte niche with three-dimensional electron microscopy. Bone. 2019;118:89–98. https://doi.org/10.1016/j.bone.2018.01.020.
8. Passegue E, Wagers AJ, Giuriato S, Anderson WC, Weissman IL. Global analysis of proliferation and cell cycle gene expression in the regulation of hematopoietic stem and progenitor cell fates. J Exp Med. 2005;202(11):1599–611. https://doi.org/10.1084/jem.20050967.
9. Lee MKS, Al-Sharea A, Dragoljevic D, Murphy AJ. Hand of FATe: lipid metabolism in hematopoietic stem cells. Curr Opin Lipidol. 2018;29(3):240–5. https://doi.org/10.1097/MOL.0000000000000500.
10. Pang WW, Price EA, Sahoo D, Beerman I, Maloney WJ, Rossi DJ, Schrier SL, Weissman IL. Human bone marrow hematopoietic stem cells are increased in frequency and myeloid-biased with age. Proc Natl Acad Sci U S A. 2011;108(50):20012–7. https://doi.org/10.1073/pnas.1116110108.
11. Pearce DJ, Anjos-Afonso F, Ridler CM, Eddaoudi A, Bonnet D. Age-dependent increase in side population distribution within hematopoiesis: implications for our understanding of the mechanism of aging. Stem Cells. 2007;25(4):828–35. https://doi.org/10.1634/stemcells.2006-0405.
12. Singer K, DelProposto J, Morris DL, Zamarron B, Mergian T, Maley N, Cho KW, Geletka L, Subbaiah P, Muir L, Martinez-Santibanez G, Lumeng CN. Diet-induced obesity promotes myelopoiesis in hematopoi-

etic stem cells. Mol Metab. 2014;3(6):664–75. https://doi.org/10.1016/j.molmet.2014.06.005.
13. Adler BJ, Green DE, Pagnotti GM, Chan ME, Rubin CT. High fat diet rapidly suppresses B lymphopoiesis by disrupting the supportive capacity of the bone marrow niche. PLoS One. 2014;9(3):e90639. https://doi.org/10.1371/journal.pone.0090639.
14. Kuranda K, Vargaftig J, de la Rochere P, Dosquet C, Charron D, Bardin F, Tonnelle C, Bonnet D, Goodhardt M. Age-related changes in human hematopoietic stem/progenitor cells. Aging Cell. 2011;10(3):542–6. https://doi.org/10.1111/j.1474-9726.2011.00675.x.
15. Shafat MS, Oellerich T, Mohr S, Robinson SD, Edwards DR, Marlein CR, Piddock RE, Fenech M, Zaitseva L, Abdul-Aziz A, Turner J, Watkins JA, Lawes M, Bowles KM, Rushworth SA. Leukemic blasts program bone marrow adipocytes to generate a protumoral microenvironment. Blood. 2017;129(10):1320–32. https://doi.org/10.1182/blood-2016-08-734798.
16. He YM, Li X, Perego M, Nefedova Y, Kossenkov AV, Jensen EA, Kagan V, Liu YF, Fu SY, Ye QJ, Zhou YH, Wei L, Gabrilovich DI, Zhou J. Transitory presence of myeloid-derived suppressor cells in neonates is critical for control of inflammation. Nat Med. 2018;24(2):224–31. https://doi.org/10.1038/nm.4467.
17. Li M, Zhu D, Wang T, Xia X, Tian J, Wang S. Roles of myeloid-derived suppressor cell subpopulations in autoimmune arthritis. Front Immunol. 2018;9:2849. https://doi.org/10.3389/fimmu.2018.02849.
18. Movahedi K, Guilliams M, Van den Bossche J, Van den Bergh R, Gysemans C, Beschin A, De Baetselier P, Van Ginderachter JA. Identification of discrete tumor-induced myeloid-derived suppressor cell subpopulations with distinct T cell-suppressive activity. Blood. 2008;111(8):4233–44. https://doi.org/10.1182/blood-2007-07-099226.
19. Youn JI, Nagaraj S, Collazo M, Gabrilovich DI. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. J Immunol. 2008;181(8):5791–802. https://doi.org/10.4049/jimmunol.181.8.5791.
20. Bronte V, Brandau S, Chen SH, Colombo MP, Frey AB, Greten TF, Mandruzzato S, Murray PJ, Ochoa A, Ostrand-Rosenberg S, Rodriguez PC, Sica A, Umansky V, Vonderheide RH, Gabrilovich DI. Recommendations for myeloid-derived suppressor cell nomenclature and characterization standards. Nat Commun. 2016;7:12150. https://doi.org/10.1038/ncomms12150.
21. Kusmartsev S, Cheng F, Yu B, Nefedova Y, Sotomayor E, Lush R, Gabrilovich D. All-trans-retinoic acid eliminates immature myeloid cells from tumor-bearing mice and improves the effect of vaccination. Cancer Res. 2003;63(15):4441–9.
22. Mirza N, Fishman M, Fricke I, Dunn M, Neuger AM, Frost TJ, Lush RM, Antonia S, Gabrilovich DI. All-trans-retinoic acid improves differentiation of myeloid cells and immune response in cancer patients. Cancer Res. 2006;66(18):9299–307. https://doi.org/10.1158/0008-5472.CAN-06-1690.
23. Millrud CR, Bergenfelz C, Leandersson K. On the origin of myeloid-derived suppressor cells. Oncotarget. 2017;8(2):3649–65. https://doi.org/10.18632/oncotarget.12278.
24. Sonda N, Simonato F, Peranzoni E, Cali B, Bortoluzzi S, Bisognin A, Wang E, Marincola FM, Naldini L, Gentner B, Trautwein C, Sackett SD, Zanovello P, Molon B, Bronte V. miR-142-3p prevents macrophage differentiation during cancer-induced myelopoiesis. Immunity. 2013;38(6):1236–49. https://doi.org/10.1016/j.immuni.2013.06.004.
25. Waight JD, Netherby C, Hensen ML, Miller A, Hu Q, Liu S, Bogner PN, Farren MR, Lee KP, Liu K, Abrams SI. Myeloid-derived suppressor cell development is regulated by a STAT/IRF-8 axis. J Clin Invest. 2013;123(10):4464–78. https://doi.org/10.1172/JCI68189.
26. Xia S, Sha H, Yang L, Ji Y, Ostrand-Rosenberg S, Qi L. Gr-1+ CD11b+ myeloid-derived suppressor cells suppress inflammation and promote insulin sensitivity in obesity. J Biol Chem. 2011;286(26):23591–9. https://doi.org/10.1074/jbc.M111.237123.
27. Marvel D, Gabrilovich DI. Myeloid-derived suppressor cells in the tumor microenvironment: expect the unexpected. J Clin Investig. 2015;125(9):3356–64. https://doi.org/10.1172/jci80005.
28. Porta C, Marino A, Consonni FM, Bleve A, Mola S, Storto M, Riboldi E, Sica A. Metabolic influence on the differentiation of suppressive myeloid cells in cancer. Carcinogenesis. 2018;39(9):1095–104. https://doi.org/10.1093/carcin/bgy088.
29. Yan D, Adeshakin AO, Xu M, Afolabi LO, Zhang G, Chen YH, Wan X. Lipid metabolic pathways confer the immunosuppressive function of myeloid-derived suppressor cells in tumor. Front Immunol. 2019;10:1399. https://doi.org/10.3389/fimmu.2019.01399.
30. Liberti MV, Locasale JW. The Warburg effect: how does it benefit cancer cells? Trends Biochem Sci. 2016;41(3):211–8. https://doi.org/10.1016/j.tibs.2015.12.001.
31. Shestov AA, Liu X, Ser Z, Cluntun AA, Hung YP, Huang L, Kim D, Le A, Yellen G, Albeck JG, Locasale JW. Quantitative determinants of aerobic glycolysis identify flux through the enzyme GAPDH as a limiting step. elife. 2014;3:e03342. https://doi.org/10.7554/eLife.03342.
32. Husain Z, Huang Y, Seth P, Sukhatme VP. Tumor-derived lactate modifies antitumor immune response: effect on myeloid-derived suppressor cells and NK cells. J Immunol. 2013;191(3):1486–95. https://doi.org/10.4049/jimmunol.1202702.
33. Yu Q, Dong L, Li Y, Liu G. SIRT1 and HIF1alpha signaling in metabolism and immune responses. Cancer Lett. 2018;418:20–6. https://doi.org/10.1016/j.canlet.2017.12.035.
34. LaGory EL, Giaccia AJ. The ever-expanding role of HIF in tumour and stromal biology. Nat Cell Biol. 2016;18(4):356–65. https://doi.org/10.1038/ncb3330.
35. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C,

Bernard NJ, Kelly B, Foley NH, Zheng L, Gardet A, Tong Z, Jany SS, Corr SC, Haneklaus M, Caffrey BE, Pierce K, Walmsley S, Beasley FC, Cummins E, Nizet V, Whyte M, Taylor CT, Lin H, Masters SL, Gottlieb E, Kelly VP, Clish C, Auron PE, Xavier RJ, O'Neill LA. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. Nature. 2013;496(7444):238–42. https://doi.org/10.1038/nature11986.

36. Goffaux G, Hammami I, Jolicoeur M. A dynamic metabolic flux analysis of myeloid-derived suppressor cells confirms immunosuppression-related metabolic plasticity. Sci Rep. 2017;7(1):9850. https://doi.org/10.1038/s41598-017-10464-1.
37. Wu T, Zhao Y, Wang H, Li Y, Shao L, Wang R, Lu J, Yang Z, Wang J, Zhao Y. mTOR masters monocytic myeloid-derived suppressor cells in mice with allografts or tumors. Sci Rep. 2016;6:20250. https://doi.org/10.1038/srep20250.
38. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, Mellor AL. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. Immunity. 2005;22(5):633–42. https://doi.org/10.1016/j.immuni.2005.03.013.
39. Sica A, Strauss L, Consonni FM, Travelli C, Genazzani A, Porta C. Metabolic regulation of suppressive myeloid cells in cancer. Cytokine Growth Factor Rev. 2017;35:27–35. https://doi.org/10.1016/j.cytogfr.2017.05.002.
40. Srivastava MK, Sinha P, Clements VK, Rodriguez P, Ostrand-Rosenberg S. Myeloid-derived suppressor cells inhibit T-cell activation by depleting cystine and cysteine. Cancer Res. 2010;70(1):68–77. https://doi.org/10.1158/0008-5472.CAN-09-2587.
41. Biswas SK. Metabolic reprogramming of immune cells in cancer progression. Immunity. 2015;43(3):435–49. https://doi.org/10.1016/j.immuni.2015.09.001.
42. Rodriguez PC, Hernandez CP, Morrow K, Sierra R, Zabaleta J, Wyczechowska DD, Ochoa AC. L-arginine deprivation regulates cyclin D3 mRNA stability in human T cells by controlling HuR expression. J Immunol. 2010;185(9):5198–204. https://doi.org/10.4049/jimmunol.1001224.
43. Zea AH, Rodriguez PC, Culotta KS, Hernandez CP, DeSalvo J, Ochoa JB, Park HJ, Zabaleta J, Ochoa AC. L-arginine modulates CD3zeta expression and T cell function in activated human T lymphocytes. Cell Immunol. 2004;232(1–2):21–31. https://doi.org/10.1016/j.cellimm.2005.01.004.
44. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12(4):253–68. https://doi.org/10.1038/nri3175.
45. Grohmann U, Puccetti P. The coevolution of IDO1 and AhR in the emergence of regulatory T-cells in mammals. Front Immunol. 2015;6:58. https://doi.org/10.3389/fimmu.2015.00058.
46. Molon B, Ugel S, Del Pozzo F, Soldani C, Zilio S, Avella D, De Palma A, Mauri P, Monegal A, Rescigno M, Savino B, Colombo P, Jonjic N, Pecanic S, Lazzarato L, Fruttero R, Gasco A, Bronte V, Viola A. Chemokine nitration prevents intratumoral infiltration of antigen-specific T cells. J Exp Med. 2011;208(10):1949–62. https://doi.org/10.1084/jem.20101956.
47. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324(5930):1029–33. https://doi.org/10.1126/science.1160809.
48. Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. Cancer Cell. 2012;21(3):297–308. https://doi.org/10.1016/j.ccr.2012.02.014.
49. Rohrig F, Schulze A. The multifaceted roles of fatty acid synthesis in cancer. Nat Rev Cancer. 2016;16(11):732–49. https://doi.org/10.1038/nrc.2016.89.
50. Balaban S, Lee LS, Schreuder M, Hoy AJ. Obesity and cancer progression: is there a role of fatty acid metabolism? Biomed Res Int. 2015;2015:274585. https://doi.org/10.1155/2015/274585.
51. Ye H, Adane B, Khan N, Sullivan T, Minhajuddin M, Gasparetto M, Stevens B, Pei S, Balys M, Ashton JM, Klemm DJ, Woolthuis CM, Stranahan AW, Park CY, Jordan CT. Leukemic stem cells evade chemotherapy by metabolic adaptation to an adipose tissue niche. Cell Stem Cell. 2016;19(1):23–37. https://doi.org/10.1016/j.stem.2016.06.001.
52. Hossain F, Al-Khami AA, Wyczechowska D, Hernandez C, Zheng L, Reiss K, Valle LD, Trillo-Tinoco J, Maj T, Zou W, Rodriguez PC, Ochoa AC. Inhibition of fatty acid oxidation modulates immunosuppressive functions of myeloid-derived suppressor cells and enhances cancer therapies. Cancer Immunol Res. 2015;3(11):1236–47. https://doi.org/10.1158/2326-6066.CIR-15-0036.
53. Du H, Ding X, Yan C. Metabolic reprogramming of myeloid-derived suppressive cells. Onco Targets Ther. 2017;4(3–4):29–30. https://doi.org/10.18632/oncoscience.349.
54. Sica A, Strauss L. Energy metabolism drives myeloid-derived suppressor cell differentiation and functions in pathology. J Leukoc Biol. 2017;102(2):325–34. https://doi.org/10.1189/jlb.4MR1116-476R.
55. Clements VK, Long T, Long R, Figley C, Smith DMC, Ostrand-Rosenberg S. Frontline science: high fat diet and leptin promote tumor progression by inducing myeloid-derived suppressor cells. J Leukoc Biol. 2018;103(3):395–407. https://doi.org/10.1002/JLB.4HI0517-210R.
56. Ostrand-Rosenberg S. Myeloid derived-suppressor cells: their role in cancer and obesity. Curr Opin Immunol. 2018;51:68–75. https://doi.org/10.1016/j.coi.2018.03.007.
57. Veglia F, Tyurin VA, Blasi M, De Leo A, Kossenkov AV, Donthireddy L, To TKJ, Schug Z, Basu S, Wang F, Ricciotti E, DiRusso C, Murphy ME, Vonderheide RH, Lieberman PM, Mulligan C, Nam B, Hockstein

N, Masters G, Guarino M, Lin C, Nefedova Y, Black P, Kagan VE, Gabrilovich DI. Fatty acid transport protein 2 reprograms neutrophils in cancer. Nature. 2019;569(7754):73–8. https://doi.org/10.1038/s41586-019-1118-2.
58. Zelenay S, van der Veen AG, Bottcher JP, Snelgrove KJ, Rogers N, Acton SE, Chakravarty P, Girotti MR, Marais R, Quezada SA, Sahai E, Reis e Sousa C. Cyclooxygenase-dependent tumor growth through evasion of immunity. Cell. 2015;162(6):1257–70. https://doi.org/10.1016/j.cell.2015.08.015.
59. Sinha P, Clements VK, Fulton AM, Ostrand-Rosenberg S. Prostaglandin E2 promotes tumor progression by inducing myeloid-derived suppressor cells. Cancer Res. 2007;67(9):4507–13. https://doi.org/10.1158/0008-5472.CAN-06-4174.
60. Fujita M, Kohanbash G, Fellows-Mayle W, Hamilton RL, Komohara Y, Decker SA, Ohlfest JR, Okada H. COX-2 blockade suppresses gliomagenesis by inhibiting myeloid-derived suppressor cells. Cancer Res. 2011;71(7):2664–74. https://doi.org/10.1158/0008-5472.CAN-10-3055.
61. Wu H, Weidinger C, Schmidt F, Keye J, Friedrich M, Yerinde C, Willimsky G, Qin Z, Siegmund B, Glauben R. Oleate but not stearate induces the regulatory phenotype of myeloid suppressor cells. Sci Rep. 2017;7(1):7498. https://doi.org/10.1038/s41598-017-07685-9.
62. Tavazoie MF, Pollack I, Tanqueco R, Ostendorf BN, Reis BS, Gonsalves FC, Kurth I, Andreu-Agullo C, Derbyshire ML, Posada J, Takeda S, Tafreshian KN, Rowinsky E, Szarek M, Waltzman RJ, McMillan EA, Zhao C, Mita M, Mita A, Chmielowski B, Postow MA, Ribas A, Mucida D, Tavazoie SF. LXR/ApoE activation restricts innate immune suppression in cancer. Cell. 2018;172(4):825–840.e818. https://doi.org/10.1016/j.cell.2017.12.026.
63. Poulsen L, Siersbaek M, Mandrup S. PPARs: fatty acid sensors controlling metabolism. Semin Cell Dev Biol. 2012;23(6):631–9. https://doi.org/10.1016/j.semcdb.2012.01.003.
64. Wahli W, Michalik L. PPARs at the crossroads of lipid signaling and inflammation. Trends Endocrinol Metab. 2012;23(7):351–63. https://doi.org/10.1016/j.tem.2012.05.001.
65. Chakraborty K, Raundhal M, Chen BB, Morse C, Tyurina YY, Khare A, Oriss TB, Huff R, Lee JS, St Croix CM, Watkins S, Mallampalli RK, Kagan VE, Ray A, Ray P. The mito-DAMP cardiolipin blocks IL-10 production causing persistent inflammation during bacterial pneumonia. Nat Commun. 2017;8:13944. https://doi.org/10.1038/ncomms13944.
66. Zhang Y, Kurupati R, Liu L, Zhou XY, Zhang G, Hudaihed A, Filisio F, Giles-Davis W, Xu X, Karakousis GC, Schuchter LM, Xu W, Amaravadi R, Xiao M, Sadek N, Krepler C, Herlyn M, Freeman GJ, Rabinowitz JD, Ertl HCJ. Enhancing CD8(+) T cell fatty acid catabolism within a metabolically challenging tumor microenvironment increases the efficacy of melanoma immunotherapy. Cancer Cell. 2017;32(3):377–391.e379. https://doi.org/10.1016/j.ccell.2017.08.004.
67. Chinetti G, Lestavel S, Bocher V, Remaley AT, Neve B, Torra IP, Teissier E, Minnich A, Jaye M, Duverger N, Brewer HB, Fruchart JC, Clavey V, Staels B. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. Nat Med. 2001;7(1):53–8. https://doi.org/10.1038/83348.
68. Chawla A, Boisvert WA, Lee CH, Laffitte BA, Barak Y, Joseph SB, Liao D, Nagy L, Edwards PA, Curtiss LK, Evans RM, Tontonoz P. A PPAR gamma-LXR-ABCA1 pathway in macrophages is involved in cholesterol efflux and atherogenesis. Mol Cell. 2001;7(1):161–71. https://doi.org/10.1016/s1097-2765(01)00164-2.
69. Ishdorj G, Graham BA, Hu X, Chen J, Johnston JB, Fang X, Gibson SB. Lysophosphatidic acid protects cancer cells from histone deacetylase (HDAC) inhibitor-induced apoptosis through activation of HDAC. J Biol Chem. 2008;283(24):16818–29. https://doi.org/10.1074/jbc.M710177200.
70. Peng M, Yin N, Chhangawala S, Xu K, Leslie CS, Li MO. Aerobic glycolysis promotes T helper 1 cell differentiation through an epigenetic mechanism. Science. 2016;354(6311):481–4. https://doi.org/10.1126/science.aaf6284.
71. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. Science. 2009;324(5930):1076–80. https://doi.org/10.1126/science.1164097.
72. Cao J, Sun L, Aramsangtienchai P, Spiegelman NA, Zhang X, Huang W, Seto E, Lin H. HDAC11 regulates type I interferon signaling through defatty-acylation of SHMT2. Proc Natl Acad Sci U S A. 2019;116(12):5487–92. https://doi.org/10.1073/pnas.1815365116.
73. Sahakian E, Powers JJ, Chen J, Deng SL, Cheng F, Distler A, Woods DM, Rock-Klotz J, Sodre AL, Youn JI, Woan KV, Villagra A, Gabrilovich D, Sotomayor EM, Pinilla-Ibarz J. Histone deacetylase 11: a novel epigenetic regulator of myeloid derived suppressor cell expansion and function. Mol Immunol. 2015;63(2):579–85. https://doi.org/10.1016/j.molimm.2014.08.002.
74. Condamine T, Dominguez GA, Youn JI, Kossenkov AV, Mony S, Alicea-Torres K, Tcyganov E, Hashimoto A, Nefedova Y, Lin C, Partlova S, Garfall A, Vogl DT, Xu X, Knight SC, Malietzis G, Lee GH, Eruslanov E, Albelda SM, Wang X, Mehta JL, Bewtra M, Rustgi A, Hockstein N, Witt R, Masters G, Nam B, Smirnov D, Sepulveda MA, Gabrilovich DI. Lectin-type oxidized LDL receptor-1 distinguishes population of human polymorphonuclear myeloid-derived suppressor cells in cancer patients. Sci Immunol. 2016;1(2):aaf8943. https://doi.org/10.1126/sciimmunol.aaf8943.
75. Munder M, Mollinedo F, Calafat J, Canchado J, Gil-Lamaignere C, Fuentes JM, Luckner C, Doschko G, Soler G, Eichmann K, Muller FM, Ho AD, Goerner

M, Modolell M. Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity. Blood. 2005;105(6):2549–56. https://doi.org/10.1182/blood-2004-07-2521.

76. Masson D, Jiang XC, Lagrost L, Tall AR. The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis. J Lipid Res. 2009;50(Suppl):S201–6. https://doi.org/10.1194/jlr.R800061-JLR200.
77. Ding S, Li S, Zhang S, Li Y. Genetic alterations and checkpoint expression: mechanisms and models for drug discovery. Adv Exp Med Biol. 2020;1248:227–50. https://doi.org/10.1007/978-981-15-3266-5_10.
78. Li Y, Di Santo JP. Modeling infectious diseases in mice with a "humanized" immune system. Microbiol Spectr. 2019;7(2) https://doi.org/10.1128/microbiolspec.BAI-0019-2019.
79. Meraz IM, Majidi M, Meng F, Shao R, Ha MJ, Neri S, Fang B, Lin SH, Tinkey PT, Shpall EJ, Morris J, Roth JA. An improved patient-derived xenograft humanized mouse model for evaluation of lung cancer immune responses. Cancer Immunol Res. 2019;7(8):1267–79. https://doi.org/10.1158/2326-6066.CIR-18-0874.
80. Zhao Y, Shuen TWH, Toh TB, Chan XY, Liu M, Tan SY, Fan Y, Yang H, Lyer SG, Bonney GK, Loh E, Chang KTE, Tan TC, Zhai W, Chan JKY, Chow EK, Chee CE, Lee GH, Dan YY, Chow PK, Toh HC, Lim SG, Chen Q. Development of a new patient-derived xenograft humanised mouse model to study human-specific tumour microenvironment and immunotherapy. Gut. 2018;67(10):1845–54. https://doi.org/10.1136/gutjnl-2017-315201.
81. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol. 2004;172(5):2731–8. https://doi.org/10.4049/jimmunol.172.5.2731.
82. Day CP, Merlino G, Van Dyke T. Preclinical mouse cancer models: a maze of opportunities and challenges. Cell. 2015;163(1):39–53. https://doi.org/10.1016/j.cell.2015.08.068.

8 Lipid Metabolism in Tumor-Associated Fibroblasts

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Abstract

Tumor- or cancer-associated fibroblasts (TAFs), one of the most abundant stromal cell types in various carcinomas, consist of a heterogeneous cell population. Typically, TAFs are assigned with pro-tumor activities to promote tumor growth and progression. One of the key features of solid tumors is the metabolic reprogramming that induces alterations of bioenergetics and biosynthesis in both tumor cells and TAFs. Therefore, this review emphasizes TAFs lipid metabolism related to both TAFs differentiation process and TAFs crosstalk with cancer cells. We hope that this review will help understand lipid metabolism in tumor microenvironment, and support the rational design of metabolism-based approaches to improve the efficacy of cancer therapy.

Keywords

Tumor-associated fibroblasts · Metabolism Lipid metabolism · Cancer cells · Tumor microenvironment

8.1 Introduction

Fibroblasts comprise the most profuse population within the family of connective-tissue cells. They exhibit the physiological role such as synthesizing ECM components and regulating tissue homeostasis and surrounding cells differentiation [1, 2]. Fibroblasts are accountable for matrix metalloproteinases (MMPs) production that is implicated in matrix remodeling, cell motility, proliferation, and death, further highlighting the effect of fibroblasts on the maintenance of ECM homeostasis [3]. Furthermore, another critical function of fibroblasts is to release diverse growth factors, which facilitates communication between epithelial and mesenchymal cells [4, 5].

Since cancer was regarded as a wound that never heals 30 years ago [6], the essential role of fibroblasts in cancer progression has aroused. Fibroblasts in cancer tissues, also known as tumor-associated fibroblasts (TAFs) or cancer-associated fibroblasts (CAFs), are the majority of stromal cells in the tumor microenvironment (TME), especially in solid tumors such as breast, prostate, and pancreatic cancers. A growing body

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of evidence shows that TAFs are key players in tumorigenesis. During tumor progression, TAFs enhance tumor cell proliferation and survival, angiogenesis, metastasis, immunogenicity, and resistance to therapies by secreting diverse inflammatory cytokines and growth factors, and remodeling the extracellular matrix [7, 8]. In addition, the crosstalk between TAFs and cancer cells, immune cells, or other cells in TME is correlated with cell metabolic reprogramming that subsidizes TAFs activation, and cancer progression and evasion from antitumor therapies [9]. In this regard, the investigations on TAFs metabolic reprogramming could contribute to better understanding TAFs activation, the cell interaction in TME, and could offer pioneering methods to improve new therapeutic strategies targeting the pro-tumorigenic activity of TAFs.

8.2 Origin and Markers of TAFs

"Fibroblasts" were first defined as cells in the connective tissues that synthesized collagen. Fibroblasts in normal tissues are generally single cells present in the interstitial space or occasionally near a capillary, without any correlation with a basement membrane but embedded within fibrillar ECM of the interstitium. Fibroblasts are part of the diverse connective tissue components and are non-epithelial, nonimmune cells with a likely mesenchymal lineage origin. Fibroblasts display classic spindle-shaped morphology with a potential for planar polarity. Fibroblasts in normal tissues are generally regarded as indolent with negligible metabolic and transcriptomic activities. Intrinsically, they are speculated to be in a hibernating, quiescent, or resting state [1, 2]. TAFs are a heterogeneous population of cells, while so far there are no precise markers that can evidently differentiate TAFs from normal, resting fibroblasts. TAFs can originate from activated resident fibroblasts or other precursor cells exemplified by bone marrow-derived mesenchymal stem cells, endothelial cells, epithelial cells, adipocytes, fibrocytes, pericytes, smooth muscle cells, carcinoma cells, stellate cells in pancreas and liver, myoepithelial cells in breast tissues, and pericryptal myofibroblasts from the gastrointestinal tract [7, 8] (Fig. 8.1). TAF activation is triggered by a range of cytokines including cancer cell-derived Transforming Growth Factor-β1 (TGF-β1), platelet-derived growth factor α (PDGFα), PDGFβ, basic fibroblast growth factor (bFGF), and interleukin-6 (IL-6) and environmental stimuli such as oxidative stress, hypoxia, and matrix stiffness [4, 10]. All these stimuli may collaborate to determine different TAF phenotypes further favoring their heterogeneity. Upon activation, TAFs secrete a huge repertoire of growth factors such as hepatocyte growth factor (HGF), epidermal growth factor (EGF), connective tissue growth factor (CTGF) and insulin-like growth factor (IGF), cytokines including CXCL12 and IL-6, extracellular vesicles (EVs), metabolites, ECM components particularly collagens, fibronectin and tenascin-C (TNC), and ECM-remodeling enzymes such as MMPs and lysyl oxidases (LOXs). All these factors directly affect the behavior of surrounding cells and remodel the ECM. Therefore, TAFs aid tumor development, from the early phases of tumorigenesis until cancer cells colonize distant organs to form metastasis [8, 11, 12] (Fig. 8.1).

The most common markers used to identify TAFs are α-smooth muscle actin (α-SMA), fibroblast activation protein alpha (FAP), fibroblast-specific protein 1 (FSP1 also known as S100A4), desmin and discoidin domain-containing receptor 2 (DDR2), and PDGFRα/β [9]. However, their expression level diverges considerably depending on the tumor type studied, and normal fibroblasts and even other cell types such as endothelial cells, pericytes, mesenchymal stem cells, and immune cells also express most of these markers [13]. Among these markers, α-SMA presented a huge labeling pattern and has been acknowledged as the most dependable marker for recognizing activated fibroblast in TAFs [14]. Cancer cell-secreted growth factors such as TGF-β [15], PDGF and FGF-2 [16], Wnt7a [17], sonic hedgehog [18], and exosomes [19, 20] can induce transformation of progenitor lacking α-SMA expression to α-SMA-positive TAFs. On the other hand, it is evidenced that leukemia inhibitory factor (LIF) renovates progeni-

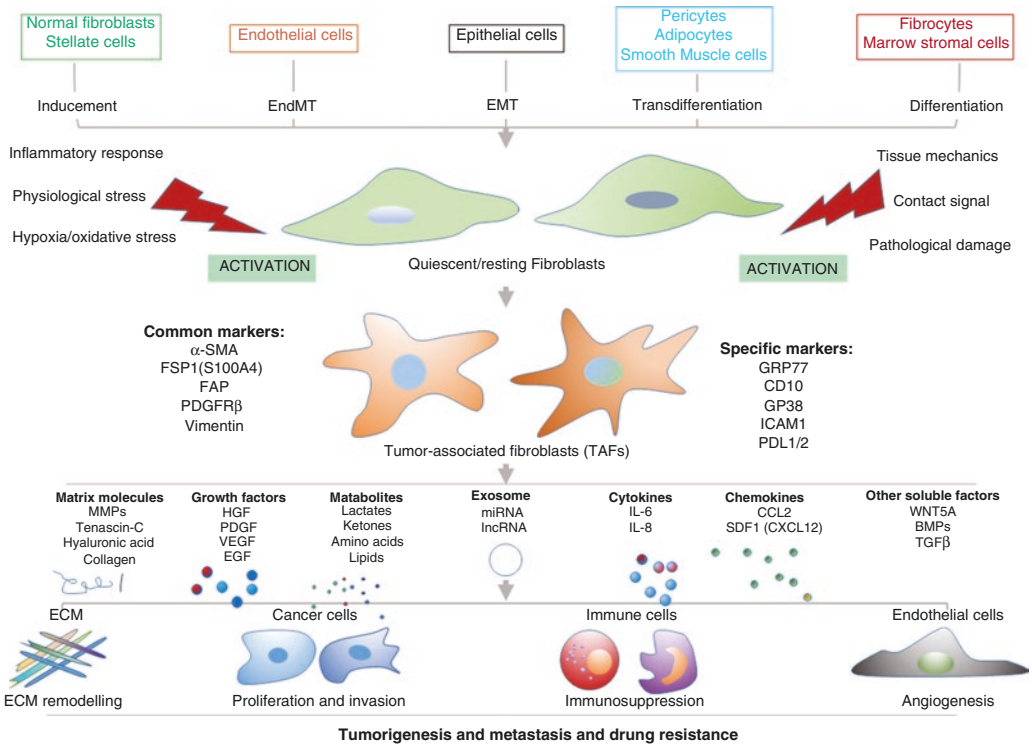

Fig. 8.1 The origin and main markers of TAFs. This schematic diagram describes the cellular origin, activation, secreted factors, and biological functions of TAFs. *ECM* extracellular matrix, *EMT* epithelial-to-mesenchymal transition, *EndMT* endothelial-to-mesenchymal transition, *α-SMA* alpha-smooth muscle actin, *FAP* fibroblast activation protein, *FSP1* fibroblast-specific protein 1, *HGF* hepatocyte growth factor, *PDGFR* platelet-derived growth factor receptor beta, *GPR77* G protein-coupled receptor 77, *BMP4* bone morphogenetic protein 4, *SDF1* stromal-derived factor 1, *MMPs* matrix metalloproteinase, *EGF* epithelial growth factor, *VEGF* vascular endothelial growth factor, *TGF-β* transforming growth factor-beta, *PDGF* platelet-derived growth factor

tor state to pro-invasive fibroblast in α-SMA independent manner [21]. Therefore, the progenitor and quiescent precursor of TAFs donates favorable tumor microenvironment to some degree for tumor development after obtaining phenotypic variations (Fig. 8.1).

8.3 Overview of Metabolism Reprogramming in TAFs

In 1927, Warburg et al. stated that glucose metabolism was considerably improved in tumor tissues compared with normal tissues, even in the existence of abundant oxygen [22, 23]. This process, well-known as the "Warburg effect," is the primary and metabolic feature of cancer and is linked to metabolic reprogramming of tumor cells [24]. Furthermore, other metabolic alterations have also been found in tumor tissues, including the use of alternative carbon sources and metabolic interactions between tumor and stromal cells signified by the "reverse Warburg effect" [24, 25]. Thus, solid tumors where several energetic pathways in TME cooperate can be regarded as metabolically heterogeneous diseases [26, 27]. As the major tumor stromal cells, TAFs adapt in a dynamic manner to the metabolic needs of cancer cells, associated with tumorigenesis and resistance to cancer treatments. Notably, TAFs could directly "feed" cancer cells essential nutrients and energy-rich metabolites, including lactate, ketone bodies, glutamine, fatty acids,

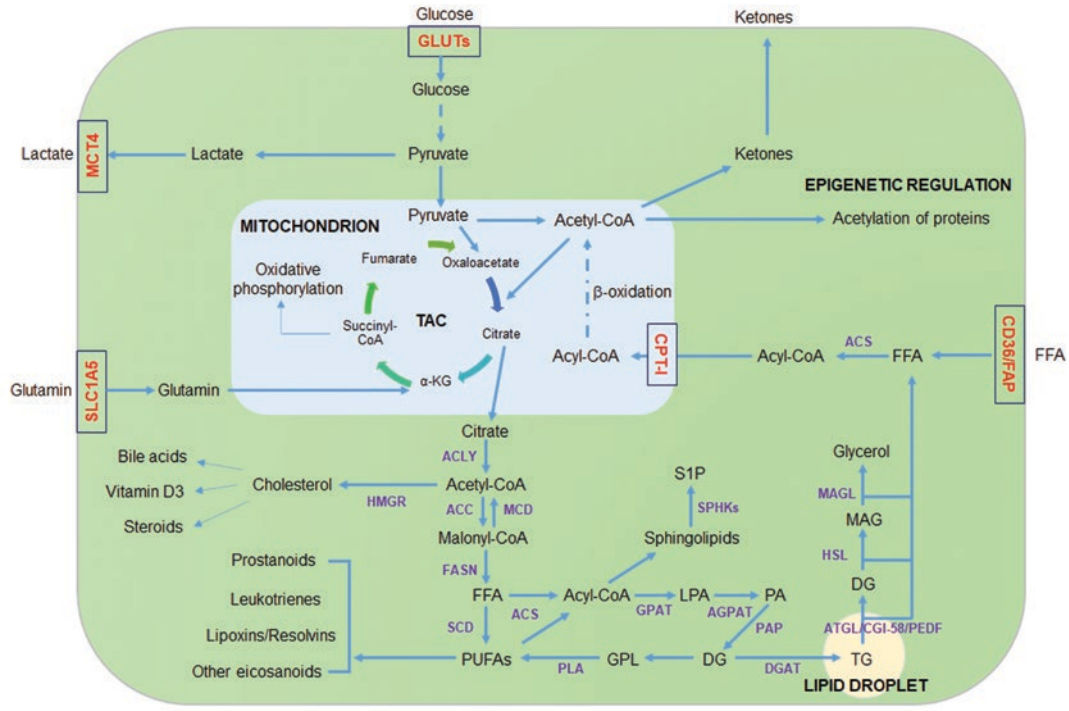

Fig. 8.2 The overview metabolism reprogramming of TAFs. *ACC* Acetyl-CoA carboxylase, *ACLY* ATP citrate lyase, *ACS* Acyl-CoA synthase, *AGPAT* 1-acylglycerol-3-phosphate acyltransferase, *ATGL* adipose triglyceride lipase, *CGI-58* comparative gene identification-58, *CPT-1* carnitine acyl transferase-1, *DG* diacylglycerol, *DGAT* diacylglycerol acyltransferase, *FAP* fatty acid transporter, *FASN* fatty acid synthase, *FFA* free fatty acid, *GLUTs* glucose transporters, *GPATs* glycerol-3-phosphate acyltransferases, *GPL* glycerophospholipids, *HMGR* HMG-CoA reductase, *HSL* hormone sensitive triglyceride lipase, *LPA* lysophophatidic acid, *MAG* monoacylglycerol, *MAGL* monoacylglycerol lipase, *MCD* malonyl-CoA decarboxylase, *MCT* monocarboxylate transporter, *PA* phosphatidic acid, *PAP* phosphatidic acid phosphohydrolase, *PEDF* pigment epithelium-derived factor, *PLA* phospholipase A, *PUFAs* polyunsaturated fatty acids, *SCD* stearoyl–CoA desaturase, *S1P* sphingosine-1-phosphate, *SPHKs* sphingosine kinases, *TAC* tricarboxylic acid cycle, *TG* triglyceride

and other amino acids through the induction of autophagy in a host–parasite pattern, to contribute to tumor growth and metastasis [9, 28]. Current studies on the metabolic reprogramming of TAFs mainly pay attention to glycol and amino acid metabolism reprogramming (Fig. 8.2). It is shown that TAFs have an improved aerobic glycolysis and reduced oxidative phosphorylation, compared with NFs. As a consequence of glycolysis, TAFs could secrete a lot of lactate to the ECM, which leads to an acidic microenvironment favoring the metastatic potential of tumor cells [29]. Studies focusing on amino acid metabolism in TAFs have shown that a diversity of metabolic substrates such as glutamine are produced by TAFs for cancer cells. Glutamate ammonia ligase, the key enzyme in glutamine synthesis, is upregulated in TAFs. The glutamine importers of cancer cells such as SLC6A14 are upregulated in a glutamine concentration-dependent manner in the existence of fibroblasts [30]. In addition to well-known glycolysis and glutamine metabolism, there is growing evidence that lipids exert a great effect on tumorigenesis and cancer development. Modifications in lipid metabolism have an impact on many cancer processes, including cell growth, motility, proliferation, autophagy, and apoptosis [31, 32]. In summary, to define the reciprocal metabolic interplay between TAFs and cancer cells will provide a better understanding of molecular mechanisms

by which the treatment resistance ensues, and benefit the strategy to improve cancer therapy.

8.4 The Role of Lipid Metabolism for TAFs

Not like proteins, nucleic acids, and polysaccharides which are large macromolecular polymers, lipids are hydrophobic or amphipathic small molecules. Lipids are normally defined as naturally occurring compounds that are soluble in nonpolar solvents but insoluble in water [31]. According to the International Lipid Classification and Nomenclature Committee, lipids are currently classified into eight categories: (1) fatty acids; (2) glycerolipids; (3) glycerophospholipids; (4) sphingolipids; (5) sterol lipids; (6) prenol lipids; (7) saccharolipids; and (8) polyketides [31, 33]. Lipids are important components of all membranes and are extensively distributed in cellular organelles. Besides their role as structural constituents, lipids in membranes also serve essential functions of diverse organelles [34, 35]. Lipids could function as second messengers to transduce signals within cells, and serve as important energy sources when nutrients are limited. In the cells, lipids structure controls their function and metabolic fate [31]. Lipid metabolism is a complicated process including lipid uptake, transport, synthesis, and degradation (Fig. 8.2). Biosynthesis and degradation of different lipids are modulated by different signaling pathways, and even the same lipid can be controlled by diverse signaling pathways in different cells and tissues under physiological or therapeutic stimulus. Cell needs and replies to environmental conditions are responsible for the regulation of these signaling pathways, which connects lipid metabolism in the modulation of cell process including cell growth, differentiation, proliferation, motility, apoptosis, and membrane homeostasis [36–38]. In the meantime, membrane composition and permeability can be changed by lipid metabolism, which promotes the progression and development of many diseases including kinds of cancers [39]. Lipids that are now regarded as most related to cancer progression and treatment are fatty acids, glycerolipids, sphingolipids, glycerophospholipids, and sterol lipids [40, 41] (Fig. 8.2). Here we highlight the role of lipid metabolic reprogramming in TAFs differentiation and function and remark lipid metabolism involved in crosstalk between TAFs and cancer cells.

8.4.1 Lipid Metabolism Reprogramming Involved in TAFs Differentiation and Proliferation

TAFs differentiation is an essential step for cancer initiation and progression, which can either ensue at the early stage of cancer or unpredictably occur before the genetic alterations of epithelial cells to initiate the malignant conversion of neighboring cells [42, 43]. The constitutive activation of tumor stroma resulting in TAFs differentiation is connected to signaling pathways, regulated mainly by tumor cells, with autocrine loops and with TAFs metabolic reprogramming shown in various solid cancers [44–48]. Particularly, TAFs differentiation can be induced by tumor cell-derived TGF, EGF, PDGF, bFGF, IL-6, and IL-1 [8, 49].

Intracellular lipid metabolism and homeostasis are modulated through selective proteins and highly dynamic organelles named lipid droplets (LDs). LDs are locations for lipid storage, membrane synthesis, and cargo protein trafficking through the cytoplasmic compartment [50]. LDs are formed by various neutral lipids containing triacylglycerol (TAG) and cholesterol esters (CEs) which are enclosed by a phospholipid monolayer with proteins either embedded in the monolayer or attached to its surface [50, 51]. LDs take part in lipid flux by experiencing an active cycle of lipolysis which involves several proteins localized on the LD surface. This cluster of surface proteins includes adipose triglyceride lipase (ATGL), comparative gene identification 58 (CGI-58), and pigment epithelium-derived factor (PEDF) [52–54], which stimulate lipolysis and the secretion of free fatty acids. Factors that regulate lipogenesis are inclined to reside in the cytoplasm, but crosstalk among other TG pathway

members is critical to sustain the net lipid balance in normal cells [50–56]. Many tumors, including prostate cancer, have a significantly lower level of PEDF, while some head and neck tumors have a mutation in G0/G1 switch protein 2 (G0S2) that acts as a potent inhibitor of ATGL, suggesting that the pathologic imbalance in the TG pathway is a common mechanism for dysregulated lipid metabolism [57, 58]. TAFs were found to store more lipids than normal fibroblasts (NFs). ATGL and PEDF were strongly expressed in NFs, while TAFs had minimal to undetectable levels of ATGL or PEDF protein. At baseline, TAFs showed microtubule-organizing centers (MTOCs) amplification when compared to 1–2 perinuclear MTOCs consistently observed in NFs. PEDF treatment or lipogenesis blockade inhibited lipid content and MTOC number. These data support that TAFs have developed a tumor-like phenotype by reprogramming lipid metabolism and amplifying MTOCs [59].

Accumulating studies suggest that sphingolipid metabolism is changed in human cancers and contributes to cancer progression, metastasis, and chemoresistance [60, 61]. Sphingosine kinases (SPHK1 and SPHK2) are homologous isoenzymes that catalyze the phosphorylation of sphingosine to produce the bioactive metabolite sphingosine-1-phosphate (S1P). SPHK1 is identified to be highly expressed in many cancers. It was reported that SPHK1 is highly expressed in the tumor stroma of high-grade serous ovarian cancer (HGSC), and is needed for the differentiation and tumor-promoting function of TAFs. Knockout or pharmacological inhibition of SPHK1 in ovarian fibroblasts mitigated TGF-β-induced expression of TAF markers and suppressed their ability to promote the metastatic potential of cancer cells in a coculture system. Mechanistically, SPHK1 mediates TGF-β signaling via the transactivation of S1P receptors (S1PR2 and S1PR3), resulting in p38 MAPK phosphorylation. The importance of stromal SPHK1 in tumorigenesis was also verified in vivo, by showing a significant decrease in tumor growth and metastasis in SPHK1 knockout mice. In addition, it was demonstrated that a paracrine mechanism whereby Hepatocellular carcinoma (HCC) cells secrete lysophosphatidic acid (LPA), which promotes trans-differentiation of peritumoral tissue fibroblasts (PTFs) to a TAF-like myofibroblastic phenotype. This effect is mediated by the upregulation of specific genes related to a myo/contractile phenotype. After trans-differentiation, PTFs expressed α-SMA and enhanced HCC cell proliferation, migration, and invasion [62]. This finding is further confirmed in another study, where ovarian cancer cell-conditioned medium (OCC-CM) or LPA induced TAF-phenotype in MRC5 lung fibroblasts. Further analysis indicated that LPA upregulated hypoxia-inducible factor 1α (HIF1α) expression and inhibiting HIF1α lessened LPA-induced glycolysis in both ovarian fibroblasts and TAFs. These data inaugurate LPA-mediated glycolytic shift as the earliest, theoretically priming event, in NFs to TAFs transition, and recognize that LPA-LPAR-HIF1α signaling hub plays an important role in maintaining glycolytic-phenotype in TAFs [63].

In addition to the role in membrane composition and energy storage, lipids are also critical signaling factors in physiology and pathophysiology. Among them, the most noticeable signaling lipids are polyunsaturated fatty acid derivatives including leukotrienes, prostanoids, and other eicosanoids. Prostanoids are not only implicated in the physiology of the digestive, reproductive, and vascular systems, but also have a dramatic impact on acute and chronic inflammation [76]. Prostaglandin E_2 (PGE_2), the product of arachidonic acid catalyzed by cyclooxygenases (COX) and PGE_2 synthases, exerts an important role in the initiation and resolution of inflammation. Since clinical observations presented that COX2/PGE_2 expression on pulmonary fibroblasts from idiopathic pulmonary fibrosis (IPF) patients was downregulated compared to healthy controls, the effect of PGE_2 on fibroblasts was broadly investigated in lung fibrosis models [65, 77]. PGE_2 was described as an inhibitor of fibroblast proliferation, following that fibroblast apoptosis is a crucial issue to halt chronic wound healing and start resolution. Apart from influencing myofibroblast proliferation and survival, some studies indicated that PGE_2 inhibits TGF-β-induced transition from fibroblast to myofibroblast in human lung fibroblasts by EP2, as measured by downregu-

lated α-SMA protein expression [78, 79]. SMAD-independent but PKA-dependent, signaling pathway triggers this effect through cAMP accumulation. PGE_2 suppresses nuclear import of myocardin-related transcription factor-A (MRTF-A), avoiding nuclear accumulation of serum-response factor -MRTF-A complexes and α-SMA promoter activity through phosphorylating vasodilator-stimulated phosphoprotein and dephosphorylating cofilin-1 [80–82]. PGE_2 seems to inhibit all hallmarks of pro-fibrotic fibroblast activity and exerts a negative impact on multiple aspects of TGF-β signaling to reform the ECM. Furthermore, PGE_2 was also demonstrated to inhibit NF-κB activation in human synovial fibroblasts, showing the PGE_2 might be a more universal negative regulator on fibroblast activation [66]. Remarkably, a few studies showed that pro-fibrotic fibroblast activation depended on the activation of the PGE_2 receptor downstream. For example, PGE_2 linking mainly to EP1 urged the proliferation of rat neonatal ventricular fibroblast, while signaling through EP2–4 enhanced intestine-derived fibroblasts migration, suggesting that the effect of PGE_2 on fibroblast may be context-specific [83, 84]. In view of that PGE_2 may inhibit myofibroblast phenotype, and considering that TAFs resemble myofibroblasts to a certain range, it sounds reasonable that PGE_2 mitigates TAF generation and/or activity. Rather than scarce studies on the effect of PGE_2 on TAFs, more studies showed that TAFs were recognized as a key source of PGE_2. It was shown that inflammatory mediators stimulated human TAFs to produce PGE_2 in breast cancers, while other studies showed that human breast cancer cells secreted PGE_2 to impact adjacent TAFs in a paracrine manner [85]. PGE_2 released from breast cancer cells prompted aromatase expression in adipose fibroblasts via enhancing promoter switching [67]. In mice, zinc finger protein SNAIL induced PGE_2 production from mesenchymal stem cells and TAFs in a TGF-β-dependent manner, which in turn supported breast cancer cell metastasis [86]. Furthermore, head and neck squamous cell carcinoma cells triggered neighboring stromal fibroblasts to produce mPGES-1-derived PGE_2 [87]. TAF markers were mainly expressed in mPGES-1 positive cells in neuroblastoma tumors, where cancer cells did not secrete PGE_2. Inhibiting PGE_2 production suppressed tumor growth in neuroblastoma models, probably through boosting angiogenesis, cancer cell proliferation, and reactivating antitumor immunity [88, 89]. These evidences propose that the pro-tumor function of TAFs is partially attributed to their capacity to secrete PGE_2. Nevertheless, the role of PGE_2 in the transition of resident fibroblast to a TAF phenotype or in the regulation of established TAFs function is not clear. Several studies regarding the impact of PGE_2 signaling on TAFs indicate that the role of PGE_2 in fibroblast activation in tumor is also context-dependent. Bone marrow fibroblasts from lung or breast cancer patients exhibited a reduced proliferative ability related to higher PGE_2 compared to fibroblasts from healthy donors [68], which agrees with the findings that PGE_2 induced fibroblasts apoptosis in fibrosis models. It was reported that cancer cells-derived PGE_2 induced indoleamine 2,3-dioxygenase (IDO)/kynurenine in fibroblasts by an EP4/STAT3-dependent pathway, which led to the degradation of E-cadherin and then promoting breast cancer metastasis [69]. On the other hand, no significant changes in PGE_2 and IDO expression between TAFs and fibroblasts surrounding tumor tissues were shown in human hepatocellular carcinoma [90]. Lastly, suppressing PGE_2 production or an EP4 antagonist can block the migration of IL-1-induced dermal fibroblasts toward neuroblastoma cells, suggesting that PGE_2 may stimulate fibroblast migration in certain sceneries [88]. Therefore, the role of PGE_2 in TAF activation is yet to be clearly acknowledged, although the association between tissue fibrosis and malignancy is well-known, and PGE_2 occurs as a negative regulator to promote fibrosis. Nevertheless, it is plausible that TAFs-derived PGE_2 enhances tumor progression by inducing cancer cell proliferation and metastatic potential, while negatively modulating PGE_2-producing cells at the same time in a homeostatic manner. Interestingly, few studies showed lipids-mediated inhibitory effects on TAFs differentiation. For example, Lipoxin A4 (LXA4), an endogenous bioactive lipid, was shown to potentially inhibit the differentiation of human pancreatic stellate cells (PSCs) (hPSCs)

into TAF-like myofibroblasts and thereby hPSC-induced pro-tumorigenic effects. LXA4 significantly suppressed TGF-β-mediated differentiation of hPSCs by inhibiting pSmad2/3 signaling. Moreover, LXA4 treatment abolished the paracrine pro-tumoral effects of hPSCs in vitro and in vivo. Fascinatingly, LXA4 significantly abolished the tumor growth, attributed to a dramatic reduction in fibrosis, which suggests LXA4 can be applied to reprogram tumor stroma in order to treat [70].

The formation of new membranes is required for cellular proliferation. It is often supposed that the lipids desired for these membranes are synthesized generally de novo. In addition to PGE_2 involved in fibroblast proliferation, it was demonstrated that proliferating fibroblasts preferred to taking up palmitate from the extracellular environment over synthesizing it de novo. Compared with quiescent fibroblasts, proliferating fibroblasts increased palmitate uptake, inhibited the degradation of fatty acid, and alternatively directed more palmitate to membrane lipids. Inhibiting fatty acid uptake suppressed fibroblasts proliferation while adding exogenous palmitate led to reduced glucose uptake and made cells lose sensitivity to glycolytic inhibition [71] (Table 8.1).

8.4.2 TAFs Serve as Lipids Source to Promote Cancer Progression

Metabolic communication between TAFs and cancer cells plays a key role in cancer progression. TAFs are also verified as lipids source to support cancer cell growth and metastasis. It was uncovered that TAFs conditioned medium (CM) promoted colorectal cancer (CRC) cell migration compared with CM from normal fibroblasts. More fatty acids and phospholipids are accumulated as TAFs undertake a lipidomic reprogramming. It was confirmed that CRC cells took up the lipids metabolites released from TAFs, as TAFs CM still promoted the CRC cells migration after protein deprivation. Inhibiting fatty acids synthase (FASN), a key enzyme in fatty acids synthesis, or suppressing the uptake of fatty acids by CRC cells, can abolish TAFs-induced CRC cell migration. These data provide a new understanding of the mechanism of CRC metastasis and indicate FASN in TAFs may be potential targets for anti-metastasis therapy [91]. In another study, TAFs have also been shown to enhance lipid synthesis due to the overexpression of FASN. Cancer cells overexpress fatty acid transporter protein 1, which mediates uptake of fatty acid from TAFs [92].

Ketones are end products of aerobic glycolysis and intermediate products generated by fatty acid catabolism in the liver of normal time, and to a lesser extent, originated from the catabolism of the ketogenic amino acids leucine, lysine, and tryptophan. Ketones can be transferred to the outside of the liver to sustain the survival of the organism under starvation, particularly during prolonged starvation [28]. Tumorigenesis is thought of as a nutrient deprivation, where ketones are mainly originated from lipid metabolism in poor glucose or rich lipids tissues. TAFs produce a large amount of ketone bodies that are then reutilized by tumor cells for anabolic metabolism or oxidative phosphorylation (OXPHOS) like lactate. Cav-1 deficiency can enhance stromal autophagy and support the stromal generation of ketone bodies [93]. Ketones are more powerful fuels for mitochondria, generating more energy and decreasing oxygen consumption, as compared with lactate. Of those, β-hydroxybutyrate promotes cancer cell proliferation approximately threefold, while lactate enhances cancer cell metastasis [94].

Moreover, it was shown that TAFs were also capable to transfer a large amount of lipids and proteins to adjacent tumor cells via using microvesicles (MVs) as cargo, thus contributing to maintaining high tumor cell proliferation [95]. As a number of transferred proteins are metabolic enzymes, MVs exhibit a critical role in the metabolic reprogramming of tumor cells owing to TAFs contact. In addition, TAFs-derived exosomes are also able to prompt a metabolic reprogramming in tumor cells [96]. Furthermore, it was demonstrated that TAFs-derived exosomes encompassed intact metabolites, such as amino acids, TCA-cycle intermediates, and lipids, which are vehiculated in tumor cells to promote their growth [97]. It is worth mentioning that FASN

Table 8.1 The role of secreted lipid metabolites on TAFs

Lipids	Origin	Cancer type	Function	References
S1P	Cancer cells	Ovarian cancer	Mediated TGFb-induced TAFs activation via the transactivation of S1PR2/3, resulting in p38 phosphorylation	Saddoughi et al. [61]
LPA	Cancer cells	Hepatocellular carcinoma	Promoted trans-differentiation of peritumoral tissue fibroblasts (PTFs) to a TAF-like myofibroblastic phenotype	Wen et al. [64]
	Cancer cells	Ovarian cancer	Induced TAF-phenotype in MRC5 lung fibroblasts by upregulation of HIF-1α	Radhakrishnan et al. [63]
PGE_2	Pulmonary fibroblasts	Idiopathic pulmonary fibrosis	Inhibited myofibroblast proliferation and survival	Vancheri et al. [65]
	Autocrine	Rheumatoid arthritis patients	Attenuated the activation of NF-κB in human synovial fibroblasts fr	Gomez et al. [66]
	Cancer cells	Breast cancer	Induced aromatase expression in adipose fibroblasts by enhancing promoter switching	Leclerc et al. [67]
	Autocrine	Lung cancer Breast cancer	Exhibited a defective proliferative capacity in bone marrow fibroblasts	Honegger et al. [68]
	Cancer cells	Breast cancer	Upregulated indoleamine 2,3-dioxygenase (IDO) expression in fibroblasts through an EP4/signal transducer and activator of transcription 3 (STAT3)-dependent pathway	Chen et al. [69]
Lipoxin A4	Exogenous	Human pancreatic stellate cells Pancreatic cancer	Inhibited the differentiation of human pancreatic stellate cells (PSCs) (hPSCs) into TAF-like myofibroblasts and thereby hPSC-induced pro-tumorigenic effects	Schnittert et al. [70]
Palmitate	ECM	Fibroblasts	Inhibited fatty acid degradation, and instead directed more palmitate to membrane lipids	Yao et al. [71]
Resolvin D1	Exogenous	Hepatocellular carcinoma	Inhibited paracrine of TAFs-derived cartilage oligomeric matrix protein to prevent epithelial–mesenchymal transition (EMT) and cancer stemness	Sun et al. [72]
Estrogen	Exogenous	Gastric cancer	Facilitated gastric cancer cell proliferation and invasion through promoting the secretion of interleukin-6 of TAFs	Zhang et al. [73]
12(*S*)-HETE	Cancer cells	Colorectal cancer	Induced the retraction of TAFs via MLC2, RHO/ROCK, and Ca^{2+} signaling	Stadler et al. [74]
Omega-3 PUFA	Exogenous	Lung cancer	Suppressed MMP9 production in TAFs	Taguchi et al. [75]

expression has been found elevated in numerous types of cancer [98]. Even though there is still no direct link between TAFs and FASN overexpression, this could be the result of the coevolution of stroma and cancer. However, more research is required before conclusions can be drawn.

8.4.3 The Effect of TAFs on Lipid Metabolism of Cancer Cells

Compared to nonmalignant cells, cancer cells display significant metabolic alterations. While normal cells modify anabolic and catabolic pathways in response to alterations in nutrient availability, cancer cells demonstrate increased growth even under nutrient scarcity. Although most of the studies regarding metabolic dysregulation in cancer focuses on carbohydrates, the importance of changes associated with lipid metabolism is starting to be acknowledged and the augmented in de novo lipogenesis is considered a new hallmark in many aggressive cancers [99]. Furthermore, cancer cells show increased levels of de novo adipogenesis via improved expression of key lipogenesis regulators like sterol regulatory element-binding proteins (SREBPs), acetyl-CoA carboxylase (ACC), FASN, and stearoyl–CoA desaturase 1

(SCD1) [40]. Additionally, current studies have shown that constitutive activation of pro-growth pathways led to a dependence on unsaturated fatty acids (FA) for survival under oxygen deprivation. The TME can be acidic, hypoxic, and deficient in nutrients, which can cause the remodeled metabolism of tumor cells and neighboring stromal cells to assist tumor cell survival, proliferation, and metastasis. Communication between tumor cells and stromal cells modulates the high metabolic requirements of the tumor. Fatty acid turnover is high in tumor cells to meet the energy and synthetic needs of the growing tumor [100].

Gene expression analysis of coculture spheroids demonstrated that TAFs induced a significant upregulation of cholesterol and steroid biosynthesis pathways in prostate cancer cells. Cytokine profiling showed high amounts of pro-inflammatory, pro-migratory, and pro-angiogenic factors in the TAFs supernatant. Especially, two enzymes, 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (HMGCS2) and aldo-keto reductase family 1 member C3 (AKR1C3), were considerably upregulated in prostate cancer cells upon coculture with TAFs. Both enzymes were also significantly increased in human prostate cancer compared to benign tissues. Suppressing HMGCS2 and AKR1C3 led to significant growth delay of coculture spheroids and enzalutamide resistant cell lines in 2D and 3D culture, underlining their presumed role in prostate cancer. Notably, dual targeting of cholesterol and steroid biosynthesis with simvastatin, a generally prescribed cholesterol synthesis inhibitor, and an inhibitor against AKR1C3 had the strongest growth inhibitory effect. These data suggest that TAFs induce an upregulation of cholesterol and steroid biosynthesis in prostate cancer cells, driving them into AR targeted therapy resistance. Hindering both pathways with simvastatin and an AKR1C3 inhibitor may therefore be a hopeful tool to overcome resistance to AR targeted treatment in prostate cancer [101].

Furthermore, several studies demonstrate that TAFs activate integrin signaling and tyrosine kinases which are critical in regulating lipid metabolism, even there is no direct evidence linking TAFs-mediated signal pathways activation to lipid metabolism of cancer cells. For example, cellular adhesion and signaling transmission can be governed by integrin signaling to activate intracellular signaling pathways, which aim to prevent apoptosis. This modulation is related to lipid metabolism. Moreover, integrin-associated Lyn kinase can support cell survival by inhibiting the activity of acid sphingomyelinase [102]. Additionally, large integrin-associated intracellular protein complexes linked to integrin signaling serve as anchors for cytoskeleton and signaling hotspots and modulate the trafficking of cholesterol-enriched membrane microdomains which are known as lipid rafts [103, 104]. TAFs-derived IL32 or lumican can activate integrin β3-p38 MAPK or integrin β1-FAK signaling pathway in cancer cells. In addition, cytokines and growth factors bind to specific cell surface receptors on target cells to show their biological activities. The majority of these surface receptors exert a tyrosine kinase activity domain that is localized at the cytoplasmic fragment of the molecule [105, 106]. The interaction between cytokines/growth factors and receptors increases the kinase activities of receptors, and further triggers downstream effectors including enzyme activation and protein phosphorylation. The innate immunity and inflammation responsive to cytokine signaling pathways can induce phospholipases, sphingomyelinases, and the enzymes that modulate cholesterol metabolism [107]. As an important source for cytokines and growth factors, the connection of TAFs to the lipid metabolism of cancer cells needs further investigation.

8.5 The Challenges and Perspective of Lipid Metabolism Study on TAFs

Metabolic reprogramming of the tumor microenvironment is required for tumor initiation and progression. In addition, solid tumors can be regarded as metabolically heterogeneous diseases where a diversity of cell types and energetic pathways coincide and cooperate to guarantee cell growth and disease progression. In this biological scenario, TAFs could symbolize the main

cell type modulating the homeostasis and crosstalk with cancer cells. Recently, increasing evidences have demonstrated that TAFs crosstalk with cancer cells by releasing growth factors and manufacturing ECM-degrading proteases, suggesting that TAFs metabolic reprogramming is critical for tumor progress [9]. Although many works have been placed on metabolic communication between TAFs and tumor cells, most of them paid attention to glucose and glutamine metabolism, few considered lipid metabolism, which needs more studies to clarify the detailed mechanism involved. Additionally, the majority of findings about TAFs metabolism were obtained in vitro, which cannot truly reflect the real-time metabolic state in vivo. This is partly due to the heterogeneity of fibroblasts cell population lacking specific markers, limiting effective research methods to study in vivo. Furthermore, the most studies on TAFs were focused on the pro-tumor activity of TAFs. However, several studies suggest that specific subset(s) of TAFs exhibit cancer-restraining roles, indicating that TAFs are functionally and molecularly heterogeneous, which is also supported by single-cell transcriptome analyses [108–110]. How to understand the metabolism involved in functional heterogeneity of TAFs is still a challenge. Collectively, the metabolic slavery of TAFs within tumor microenvironment is a key topic in tumor research field. The development of approaches aimed to disarm TAFs myofibroblastic capacity and to cut off the metabolic crosstalk between TAFs and tumor cells could help eliminate pro-tumorigenic abilities of TAFs in cancer network.

References

1. Kalluri R. The biology and function of fibroblasts in cancer. Nat Rev Cancer. 2016;16:582–98.
2. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol. 2002;3:349–63.
3. Wang X, Khalil RA. Matrix metalloproteinases, vascular remodeling, and vascular disease. Adv Pharmacol. 2018;81:241–330.
4. Kuzet SE, Gaggioli C. Fibroblast activation in cancer: when seed fertilizes soil. Cell Tissue Res. 2016;365:607–19.
5. Wiseman BS, Werb Z. Stromal effects on mammary gland development and breast cancer. Science. 2002;296:1046–9.
6. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med. 1986;315:1650–9.
7. Liao Z, Tan ZW, Zhu P, Tan NS. Cancer-associated fibroblasts in tumor microenvironment—accomplices in tumor malignancy. Cell Immunol. 2019;343:103729.
8. Santi A, Kugeratski FG, Zanivan S. Cancer associated fibroblasts: the architects of stroma remodeling. Proteomics. 2018;18:e1700167.
9. Avagliano A, Granato G, Ruocco MR, Romano V, Belviso I, Carfora A, Montagnani S, Arcucci A. Metabolic reprogramming of cancer associated fibroblasts: the slavery of stromal fibroblasts. Biomed Res Int. 2018;2018:6075403.
10. Caja L, Dituri F, Mancarella S, Caballero-Diaz D, Moustakas A, Giannelli G, Fabregat I. TGF-beta and the tissue microenvironment: relevance in fibrosis and cancer. Int J Mol Sci. 2018;19:1294.
11. Cirri P, Chiarugi P. Cancer associated fibroblasts: the dark side of the coin. Am J Cancer Res. 2011;1:482–97.
12. Marsh T, Pietras K, McAllister SS. Fibroblasts as architects of cancer pathogenesis. Biochim Biophys Acta. 2013;1832:1070–8.
13. Bu L, Baba H, Yoshida N, Miyake K, Yasuda T, Uchihara T, Tan P, Ishimoto T. Biological heterogeneity and versatility of cancer-associated fibroblasts in the tumor microenvironment. Oncogene. 2019;38:4887–901.
14. Sugimoto H, Mundel TM, Kieran MW, Kalluri R. Identification of fibroblast heterogeneity in the tumor microenvironment. Cancer Biol Ther. 2006;5:1640–6.
15. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005;121:335–48.
16. Elenbaas B, Weinberg RA. Heterotypic signaling between epithelial tumor cells and fibroblasts in carcinoma formation. Exp Cell Res. 2001;264:169–84.
17. Avgustinova A, Iravani M, Robertson D, Fearns A, Gao Q, Klingbeil P, Hanby AM, Speirs V, Sahai E, Calvo F, Isacke CM. Tumour cell-derived Wnt7a recruits and activates fibroblasts to promote tumour aggressiveness. Nat Commun. 2016;7:10305.
18. Bailey JM, Swanson BJ, Hamada T, Eggers JP, Singh PK, Caffery T, Ouellette MM, Hollingsworth MA. Sonic hedgehog promotes desmoplasia in pancreatic cancer. Clin Cancer Res. 2008;14:5995–6004.
19. Paggetti J, Haderk F, Seiffert M, Janji B, Distler U, Ammerlaan W, Kim YJ, Adam J, Lichter P, Solary E, Berchem G, Moussay E. Exosomes released by chronic lymphocytic leukemia cells induce the transition of stromal cells into cancer-associated fibroblasts. Blood. 2015;126:1106–17.

20. Webber JP, Spary LK, Sanders AJ, Chowdhury R, Jiang WG, Steadman R, Wymant J, Jones AT, Kynaston H, Mason MD, Tabi Z, Clayton A. Differentiation of tumour-promoting stromal myofibroblasts by cancer exosomes. Oncogene. 2015;34:290–302.
21. Albrengues J, Bourget I, Pons C, Butet V, Hofman P, Tartare-Deckert S, Feral CC, Meneguzzi G, Gaggioli C. LIF mediates proinvasive activation of stromal fibroblasts in cancer. Cell Rep. 2014;7:1664–78.
22. Warburg O. On the origin of cancer cells. Science. 1956;123:309–14.
23. Warburg O, Wind F, Negelein E. The metabolism of tumors in the body. J Gen Physiol. 1927;8:519–30.
24. Yoshida GJ. Metabolic reprogramming: the emerging concept and associated therapeutic strategies. J Exp Clin Cancer Res. 2015;34:111.
25. Gentric G, Mieulet V, Mechta-Grigoriou F. Heterogeneity in cancer metabolism: new concepts in an old field. Antioxid Redox Signal. 2017;26:462–85.
26. Min HY, Lee HY. Oncogene-driven metabolic alterations in cancer. Biomol Ther (Seoul). 2018;26:45–56.
27. Xing Y, Zhao S, Zhou BP, Mi J. Metabolic reprogramming of the tumour microenvironment. FEBS J. 2015;282:3892–8.
28. Wu D, Zhuo L, Wang X. Metabolic reprogramming of carcinoma-associated fibroblasts and its impact on metabolic heterogeneity of tumors. Semin Cell Dev Biol. 2017;64:125–31.
29. Fiaschi T, Giannoni E, Taddei ML, Cirri P, Marini A, Pintus G, Nativi C, Richichi B, Scozzafava A, Carta F, Torre E, Supuran CT, Chiarugi P. Carbonic anhydrase IX from cancer-associated fibroblasts drives epithelial-mesenchymal transition in prostate carcinoma cells. Cell Cycle. 2013;12:1791–801.
30. Ko YH, Lin Z, Flomenberg N, Pestell RG, Howell A, Sotgia F, Lisanti MP, Martinez-Outschoorn UE. Glutamine fuels a vicious cycle of autophagy in the tumor stroma and oxidative mitochondrial metabolism in epithelial cancer cells: implications for preventing chemotherapy resistance. Cancer Biol Ther. 2011;12:1085–97.
31. Huang C, Freter C. Lipid metabolism, apoptosis and cancer therapy. Int J Mol Sci. 2015;16:924–49.
32. Rabinowitz JD, White E. Autophagy and metabolism. Science. 2010;330:1344–8.
33. Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CR, Shimizu T, Spener F, van Meer G, Wakelam MJ, Dennis EA. Update of the LIPID MAPS comprehensive classification system for lipids. J Lipid Res. 2009;50(Suppl):S9–14.
34. Cullis PR, de Kruijff B. Lipid polymorphism and the functional roles of lipids in biological membranes. Biochim Biophys Acta. 1979;559:399–420.
35. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol. 2008;9:112–24.
36. Hannun YA, Obeid LM. Principles of bioactive lipid signalling: lessons from sphingolipids. Nat Rev Mol Cell Biol. 2008;9:139–50.
37. Krycer JR, Sharpe LJ, Luu W, Brown AJ. The Akt-SREBP nexus: cell signaling meets lipid metabolism. Trends Endocrinol Metab. 2010;21:268–76.
38. Zechner R, Zimmermann R, Eichmann TO, Kohlwein SD, Haemmerle G, Lass A, Madeo F. FAT SIGNALS--lipases and lipolysis in lipid metabolism and signaling. Cell Metab. 2012;15:279–91.
39. Santos CR, Schulze A. Lipid metabolism in cancer. FEBS J. 2012;279:2610–23.
40. Cheng C, Geng F, Cheng X, Guo D. Lipid metabolism reprogramming and its potential targets in cancer. Cancer Commun (Lond). 2018;38:27.
41. Maan M, Peters JM, Dutta M, Patterson AD. Lipid metabolism and lipophagy in cancer. Biochem Biophys Res Commun. 2018;504:582–9.
42. Buchsbaum RJ, Oh SY. Breast cancer-associated fibroblasts: where we are and where we need to go. Cancers (Basel). 2016;8:19.
43. Qiao A, Gu F, Guo X, Zhang X, Fu L. Breast cancer-associated fibroblasts: their roles in tumor initiation, progression and clinical applications. Front Med. 2016;10:33–40.
44. Augsten M. Cancer-associated fibroblasts as another polarized cell type of the tumor microenvironment. Front Oncol. 2014;4:62.
45. Bremnes RM, Donnem T, Al-Saad S, Al-Shibli K, Andersen S, Sirera R, Camps C, Marinez I, Busund LT. The role of tumor stroma in cancer progression and prognosis: emphasis on carcinoma-associated fibroblasts and non-small cell lung cancer. J Thorac Oncol. 2011;6:209–17.
46. Costa A, Scholer-Dahirel A, Mechta-Grigoriou F. The role of reactive oxygen species and metabolism on cancer cells and their microenvironment. Semin Cancer Biol. 2014;25:23–32.
47. Tang S, Yang L, Tang X, Liu M. The role of oxidized ATM in the regulation of oxidative stress-induced energy metabolism reprogramming of CAFs. Cancer Lett. 2014;353:133–44.
48. Yu T, Yang G, Hou Y, Tang X, Wu C, Wu XA, Guo L, Zhu Q, Luo H, Du YE, Wen S, Xu L, Yin J, Tu G, Liu M. Cytoplasmic GPER translocation in cancer-associated fibroblasts mediates cAMP/PKA/CREB/glycolytic axis to confer tumor cells with multidrug resistance. Oncogene. 2017;36:2131–45.
49. Ziani L, Chouaib S, Thiery J. Alteration of the anti-tumor immune response by cancer-associated fibroblasts. Front Immunol. 2018;9:414.
50. Zehmer JK, Huang Y, Peng G, Pu J, Anderson RG, Liu P. A role for lipid droplets in inter-membrane lipid traffic. Proteomics. 2009;9:914–21.
51. Khor VK, Shen WJ, Kraemer FB. Lipid droplet metabolism. Curr Opin Clin Nutr Metab Care. 2013;16:632–7.
52. Borg ML, Andrews ZB, Duh EJ, Zechner R, Meikle PJ, Watt MJ. Pigment epithelium-derived factor regulates lipid metabolism via adipose triglyceride lipase. Diabetes. 2011;60:1458–66.
53. Chung C, Doll JA, Gattu AK, Shugrue C, Cornwell M, Fitchev P, Crawford SE. Anti-angiogenic pigment epithelium-derived factor regulates hepatocyte

triglyceride content through adipose triglyceride lipase (ATGL). J Hepatol. 2008;48:471–8.
54. Zhang J, Xu D, Nie J, Han R, Zhai Y, Shi Y. Comparative gene identification-58 (CGI-58) promotes autophagy as a putative lysophosphatidylglycerol acyltransferase. J Biol Chem. 2014;289:33044–53.
55. Boeszoermenyi A, Nagy HM, Arthanari H, Pillip CJ, Lindermuth H, Luna RE, Wagner G, Zechner R, Zangger K, Oberer M. Structure of a CGI-58 motif provides the molecular basis of lipid droplet anchoring. J Biol Chem. 2015;290:26361–72.
56. Young SG, Zechner R. Biochemistry and pathophysiology of intravascular and intracellular lipolysis. Genes Dev. 2013;27:459–84.
57. Halin S, Rudolfsson SH, Doll JA, Crawford SE, Wikstrom P, Bergh A. Pigment epithelium-derived factor stimulates tumor macrophage recruitment and is downregulated by the prostate tumor microenvironment. Neoplasia. 2010;12:336–45.
58. Tokumaru Y, Yamashita K, Osada M, Nomoto S, Sun DI, Xiao Y, Hoque MO, Westra WH, Califano JA, Sidransky D. Inverse correlation between cyclin A1 hypermethylation and p53 mutation in head and neck cancer identified by reversal of epigenetic silencing. Cancer Res. 2004;64:5982–7.
59. Nardi F, Fitchev P, Franco OE, Ivanisevic J, Scheibler A, Hayward SW, Brendler CB, Welte MA, Crawford SE. PEDF regulates plasticity of a novel lipid-MTOC axis in prostate cancer-associated fibroblasts. J Cell Sci. 2018;131:jcs213579.
60. Pyne NJ, Pyne S. Sphingosine 1-phosphate and cancer. Nat Rev Cancer. 2010;10:489–503.
61. Saddoughi SA, Song P, Ogretmen B. Roles of bioactive sphingolipids in cancer biology and therapeutics. Subcell Biochem. 2008;49:413–40.
62. Beach JA, Aspuria PJ, Cheon DJ, Lawrenson K, Agadjanian H, Walsh CS, Karlan BY, Orsulic S. Sphingosine kinase 1 is required for TGF-beta mediated fibroblastto- myofibroblast differentiation in ovarian cancer. Oncotarget. 2016;7:4167–82.
63. Radhakrishnan R, Ha JH, Jayaraman M, Liu J, Moxley KM, Isidoro C, Sood AK, Song YS, Dhanasekaran DN. Ovarian cancer cell-derived lysophosphatidic acid induces glycolytic shift and cancer-associated fibroblast-phenotype in normal and peritumoral fibroblasts. Cancer Lett. 2019;442:464–74.
64. Wen Z, Liu H, Li M, Li B, Gao W, Shao Q, Fan B, Zhao F, Wang Q, Xie Q, Yang Y, Yu J, Qu X. Increased metabolites of 5-lipoxygenase from hypoxic ovarian cancer cells promote tumor-associated macrophage infiltration. Oncogene. 2015;34:1241–52.
65. Vancheri C, Sortino MA, Tomaselli V, Mastruzzo C, Condorelli F, Bellistri G, Pistorio MP, Canonico PL, Crimi N. Different expression of TNF-alpha receptors and prostaglandin E(2)Production in normal and fibrotic lung fibroblasts: potential implications for the evolution of the inflammatory process. Am J Respir Cell Mol Biol. 2000;22:628–34.
66. Gomez PF, Pillinger MH, Attur M, Marjanovic N, Dave M, Park J, Bingham CO 3rd, Al-Mussawir H, Abramson SB. Resolution of inflammation: prostaglandin E2 dissociates nuclear trafficking of individual NF-kappaB subunits (p65, p50) in stimulated rheumatoid synovial fibroblasts. J Immunol. 2005;175:6924–30.
67. Leclerc P, Idborg H, Spahiu L, Larsson C, Nekhotiaeva N, Wannberg J, Stenberg P, Korotkova M, Jakobsson PJ. Characterization of a human and murine mPGES-1 inhibitor and comparison to mPGES-1 genetic deletion in mouse models of inflammation. Prostaglandins Other Lipid Mediat. 2013;107:26–34.
68. Honegger AE, Hofer EL, Baranao RI, Mackinlay TA, Mackinlay DA, Bullorsky EO, Bordenave RH, Chasseing NA. Interleukin-1 beta, transforming growth factor beta 1, prostaglandin E2, and fibronectin levels in the conditioned mediums of bone marrow fibroblast cultures from lung and breast cancer patients. Ann Hematol. 2002;81:80–5.
69. Chen JY, Li CF, Kuo CC, Tsai KK, Hou MF, Hung WC. Cancer/stroma interplay via cyclooxygenase-2 and indoleamine 2,3-dioxygenase promotes breast cancer progression. Breast Cancer Res. 2014;16:410.
70. Schnittert J, Heinrich MA, Kuninty PR, Storm G, Prakash J. Reprogramming tumor stroma using an endogenous lipid lipoxin A4 to treat pancreatic cancer. Cancer Lett. 2018;420:247–58.
71. Yao CH, Fowle-Grider R, Mahieu NG, Liu GY, Chen YJ, Wang R, Singh M, Potter GS, Gross RW, Schaefer J, Johnson SL, Patti GJ. Exogenous fatty acids are the preferred source of membrane lipids in proliferating fibroblasts. Cell Chem Biol. 2016;23:483–93.
72. Sun L, Wang Y, Wang L, Yao B, Chen T, Li Q, Liu Z, Liu R, Niu Y, Song T, Liu Q, Tu K. Resolvin D1 prevents epithelial-mesenchymal transition and reduces the stemness features of hepatocellular carcinoma by inhibiting paracrine of cancer-associated fibroblast-derived COMP. J Exp Clin Cancer Res. 2019; 38:170.
73. Zhang Y, Cong X, Li Z, Xue Y. Estrogen facilitates gastric cancer cell proliferation and invasion through promoting the secretion of interleukin-6 by cancer-associated fibroblasts. Int Immunopharmacol. 2020; 78:105937.
74. Stadler S, Nguyen C H, Schachner H, Milovanovic D, Holzner S, Brenner S, Eichsteininger J, Stadler M, Senfter D, Krenn L, Schmidt W M, Huttary N, Krieger S, Koperek O, Bago-Horvath Z, Brendel K A, Marian B, De Wever O, Mader R M, Giessrigl B, Jager W, Dolznig H, Krupitza G. Colon cancer cell-derived 12(S)-HETE induces the retraction of cancer-associated fibroblast via MLC2, RHO/ROCK and Ca(2+) signalling. Cell Mol Life Sci. 2017; 74:1907–21.
75. Taguchi A, Kawana K, Tomio K, Yamashita A, Isobe Y, Nagasaka K, Koga K, Inoue T, Nishida H, Kojima S, Adachi K, Matsumoto Y, Arimoto T, Wada-

Hiraike O, Oda K, Kang J X, Arai H, Arita M, Osuga Y, Fujii T. Matrix metalloproteinase (MMP)-9 in cancer-associated fibroblasts (CAFs) is suppressed by omega-3 polyunsaturated fatty acids in vitro and in vivo. PLoS One. 2014;9:e89605.
76. Elwakeel E, Brune B, Weigert A. PGE2 in fibrosis and cancer: insights into fibroblast activation. Prostaglandins Other Lipid Mediat. 2019;143:106339.
77. Wilborn J, Crofford LJ, Burdick MD, Kunkel SL, Strieter RM, Peters-Golden M. Cultured lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis have a diminished capacity to synthesize prostaglandin E2 and to express cyclooxygenase-2. J Clin Invest. 1995;95:1861–8.
78. Kolodsick JE, Peters-Golden M, Larios J, Toews GB, Thannickal VJ, Moore BB. Prostaglandin E2 inhibits fibroblast to myofibroblast transition via E. prostanoid receptor 2 signaling and cyclic adenosine monophosphate elevation. Am J Respir Cell Mol Biol. 2003;29:537–44.
79. Wettlaufer SH, Scott JP, McEachin RC, Peters-Golden M, Huang SK. Reversal of the transcriptome by prostaglandin E2 during myofibroblast dedifferentiation. Am J Respir Cell Mol Biol. 2016;54:114–27.
80. Huang SK, Wettlaufer SH, Chung J, Peters-Golden M. Prostaglandin E2 inhibits specific lung fibroblast functions via selective actions of PKA and Epac-1. Am J Respir Cell Mol Biol. 2008;39:482–9.
81. Penke LR, Huang SK, White ES, Peters-Golden M. Prostaglandin E2 inhibits alpha-smooth muscle actin transcription during myofibroblast differentiation via distinct mechanisms of modulation of serum response factor and myocardin-related transcription factor-A. J Biol Chem. 2014;289:17151–62.
82. Thomas PE, Peters-Golden M, White ES, Thannickal VJ, Moore BB. PGE(2) inhibition of TGF-beta1-induced myofibroblast differentiation is Smad-independent but involves cell shape and adhesion-dependent signaling. Am J Physiol Lung Cell Mol Physiol. 2007;293:L417–28.
83. Harding P, LaPointe MC. Prostaglandin E2 increases cardiac fibroblast proliferation and increases cyclin D expression via EP1 receptor. Prostaglandins Leukot Essent Fatty Acids. 2011;84:147–52.
84. Iwanaga K, Okada M, Murata T, Hori M, Ozaki H. Prostaglandin E2 promotes wound-induced migration of intestinal subepithelial myofibroblasts via EP2, EP3, and EP4 prostanoid receptor activation. J Pharmacol Exp Ther. 2012;340:604–11.
85. Schrey MP, Patel KV. Prostaglandin E2 production and metabolism in human breast cancer cells and breast fibroblasts. Regulation by inflammatory mediators. Br J Cancer. 1995;72:1412–9.
86. Alba-Castellon L, Olivera-Salguero R, Mestre-Farrera A, Pena R, Herrera M, Bonilla F, Casal JI, Baulida J, Pena C, Garcia de Herreros A. Snail1-dependent activation of cancer-associated fibroblast controls epithelial tumor cell invasion and metastasis. Cancer Res. 2016;76:6205–17.
87. Alcolea S, Anton R, Camacho M, Soler M, Alfranca A, Aviles-Jurado FX, Redondo JM, Quer M, Leon X, Vila L. Interaction between head and neck squamous cell carcinoma cells and fibroblasts in the biosynthesis of PGE2. J Lipid Res. 2012;53:630–42.
88. Kock A, Larsson K, Bergqvist F, Eissler N, Elfman LHM, Raouf J, Korotkova M, Johnsen JI, Jakobsson PJ, Kogner P. Inhibition of microsomal prostaglandin E Synthase-1 in cancer-associated fibroblasts suppresses neuroblastoma tumor growth. EBioMedicine. 2018;32:84–92.
89. Larsson K, Kock A, Idborg H, Arsenian Henriksson M, Martinsson T, Johnsen JI, Korotkova M, Kogner P, Jakobsson PJ. COX/mPGES-1/PGE2 pathway depicts an inflammatory-dependent high-risk neuroblastoma subset. Proc Natl Acad Sci U S A. 2015;112:8070–5.
90. Li T, Yang Y, Hua X, Wang G, Liu W, Jia C, Tai Y, Zhang Q, Chen G. Hepatocellular carcinoma-associated fibroblasts trigger NK cell dysfunction via PGE2 and IDO. Cancer Lett. 2012;318:154–61.
91. Gong J, Lin Y, Zhang H, Liu C, Cheng Z, Yang X, Zhang J, Xiao Y, Sang N, Qian X, Wang L, Cen X, Du X, Zhao Y. Reprogramming of lipid metabolism in cancer-associated fibroblasts potentiates migration of colorectal cancer cells. Cell Death Dis. 2020;11:267.
92. Lopes-Coelho F, Andre S, Felix A, Serpa J. Breast cancer metabolic cross-talk: fibroblasts are hubs and breast cancer cells are gatherers of lipids. Mol Cell Endocrinol. 2018;462:93–106.
93. Pavlides S, Tsirigos A, Migneco G, Whitaker-Menezes D, Chiavarina B, Flomenberg N, Frank PG, Casimiro MC, Wang C, Pestell RG, Martinez-Outschoorn UE, Howell A, Sotgia F, Lisanti MP. The autophagic tumor stroma model of cancer: role of oxidative stress and ketone production in fueling tumor cell metabolism. Cell Cycle. 2010;9:3485–505.
94. Bonuccelli G, Tsirigos A, Whitaker-Menezes D, Pavlides S, Pestell RG, Chiavarina B, Frank PG, Flomenberg N, Howell A, Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Ketones and lactate "fuel" tumor growth and metastasis: evidence that epithelial cancer cells use oxidative mitochondrial metabolism. Cell Cycle. 2010;9:3506–14.
95. Santi A, Caselli A, Ranaldi F, Paoli P, Mugnaioni C, Michelucci E, Cirri P. Cancer associated fibroblasts transfer lipids and proteins to cancer cells through cargo vesicles supporting tumor growth. Biochim Biophys Acta. 2015;1853:3211–23.
96. Grasso C, Jansen G, Giovannetti E. Drug resistance in pancreatic cancer: impact of altered energy metabolism. Crit Rev Oncol Hematol. 2017;114:139–52.
97. Zhao H, Yang L, Baddour J, Achreja A, Bernard V, Moss T, Marini JC, Tudawe T, Seviour EG, San Lucas FA, Alvarez H, Gupta S, Maiti SN, Cooper L, Peehl D, Ram PT, Maitra A, Nagrath D. Tumor

microenvironment derived exosomes pleiotropically modulate cancer cell metabolism. elife. 2016;5:e10250.
98. Hu JW, Sun P, Zhang DX, Xiong WJ, Mi J. Hexokinase 2 regulates G1/S checkpoint through CDK2 in cancer-associated fibroblasts. Cell Signal. 2014;26:2210–6.
99. Robey RB, Weisz J, Kuemmerle NB, Salzberg AC, Berg A, Brown DG, Kubik L, Palorini R, Al-Mulla F, Al-Temaimi R, Colacci A, Mondello C, Raju J, Woodrick J, Scovassi AI, Singh N, Vaccari M, Roy R, Forte S, Memeo L, Salem HK, Amedei A, Hamid RA, Williams GP, Lowe L, Meyer J, Martin FL, Bisson WH, Chiaradonna F, Ryan EP. Metabolic reprogramming and dysregulated metabolism: cause, consequence and/or enabler of environmental carcinogenesis? Carcinogenesis. 2015;36(Suppl 1):S203–31.
100. Ackerman D, Simon MC. Hypoxia, lipids, and cancer: surviving the harsh tumor microenvironment. Trends Cell Biol. 2014;24:472–8.
101. Neuwirt H, Bouchal J, Kharaishvili G, Ploner C, Johrer K, Pitterl F, Weber A, Klocker H, Eder IE. Cancer-associated fibroblasts promote prostate tumor growth and progression through upregulation of cholesterol and steroid biosynthesis. Cell Commun Signal. 2020;18:11.
102. Chudakova DA, Zeidan YH, Wheeler BW, Yu J, Novgorodov SA, Kindy MS, Hannun YA, Gudz TI. Integrin-associated Lyn kinase promotes cell survival by suppressing acid sphingomyelinase activity. J Biol Chem. 2008;283:28806–16.
103. Salanueva IJ, Cerezo A, Guadamillas MC, del Pozo MA. Integrin regulation of caveolin function. J Cell Mol Med. 2007;11:969–80.
104. Xiong J, Balcioglu HE, Danen EH. Integrin signaling in control of tumor growth and progression. Int J Biochem Cell Biol. 2013;45:1012–5.
105. Greenhalgh CJ, Hilton DJ. Negative regulation of cytokine signaling. J Leukoc Biol. 2001;70:348–56.
106. Perona R. Cell signalling: growth factors and tyrosine kinase receptors. Clin Transl Oncol. 2006;8:77–82.
107. Lebman DA, Spiegel S. Cross-talk at the crossroads of sphingosine-1-phosphate, growth factors, and cytokine signaling. J Lipid Res. 2008;49:1388–94.
108. Bartoschek M, Oskolkov N, Bocci M, Lovrot J, Larsson C, Sommarin M, Madsen CD, Lindgren D, Pekar G, Karlsson G, Ringner M, Bergh J, Bjorklund A, Pietras K. Spatially and functionally distinct subclasses of breast cancer-associated fibroblasts revealed by single cell RNA sequencing. Nat Commun. 2018;9:5150.
109. Elyada E, Bolisetty M, Laise P, Flynn WF, Courtois ET, Burkhart RA, Teinor JA, Belleau P, Biffi G, Lucito MS, Sivajothi S, Armstrong TD, Engle DD, Yu KH, Hao Y, Wolfgang CL, Park Y, Preall J, Jaffee EM, Califano A, Robson P, Tuveson DA. Cross-species single-cell analysis of pancreatic ductal adenocarcinoma reveals antigen-presenting Cancer-associated fibroblasts. Cancer Discov. 2019;9:1102–23.
110. Miyai Y, Esaki N, Takahashi M, Enomoto A. Cancer-associated fibroblasts that restrain cancer progression: hypotheses and perspectives. Cancer Sci. 2020;111:1047–57.

9 Lipid Metabolism in Tumor-Associated B Cells

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Abstract

Breakthroughs have been made in the cancer immunotherapy field focusing on utilizing T cells' antitumor immunity, and the lipid metabolism of tumor-associated B cells is not well studied compared to T cells. Accumulating evidence suggested that B cells also play important roles in tumor biology and antitumor immunity, especially the germinal center B cells that present in the tumor-related tertiary lymphoid structures. Due to scarce studies on lipid metabolisms of tumor-associated B cells, this chapter mainly summarized findings on B cell lipid metabolism and discussed B cell development and major transcription factors, tumor-associated B cell populations and their potential functions in antitumor immunity, fatty acid oxidation in germinal center B cells, and tumor microenvironment factors that potentially affect B cell lipid metabolism, focusing on hypoxia and nutrients competition, as well as lipid metabolites that affect B cell function, including cholesterol, geranylgeranyl pyrophosphate, oxysterols, and short-chain fatty acids.

Keywords

B cells · Lipid metabolism · Germinal center Cholesterol · Cancer

9.1 B Cell Development

B cells, derived from bone marrow (BM) hematopoietic stem cells, undergo programmed development firstly from lymphoid progenitors to pre-B cells, which express "B cell receptor (BCR)" composed of immunoglobulin (Ig) heavy chain and a surrogate light chain after Ig gene V(D)J recombination [1]. Then the pre-BCR signaling triggers proliferation and results in an increased amount of resting pre-B cells, among which Ig light-chain gene rearrangement is fulfilled and a functional BCR (IgM usually) presents, indicating development into immature B cells [1]. Accompanied by another kind of BCR (IgD) expression, mature B cells migrate to the periphery surveying for antigens (Ag). Ags and co-stimulatory molecules as toll-like receptor ligands drive the subsequent B cell proliferation and differentiation into the antibody (Ab)-secreting cells. A cohort of B cells further differentiates to memory B cells or long-lived plasma cells [2, 3]. A summary of B cell development is shown in Fig. 9.1.

To fulfill the energy and nutrient demands of humoral immunity, B cells adapt both non-

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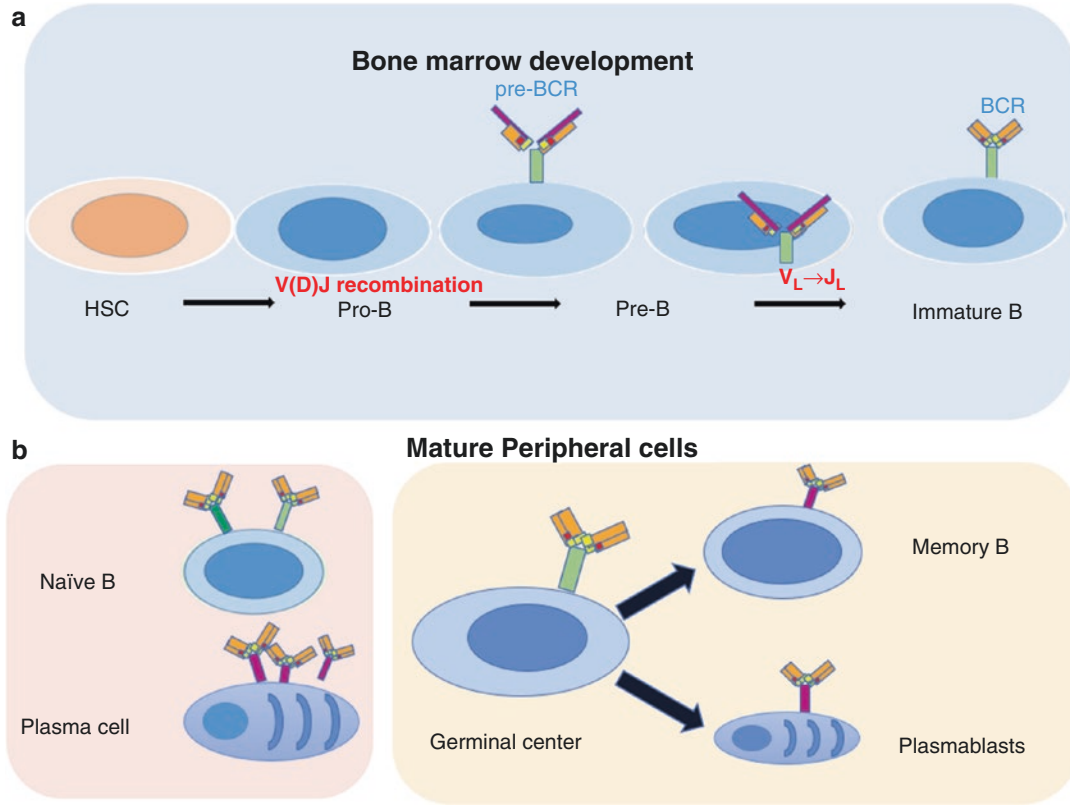

Fig. 9.1 B cell development. A schematic of B cell development from the bone marrow to the periphery. (**a**) B cells in the bone marrow undergo early stage of differentiation from hematopoietic stem cell (HSC) to immature B cells, which transit to the periphery to develop to mature circulating cells. (**b**) The naïve B cells are activated by antigen and co-stimulatory molecules and become antibody-secreting plasma cells after proliferation and differentiation. A cohort of B cells undergo further differentiation in the germinal center to become memory B cells or long-lived plasmablasts

cycling resting state and rapid proliferation state. The metabolic requirements are high when B cells are the early proliferative progenitors in the BM and fall in the pre-B and immature B cells. For the resting naïve and memory B cells, energy demands are relatively low, but raised during antigen-stimulated proliferation and differentiation, and remain high in the Ab-secreting plasma cells [4].

Glucose, glutamine, and fatty acids are the main carbon sources for B cell metabolism, among which glucose is the main carbon source for both the resting and activated B cells [5]. It is reported that resting B cells in murine spleen require glycolysis as well as oxidative phosphorylation (OXPHOS) [6]. Meanwhile, for the peripheral naïve B cells, there exists a specific loss of mature B cells in response to the absence of glucose transporter GLUT1, indicating that glucose uptake plays an especially important role for peripheral resting B cell homeostasis [6]. On the other hand, the surface expression of GLUT1 is raised after BCR engagement, and the subsequent upregulated glycolysis is PI3K-Akt-dependent [6–8]. BCR engagement also promotes OXPHOS, which is a significant difference between BCR-stimulated B cells and TCR/CD28-stimulated T cells that prefer glycolysis only [6]. Besides, LPS treatment also promotes glycolysis and OXPHOS in naïve B cells, indicated by increased GLUT1 expression and mitochondrial mass [6, 7].

For B cell proliferation and differentiation, glycolysis has been considered the primary driving power [5]. Per the increased energy needs after B cell activation, glucose uptake is elevated to enter glycolysis [6, 9, 10]. Consistently, GLUT1-deficient B cells are defective in Ab production [6]. Similarly, 2-deoxyglucose (2-DG) treatment inhibits LPS-activated B cell proliferation as well as Ab production [8]. The resultant pyruvate derived from glycolysis then enters mitochondria and undergoes Krebs (TCA) cycle and OXPHOS not only to enhance energy production as the form of ATP but also to provide a large pool of citrate for intrinsic lipogenesis catalyzed by ATP citrate lyase (ACLY). The newly produced lipids contribute to membrane synthesis during B cell growth and division [11].

Lipid and protein metabolism also fulfills energy supply and supports B cell function. Fatty acid oxidation also generates pyruvate, which enters the TCA cycle to feed OXPHOS and produce ATP [5]. Proteins can also function as a carbon source. The hydrolyzed individual amino acids participate in parts of the TCA cycle and generate ATP [5]. On the other hand, serine metabolism also contributes to lipid synthesis. Serine is generated from glucose-derived 3-phosphoglyceric acid and enters the one-carbon metabolism pathway, which means a one-carbon unit from serine is processed through methionine and folate cycles to contribute to lipid, nucleotide, and protein synthesis. This pathway also generates products that are crucial for methylation reactions as well as redox reactions. How serine and serine-derived one-carbon metabolism involves the regulation of tumor and immunity is investigated [12, 13].

B1 B cells, tissue-resident and innate-like, display distinct development and metabolic patterns from abovementioned B2 B cells [14], which is the center of humoral immunity and the main source of glycosylated Abs. B1 B cells exist from fetal and neonatal stages, and the subsequent expansion is mainly through self-renewal [15], although there is rare potential that B1 B cells originate from BM B1 progenitors [16]. Similar to B2 cells, B1 cells have a potent requirement for lipogenesis de novo, potentially from glycolytic product citrate by ACLY. However, B1 cells exhibit higher levels of glycolysis as well as OXPHOS and the subsequent TCA cycle-coupled fatty acid synthesis compared to B2 cells [14].

9.1.1 Transcriptional Regulation of B Cell Metabolism

The transcriptional regulation for peripheral B cell destiny still needs further investigation, although for early-stage differentiation, it is relatively well-known.

Among the critical B cell transcription factors, c-Myc plays an important role in B cell proliferation, clonal expansion, and fate determination [17, 18], contributing to an expression of effectors involved in nutrient uptake and mTOR activation [19]. c-Myc is essential for B cell positive selection in the germinal center (GC). c-Myc is expressed in a small portion of B cells in the light zone of the GC [20], where mesenchyme-originated follicular dendritic cells capture immune-complex and facilitate B cell recognition of antigen by B cell receptor [21]. The c-Myc-expressing cells are characterized by upregulated genes critical to glycolysis and nutrient sensing [22]. Also, c-Myc is modestly crucial for LPS-triggered glutamine oxidation increase while antagonizing LPS-mediated downregulation of fatty acid oxidation and pyruvate oxidation [6]. In addition, c-Myc induces transcription factor AP4, which is essential for T-B interaction in the light zone of GC through IL-21 signaling and for the subsequent GC B cell division in the dark zone of the GC [23].

Nuclear factor-κB (NF-κB), the key regulator of inflammatory immune response and cytokine production, is suggested to be involved in the regulation of B cell metabolism and proliferation via a TRAF3-NIK-NF-κB axis [24]. Tumor necrosis factor (TNF) receptor-associated factor-3 (TRAF3) plays a critical role in B cell metabolism. TRAF3-deficient B cells present unusually increased expression of key genes that are involved in the early phase of glycolysis, such as genes encoding GLUT1 and hexokinase-2. In addition, this kind of B cells increases mitochondrial respi-

ration, but does not increase reactive oxygen species generation [25]. B cell full activation demands co-stimulation through CD40-B-cell activating factor (BAFF) receptor pathway, which triggers the degradation of TRAF3 and the buildup of NF-κB-inducing kinase (NIK) [26]. The latter leads to translocation of NF-κB into the nucleus, where the transcription of target genes is activated. Double knockout of TRAF3 and NIK causes a low level of GLUT1 expression and reduced mature B cell counts [25]. Consistently, when activated by LPS, BAFF-exposed naïve B cells display increased glucose uptake as well as a relatively higher basal mitochondrial activity [6]. The above findings indicate that NF-κB is involved in the regulation of glucose uptake [25] and that B cell co-stimulation plays a role in B cell metabolic reprogramming [24].

The transcription factor Bcl6 is highly expressed in both GC B cells and follicular helper T (Tfh) cells, induced by multiple co-stimulatory molecules between B and T cells, including IL-21 [27–29]. It has been reported that Bcl6 suppresses glycolysis in macrophages [30], therefore may have to be overcome by c-Myc [20, 22, 31] and hypoxia-inducible factor (HIF) in the GC.

The light zone of GC provides a hypoxic environment, which is also related to B cell metabolism [32]. HIF1 and HIF2 evoke glycolysis through aldolase A, M2 isoform of pyruvate kinase, and phosphoglycerate kinase 1 [33]. Although HIF and c-Myc both actuate glycolysis [34], HIF represses c-Myc activity [23, 35]. c-Myc promotes mitochondrial biogenesis [36], while HIF inhibits Krebs cycle and respiration [37]. Given that c-Myc evokes expression of effectors related to mTOR activation [19], HIF also suppresses mTOR1 activity [23, 35]. HIF-1α not only regulates expression of genes associated with glycolysis in response to limited oxygen environment but also controls B cell development and activity in a stage-specific pattern [38, 39]. It is reported that lack of HIF-1α results in decreased expression of phosphofructokinase (Pfkfb3) and glucose transporters, which obstructs the development from pro- to pre-B cell stage in the BM [40]. Also, HIF-1α sustains the energy requirement of the Ag-exposed B cells in the GC [41]. In addition, hypoxia potentially promotes plasma cell fate determination [32], which may be due to the HIF-regulated *lrf4* gene [42].

IRF4 is critical to plasma cell differentiation and GC response [43]. It is expressed in resting B cells at a low level to promote survival, and its expression is elevated inconsistent with the strength of activation signals stimulated by Ag, cytokines, or TLR ligands. The majority of IRF4-targeted genes may be co-regulated by c-Myc since they bear AP1-IRF4 composite sites [42]. A small amount of $IRF4^{+}c\text{-}Myc^{+}$ cells exist in the GC and may be the outcome of asymmetric division to generate plasmablasts [20, 22, 44].

The c-Rel transcription factor is also expressed at a relatively high level upon B cell activation, inducing *lrf4* expression [45]. It is involved in the metabolic regulation that fulfills the energy demands of proliferating GC B cells [46]. c-Rel translocation is PI3K-dependent and only happens in a small amount of GC B cells, which may be the ones facilitated by T cells [47].

Bach2, Foxo1, and Pax5 act similarly to Bcl6 and inhibit plasma cell differentiation [48], while E2A and E2–2 are committed to GC response and plasma cell differentiation [49, 50]. In addition, Pax5 has been reported that it inhibits metabolism in early B cells [51]. The combined findings indicate that the transcription factors regulating B cell metabolism remain to be revealed.

B1 B cells exhibit significantly higher gene expression associated with glycolysis and lipid metabolism as well as lipid storage, compared to B2 B cells [14].

9.2 Tumor-Associated B Cell Populations

B cells are commonly found in tumor-draining lymph nodes, and less commonly at the invasive margin of tumors, or infiltrated into the tumor mass. A closer look at tumor-related B cells revealed that B cells exist in different forms, from nonstructured immune cell aggregates to structured ones, i.e., tertiary lymphoid structures

(TLS). TLS is induced in chronic inflammations including cancer, autoimmunity, and organ transplant and resembles follicles of the second lymphoid organs. In cancer, TLS localizes at the tumor periphery and, less frequently, inside the tumor.

9.2.1 B Cells with Antitumor Function

The presence of certain B cells in the tumor has been associated with a better prognosis. Earlier studies found that in some breast or ovarian cancer patients, tumor infiltrated B cells were associated with a good prognosis [52]. Later, TLS that contains a GC was found to be correlated with improved survival in multiple cancer types. The prognostic significance of the tumor-related TLS was reviewed in depth by Sautès-Fridman et al. [53]. Originally discovered in hepatocellular carcinoma, numerous studies found that B cells/GCs' presence in the TLS correlated with prolonged survival in other types of cancers, including non-small cell lung cancer, colorectal cancer, pancreatic cancer, oral squamous cell carcinoma, invasive breast cancer, etc. Since B cells initiate TLS formation, these discoveries highlighted B cell roles in patients' survival. The two scenarios of tumor-associated B cells are summarized in Fig. 9.2: presence of B cells with mature TLS containing GC, or with less organized cell aggregates without GC (immature TLS). It should be noted that the broad classification of TLS presented here is an oversimplified model, as there are immune "cold" tumors and more variety of tumor-associated B cells.

Very recently, the presence of B cells in the TLS has been found to correlate with immunotherapy success [54–56], indicating that B cells play a critical role in immunotherapy success. In searching for predictors for patients' outcome after immune checkpoint blockade (ICB), the B lineage signature has been found to be the strongest predictor for survival in a cohort of sarcoma patients [56]. Corroborating this finding, in another cohort of melanoma patients treated with ICB, a TLS associated gene signature predicted

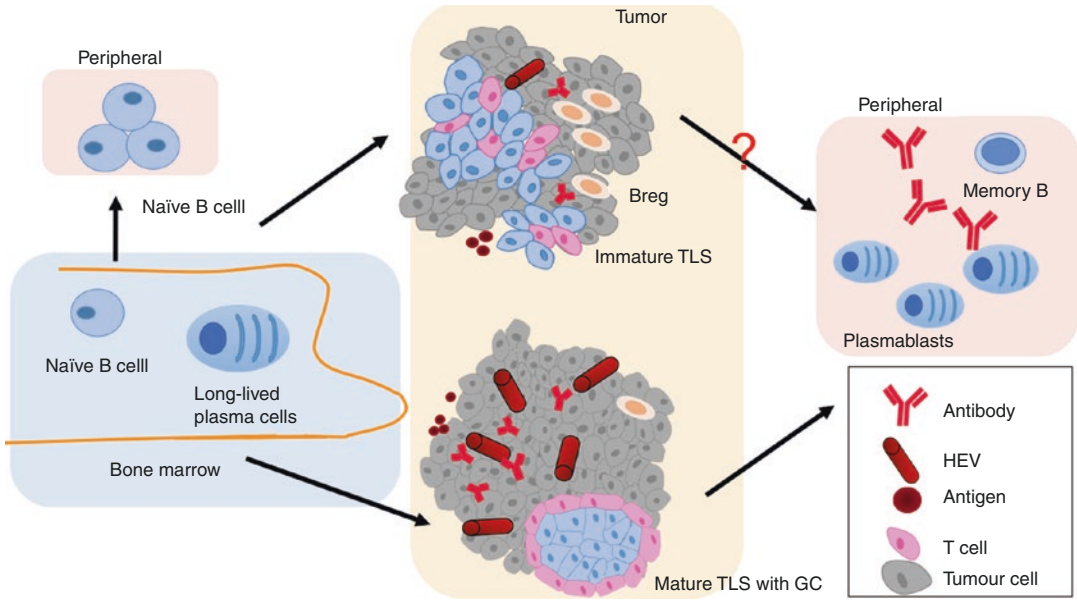

Fig. 9.2 Mature or immature TLS and tumor-associated B cells. A schematic of various tumor-associated B cell populations and TLS. The existence of mature B cells in tumors featuring germinal centers is associated with better prognosis, where B cells are selected and differentiated and correlated with better prognosis in multiple cancers. Immature TLS are unstructured cell aggregates. B cells and TLS signature are more prominent in responders versus nonresponders of immunotherapy. Memory B cells and regulatory B cells are associated with tumor, too

patients' survival [54]. Furthermore, the B cells and TLS signatures are more prominent than T cell signatures for discerning between the responders and the nonresponders to neoadjuvant ICB for melanoma [55]. These findings confirm the critical roles played by B cells/TLS in antitumor immunity and immunotherapy.

Circulating plasmablasts, a type of mature B cells that circulate back to the BM or reside in the chronic inflammation sites, were found increased in patients with metastatic but non-progressing melanoma, lung adenocarcinoma, or renal cell carcinoma. In these non-progressing cancer cases, clonal affinity matured B cells exhibited progressive class switch, and recombinant antibodies from clonal families were able to bind non-autologous tumor tissue/cell lines and caused tumor regression in syngeneic mouse tumor models [57], indicating that B cells contributed to control the disease progression in these patients.

B1a cells, an innate-like B cell population, were essential for protection induced by toll-like receptor and C-type lectin receptor agonist pairing of monophosphoryl lipid A (MPL) and trehalose-6,6′-dicorynomycolate (TDCM) treatment in a mouse model. MPL/TDCM treatment effectively inhibited tumor growth and ascites development in this mouse model of aggressive mammary cancer-induced peritoneal carcinomatosis. B1a cells were enriched in the peritoneal cavity and deficient in mice lacking CD19. MPL/TDCM treatment effects were not observed in mice lacking CD19, and adoptive transfer of B1a cells restored the protective effects [58].

Atypical B cell populations with protective effects are found in breast cancers, too. ICOSL^{+}B cells (ICOSL + CR2highIL – 10 – CD20 + CD38 + CD27 + IgA – IgD-) emerged after chemotherapy in breast cancer patients and correlated with better therapeutic effect and prognosis [59]. CD40 signals in GC B cells upregulated ICOSL in these cells, which in turn promoted interaction with follicular T cells and the GC selection process, forming a feed-forward loop [60].

All the abovementioned pieces of evidence point to certain B cell function in antitumor immunity. Producing antibodies, secreting antitumor cytokines, and serving as antigen-presenting cells are examples of how B cells can help to improve antitumor immunity. Moreover, B cells can secrete granzyme and directly kill tumor cells. The field of B cells' role in antitumor immunity is wide open and booming, and more findings on B cells' function are sure to be revealed.

9.2.2 B Cells with Pro-tumor or Unknown Function

Regulatory B cells expressing IgA and IL-10 were discovered in certain mouse cancer models. Liver-resident cells producing IgA, expressing IL-10, and PD-L1 directly suppressed CD8^{+} T cell activity [61]. Plasmocytes accumulation in this model depended on PD-L1-PD1 interaction, indicating that follicular T cells were involved, probably in TLS GC. B cells expressing IgA, IL-10, PD-L1, and FasL have been shown to suppress antitumor immunity induced by the chemotherapy drug oxaliplatin in mouse prostate cancer models with large tumors, and removal of these cells restores oxaliplatin's activity [62].

B cells accumulated in tumor-draining lymph nodes in a mouse breast cancer model and facilitated tumor metastasis to the lymph nodes. In this spontaneous metastasis model, these B cells produced IgG specifically targeting glycosylated membrane protein HSPA4. This IgG bound to HSPA4 and activated the HSPA4-binding protein ITGB5, which in turn evoked downstream Src/NF-κB pathway in tumor cells, promoting CXCR4/SDF1α-axis-mediated metastasis. High serum anti-HSPA4 IgG correlated with high tumor HSPA4 expression and poor prognosis of breast cancer subjects [63].

How B cell populations affected antitumor immunity varies in different types of cancers. Analysis of the RNA sequencing data from The Cancer Genome Atlas database revealed that gene expression signatures of B cells correlated with good prognosis in melanoma, lung adenocarcinoma, pancreatic adenocarcinoma, and head

and neck squamous cell carcinoma patients, but with poorer survival in renal tumor patients [64]. A recent review on B cells, plasma cells, and cancer was published by Sharonov et al. and provided a comprehensive summary of B cell involvement in cancers [65].

In the complex tumor microenvironment (TME), B cell populations with unknown functions have been discovered. Mature $CD27^{-}IgG^{+}$ memory B cells were found in human ovarian and liver cancer samples, expressing antigen-presenting cells surface markers (MHC Class II, CD40, CD80, and CD86), and cooperated or co-localized with $CD8^{+}$ T cells [66, 67]). Circulating memory B cells significantly increased in breast cancer patients [68], with unknown prognostic significance. In glioblastoma, lymphocytes with both T and B markers were detected [69], corroborating that the tumor microenvironment promoted aberrant immune cell development.

9.3 Fatty Acid Oxidation in Germinal Center B Cells

Just as our understanding of B cells' function in tumors is limited, our knowledge of their lipid metabolism is even scarce. Lipid metabolism in tumor-related B cells is an ongoing research topic with few published studies. To provide the readers with some clues in this subject, here we summarized mainly discoveries on normal B cell lipid metabolism, focusing on fatty acid oxidation in germinal center B cells. Cautions shall be taken when postulates from observations made in normal B cells, as tumor microenvironment poses unique challenges for B cells, including but not limited to hypoxia, possible acidosis, limited nutrients, etc. [70].

In healthy people, B cells can be activated in the secondary lymphoid organs including the spleen, lymph node, Peyer's patches, mucosal-associated lymphoid tissue, etc. In these organs, B cells are activated upon antigen binding in the primary follicles; start to proliferate, forming a secondary follicle; and then become a GC.

Quiescent B cells (Naive B, memory B, and long-lived plasma B cells) have a low energy demand and mainly adopt OXPHOS. Once activated, B cells start proliferation and greatly increase their energy demand. Glucose uptake is increased and mainly used for the synthesis of ribonucleotides. Glutamine contributes to the TCA cycle and subsequently provides building blocks by connecting to the pentose phosphate pathway. An earlier study has discovered that in the terminal differentiation phase, murine B cells express CD36, a fatty acid importer under control of the transcription factor Oct2, indicating the importing of fatty acids at the final stage of B cell differentiation [71].

The GC is a microstructure found in all secondary lymphoid organs, composed of the light zone and dark zone. In the light zone, B cells encounter the follicular dendritic cells that capture immune-complex associated antigen and compete for antigen stimulation based on their BCR affinity, followed by a competition for a limited pool of follicular T cells. B cells successfully passing the light zone selection move into the dark zone, proliferate, and induce the enzyme activation-induced cytosine deaminase (AID), and the BCR locus undergoes a high rate of somatic mutation (SHM). B cell clones with a high affinity for antigens emerge and further mature into plasmablasts or memory B cells.

Previously GC B cells have been thought to mainly adopt glycolysis pathway to fulfill their energy needs; however, a recent discovery identified fatty acids as the major fuel for GC B cells [72]. GC B cells adopted fatty acid oxidation for energy and minimally glucose uptake compared to activated splenic B cells, GC follicular T cells, and activated $CD4^{+}$ T cells. When palmitate was supplied in the culture medium, GC B cells produced a large amount of acetyl-CoA with little lactate. Cancer GC B cells are associated with better prognosis and immunotherapy success, and whether they rely on fatty acid oxidation warrants further investigation.

As introduced in Sect. 10.1, GC can be divided into the more hypoxia light zone and the less hypoxia dark zone. It's reported that FOXO1

plays a critical role in the formation and/or maintenance of the dark zone where B cells proliferate and undergo somatic hypermutation [2]. FOXO1 promotes fatty acid oxidation in cells, and whether FOXO1 also exerts similar effects in GC B cells warrants further investigation.

9.4 Factors that May Affect Tumor-Associated B Cell Lipid Metabolism

B cells adapt to the environment by responding to various factors, such as direct interaction with other immune cells, cytokines, hypoxia, and signaling molecules like oxysterols, just to name a few. Tumor microenvironment challenges B cells to differentiate and function normally: hypoxia and local deprivation of nutrients like glutamine, glucose, tryptophan, arginine, etc. could interfere with B cell maturation.

9.4.1 Hypoxia

Hypoxic gradients in GC are important for normal B cell maturation, and accumulating evidence indicate the Goldilocks conditions applied to GC B cell requirement of hypoxia: the hypoxia gradient in the GC has to be "just right" for successful B cell maturation. The main hypoxia sensors in the cell are transcription factors named hypoxia-inducible factors (HIFs). HIFs regulate multiple cellular pathways including cellular metabolisms to adapt to hypoxia stress. It's known that consistent HIF1α stabilization by B cell-specific VHL deletion results in B cell proliferation, decreased antigen-specific GC B cells, and impaired the generation of high-affinity IgG antibodies [73]. Whether/how the "right" GC hypoxia gradient is achieved in the tumor microenvironment is unknown.

HIF1α is known to induce glycolysis; increase fatty acid uptake, lipogenesis, and storage; and reduce its oxidation in cells. In the hypoxia tumor microenvironment, sustained activation of HIF1α in cancer cells inhibits fatty acid oxidation [74]. It has been reported that GC B cells increase glycolysis and mitochondria biogenesis via HIF and GSK3B, respectively [32]. The very recent discovery of fatty acid oxidation as the major energy fuel in GC B cells indicated that other transcription factors regulated the metabolic reprogramming. Both FOXO1 and Bcl6 could regulate this metabolic reprogramming, for FOXO1 is known to activate fatty acid oxidation, while Bcl6 is known to repress glycolysis in other cell types [30].

9.4.2 Nutrients Competition

Fatty acids in the tumor microenvironment are taken up by cells expressing fatty acid importers, for example, B cells and cancer cells with metastasis potential. Metabolic symbiosis exists in colon-rectal cancer regarding fatty acids: cancer-associated fibroblast stock up fatty acids and release them into the extracellular space, which are then taken up by cancer cells [75]. For GC B cells, a possible source of fatty acids is for the B cells, which undergo apoptosis due to insufficient receptor affinity. The relatively enclosed GC environment might provide some insulation for GC B cells against the metabolic competition in the tumor microenvironment. In contrast, tumor infiltrated T cells could face bigger metabolic challenges as activated T cells rely on glycolysis and must compete for glucose in the tumor microenvironment.

As an integral part of the metabolism network, fatty acid oxidation is affected by other metabolism pathways, and one of them is glutamine deficiency. Regional glutamine deficiency often occurs in tumor core and leads to a lack of α-ketoglutarate, which in turn leads to hypermethylation of histones in cancer cells, because histone demethylase JMJD3 requires α-ketoglutarate as a cofactor for removing methyl groups on H3k27 [76]. Whether this glutamine deficiency impairs the fatty acid oxidation in B cells is unknown.

9.5 Lipid Metabolites that Affect B Cell Function

9.5.1 Cholesterol

Cholesterol is synthesized in the liver and transported to other tissues as low-density lipoprotein, taken up by the cells via lipoprotein receptors. Cholesterol can be synthesized from HMG-CoA derived from acetyl-CoA, via the cholesterol biosynthesis pathway. Mevalonate is synthesized from HMG-CoA, and then farnesyl pyrophosphate (FPP) is synthesized and further converted into different signaling metabolites: (1) FPP is converted into geranylgeranyl pyrophosphate (GGPP), which prenylates important protein targets including small GTPase Rac, Rho, Rab, etc. (2) FPP is further metabolized to cholesterol and eventually generates either steroids or oxysterols, both are important signaling molecules. A summary of cholesterol metabolism and related B cell functions is shown in Fig. 9.3.

9.5.2 Geranylgeranyl Pyrophosphate

GGPP regulates cellular processes via posttranslational modification of important protein targets in B cells. GGPP drives the IL-10 production of regulatory B cells via PI3K-Akt signaling, revealing the critical roles played by cholesterol metabolism in regulatory B cells [77]. On the contrary, in autoimmunity-related disease and graft-versus-host disease, GGPP is important for CD40-mediated B cell activation [78]. How GGPP regulates tumor-associated B cells is an intriguing question, especially when considering potential cancer therapy with mevalonate pathway inhibition.

Mevalonate pathway is an important cancer therapy target as cancer cells rely on it for survival (reviewed by [79, 80]). Very recently, it's found that PTEN mutates cancer cells and *t*(4;14)-positive multiple myeloma cells generate GGPP via the mevalonate pathway, and statin kills these cells by decreasing GGPP [81, 82].

While statin starves cancer cells by decreasing GGPP, it also interferes with B cell activation [83]. Given that B cell expresses increased cholesterol biosynthesis enzymes upon activation by CD40-CD40L, and the important role of GGPP in CD40-mediated cell activation, statin's inhibitory effects in B cells might not be a direct consequence of reduced cellular cholesterol level, but of reduced GGPP. Treatment schemes have to be carefully designed to avoid statins' immune-suppressive effects when combining statin and immunotherapy in cancer treatment.

9.5.3 Oxysterol

Oxysterols are oxygenated derivatives of cholesterol and can be generated by cholesterol metabolism pathway or ingested from the diet. Oxysterols affect many cellular functions by binding to different proteins such as liver X receptors, oxysterol-binding proteins, ATP-binding cassette, etc. Accumulating evidence suggests that oxysterols play roles in cancers, including breast, prostate, colon, and bile duct cancers, which is nicely reviewed by Kloudova et al. [84].

Two oxysterols, 7α,25-dihydroxycholesterol (7a,25-HC) and 7α,27-dihydroxycholesterol (7a,27-HC), are ligands for a G protein-coupled receptor EBI2, also named as GPR183. Various immune cells express EBI2, including B cells. Oxysterols direct B cell migration via binding to EBI2 [85], and the oxysterol gradient generated by lymphoid stromal cells guides activated B cell migration [86]. EBI2 and 7α,25-HC deficiency both cause defective antibody responses. The function of the oxysterol-EBI2 axis in immune cells is comprehensively reviewed by Cyster et al. [87].

Recently, it's found that the EBI2-oxysterol axis promotes the development of intestinal lymphoid structures and colitis [88]. Since TLS resemble follicles of secondary lymphoid organs,

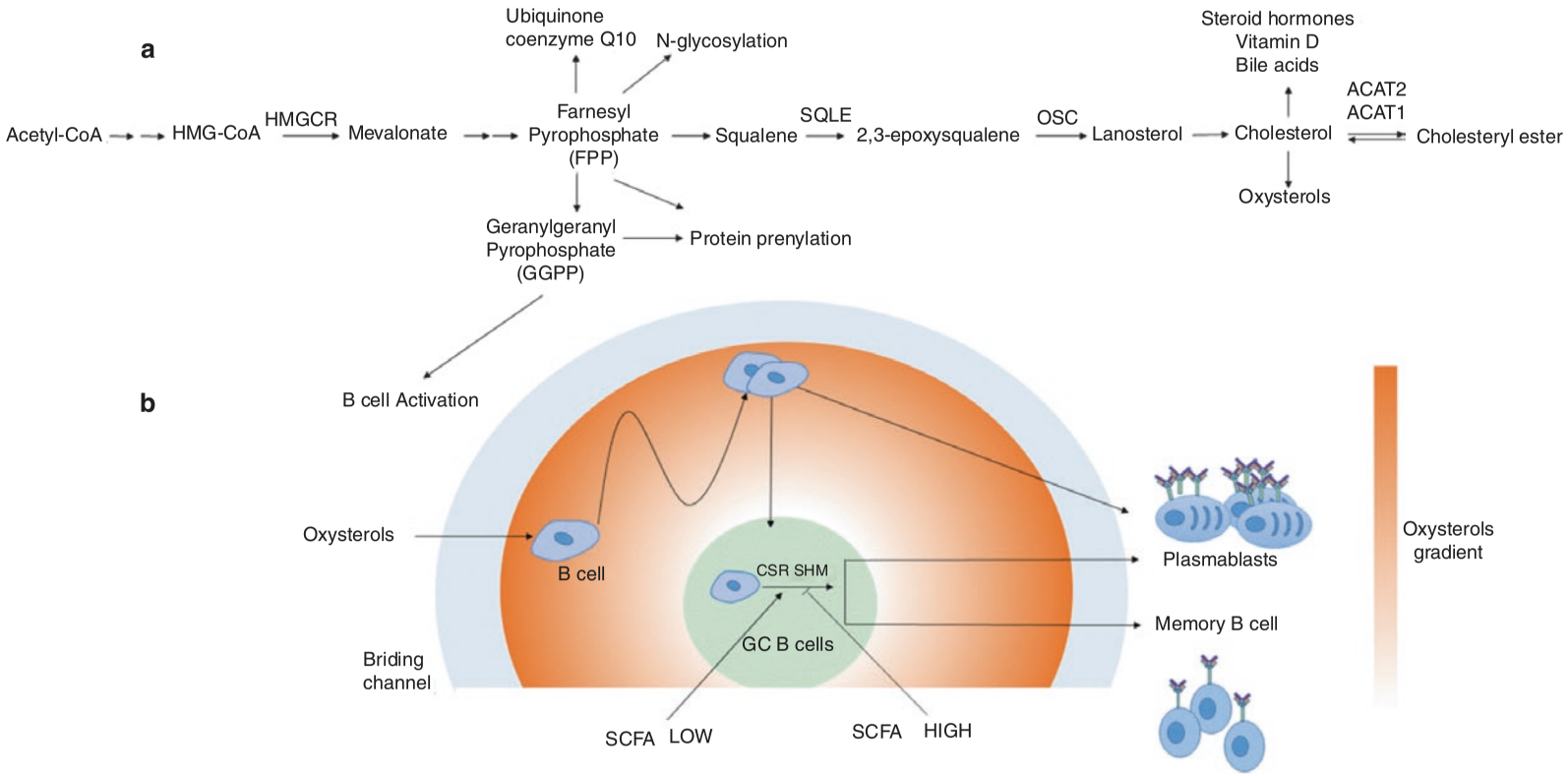

Fig. 9.3 GGPP, oxysterols, and short-chain fatty acids regulate B cell function. (**a**) Cholesterol biosynthesis and metabolism pathway generate GGPP and oxysterols. (**b**) A schematic of B cell regulation by GGPP, oxysterols, and short-chain fatty acids. GGPP regulates B cell activation; oxysterol gradient in TLS regulates B cell migration; short-chain fatty acids regulate GC B cell maturation: low dosage moderately enhances CSR, while high dosage reduces CSR, SHM, and plasma cell differentiation

the control of TLS development in cancer could be affected by oxysterols, too.

9.5.4 Short-Chain Fatty Acids

Butyrate and propionate are short-chain fatty acids generated by gut microbiota when fermenting dietary fibers, and both serve as histone deacetylase inhibitors. Previously it's reported that short-chain fatty acids increase acetyl-CoA production, glycolysis, mitochondrial respiration, and the production of lipid droplets in primary mouse B cells, indicating that these metabolic changes may aid in antibody production [89]. Recently, it's reported that by acting as HDAC inhibitors, butyrate and propionate can enhance or impair B cell antibody responses [90] in human and mouse B cells, depending on the doses. Low-dosage short-chain fatty acids moderately enhance class-switch DNA recombination (CSR), while higher doses of SCFAs decrease AID and Blimp1 expression, CSR, somatic hypermutation, and plasma cell differentiation.

9.6 Future Directions

Contrary to T cells, researches on the function and metabolisms of tumor-associated B cells only now start to gather momentum. Many questions remain open. What are the regulatory mechanisms controlling GC initiation/development in the tumor? How does the immune checkpoint blockade therapy affect tumor-associated B cells in metabolism reprogramming? In the often nutrient-depleted tumor microenvironment, how does TLS secure enough substrate to sustain its energy symbiosis? Do tumor-associated GC B cells utilize fatty acid oxidation to fulfill their energy needs? If so, how does it maintain redox balance and prevent lipid peroxidation/ferroptosis?

Technology advances have paved the way for answering these questions. Single-cell mass spectrometry (SCMS) can be applied for detecting lipid metabolites in a minimal amount of tissue/cell samples. For measuring metabolites in tumor-associated B cells, it's crucial to main the target cells in its native environment, i.e., tumor-associated GC/TLS, and quenching of the fresh cancer samples followed by mass imaging is an option. For peripheral B cells in cancer patients, MS methods established for circulating tumor cells shall be easily adopted [91]. Emphasis should be put on the metabolic symbiosis of the tumor-associated GC/TLS, as these microstructures determine immunotherapy success.

References

1. Melchers F. Checkpoints that control B cell development. J Clin Invest. 2015;125:2203–10.
2. Sander S, Chu VT, Yasuda T, Franklin A, Graf R, Calado DP, Li S, Imami K, Selbach M, Di Virgilio M, Bullinger L, Rajewsky K. PI3 kinase and FOXO1 transcription factor activity differentially control B cells in the germinal center light and dark zones. Immunity. 2015;43:1075–86.
3. Victora GD, Nussenzweig MC. Germinal centers. Annu Rev Immunol. 2012;30:429–57.
4. Aronov M, Tirosh B. Metabolic control of plasma cell differentiation—what we know and what we don't know. J Clin Immunol. 2016;36(Suppl 1):12–7.
5. Li B, Pan F. Immune metabolism in health and tumor. Cham: Springer; 2017.
6. Caro-Maldonado A, Wang R, Nichols AG, Kuraoka M, Milasta S, Sun LD, Gavin AL, Abel ED, Kelsoe G, Green DR, Rathmell JC. Metabolic reprogramming is required for antibody production that is suppressed in anergic but exaggerated in chronically BAFF-exposed B cells. J Immunol. 2014;192:3626–36.
7. Doughty CA, Bleiman BF, Wagner DJ, Dufort FJ, Mataraza JM, Roberts MF, Chiles TC. Antigen receptor-mediated changes in glucose metabolism in B lymphocytes: role of phosphatidylinositol 3-kinase signaling in the glycolytic control of growth. Blood. 2006;107:4458–65.
8. Woodland RT, Fox CJ, Schmidt MR, Hammerman PS, Opferman JT, Korsmeyer SJ, Hilbert DM, Thompson CB. Multiple signaling pathways promote B lymphocyte stimulator dependent B-cell growth and survival. Blood. 2008;111:750–60.
9. Cho SH, Ahn AK, Bhargava P, Lee CH, Eischen CM, McGuinness O, Boothby M. Glycolytic rate and lymphomagenesis depend on PARP14, an ADP ribosyltransferase of the B aggressive lymphoma (BAL) family. Proc Natl Acad Sci U S A. 2011;108:15972–7.

10. Dufort FJ, Bleiman BF, Gumina MR, Blair D, Wagner DJ, Roberts MF, Abu-Amer Y, Chiles TC. Cutting edge: IL-4-mediated protection of primary B lymphocytes from apoptosis via Stat6-dependent regulation of glycolytic metabolism. J Immunol. 2007;179:4953–7.
11. Dufort FJ, Gumina MR, Ta NL, Tao Y, Heyse SA, Scott DA, Richardson AD, Seyfried TN, Chiles TC. Glucose-dependent de novo lipogenesis in B lymphocytes: a requirement for atp-citrate lyase in lipopolysaccharide-induced differentiation. J Biol Chem. 2014;289:7011–24.
12. Locasale JW. Serine, glycine and one-carbon units: cancer metabolism in full circle. Nat Rev Cancer. 2013;13:572–83.
13. Pearce EL, Poffenberger MC, Chang CH, Jones RG. Fueling immunity: insights into metabolism and lymphocyte function. Science. 2013;342:1242454.
14. Clarke AJ, Riffelmacher T, Braas D, Cornall RJ, Simon AK. B1a B cells require autophagy for metabolic homeostasis and self-renewal. J Exp Med. 2018;215:399–413.
15. Krop I, de Fougerolles AR, Hardy RR, Allison M, Schlissel MS, Fearon DT. Self-renewal of B-1 lymphocytes is dependent on CD19. Eur J Immunol. 1996;26:238–42.
16. Barber CL, Montecino-Rodriguez E, Dorshkind K. Reduced production of B-1-specified common lymphoid progenitors results in diminished potential of adult marrow to generate B-1 cells. Proc Natl Acad Sci U S A. 2011;108:13700–4.
17. Heinzel S, Binh Giang T, Kan A, Marchingo JM, Lye BK, Corcoran LM, Hodgkin PD. A Myc-dependent division timer complements a cell-death timer to regulate T cell and B cell responses. Nat Immunol. 2017;18:96–103.
18. de Alboran IM, O'Hagan RC, Gartner F, Malynn B, Davidson L, Rickert R, Rajewsky K, DePinho RA, Alt FW. Analysis of C-MYC function in normal cells via conditional gene-targeted mutation. Immunity. 2001;14:45–55.
19. Stine ZE, Walton ZE, Altman BJ, Hsieh AL, Dang CV. MYC, metabolism, and cancer. Cancer Discov. 2015;5:1024–39.
20. Calado DP, Sasaki Y, Godinho SA, Pellerin A, Kochert K, Sleckman BP, de Alboran IM, Janz M, Rodig S, Rajewsky K. The cell-cycle regulator c-Myc is essential for the formation and maintenance of germinal centers. Nat Immunol. 2012;13:1092–100.
21. Allen CD, Okada T, Tang HL, Cyster JG. Imaging of germinal center selection events during affinity maturation. Science. 2007;315:528–31.
22. Dominguez-Sola D, Victora GD, Ying CY, Phan RT, Saito M, Nussenzweig MC, Dalla-Favera R. The proto-oncogene MYC is required for selection in the germinal center and cyclic reentry. Nat Immunol. 2012;13:1083–91.
23. Chou C, Verbaro DJ, Tonc E, Holmgren M, Cella M, Colonna M, Bhattacharya D, Egawa T. The transcription factor AP4 mediates resolution of chronic viral infection through amplification of germinal center B cell responses. Immunity. 2016;45:570–82.
24. Franchina DG, Grusdat M, Brenner D. B-cell metabolic remodeling and cancer. Trends Cancer. 2018;4:138–50.
25. Mambetsariev N, Lin WW, Wallis AM, Stunz LL, Bishop GA. TRAF3 deficiency promotes metabolic reprogramming in B cells. Sci Rep. 2016;6:35349.
26. Xiao G, Fong A, Sun SC. Induction of p100 processing by NF-kappaB-inducing kinase involves docking IkappaB kinase alpha (IKKalpha) to p100 and IKKalpha-mediated phosphorylation. J Biol Chem. 2004;279:30099–105.
27. Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, Verma NK, Smyth MJ, Rigby RJ, Vinuesa CG. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med. 2010;207:353–63.
28. Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, Kallies A, Corcoran LM, Godfrey DI, Toellner KM, Smyth MJ, Nutt SL, Tarlinton DM. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med. 2010;207:365–78.
29. Cannons JL, Qi H, Lu KT, Dutta M, Gomez-Rodriguez J, Cheng J, Wakeland EK, Germain RN, Schwartzberg PL. Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84. Immunity. 2010;32:253–65.
30. Oestreich KJ, Read KA, Gilbertson SE, Hough KP, McDonald PW, Krishnamoorthy V, Weinmann AS. Bcl-6 directly represses the gene program of the glycolysis pathway. Nat Immunol. 2014;15:957–64.
31. Shaffer AL, Yu X, He Y, Boldrick J, Chan EP, Staudt LM. BCL-6 represses genes that function in lymphocyte differentiation, inflammation, and cell cycle control. Immunity. 2000;13:199–212.
32. Abbott RK, Thayer M, Labuda J, Silva M, Philbrook P, Cain DW, Kojima H, Hatfield S, Sethumadhavan S, Ohta A, Reinherz EL, Kelsoe G, Sitkovsky M. Germinal center hypoxia potentiates immunoglobulin class switch recombination. J Immunol. 2016;197:4014–20.
33. Semenza GL, Roth PH, Fang HM, Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J Biol Chem. 1994;269:23757–63.
34. Gordan JD, Thompson CB, Simon MC. HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. Cancer Cell. 2007;12:108–13.
35. Zhang H, Gao P, Fukuda R, Kumar G, Krishnamachary B, Zeller KI, Dang CV, Semenza GL. HIF-1 inhibits mitochondrial biogenesis and cellular respiration in VHL-deficient renal cell carcinoma by repression of C-MYC activity. Cancer Cell. 2007;11:407–20.
36. Li F, Wang Y, Zeller KI, Potter JJ, Wonsey DR, O'Donnell KA, Kim JW, Yustein JT, Lee LA, Dang CV. Myc stimulates nuclearly encoded mitochondrial

genes and mitochondrial biogenesis. Mol Cell Biol. 2005;25:6225–34.
37. Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab. 2006;3:177–85.
38. Kojima H, Gu H, Nomura S, Caldwell CC, Kobata T, Carmeliet P, Semenza GL, Sitkovsky MV. Abnormal B lymphocyte development and autoimmunity in hypoxia-inducible factor 1alpha -deficient chimeric mice. Proc Natl Acad Sci U S A. 2002;99:2170–4.
39. Kojima H, Sitkovsky MV, Cascalho M. HIF-1 alpha deficiency perturbs T and B cell functions. Curr Pharm Des. 2003;9:1827–32.
40. Kojima H, Kobayashi A, Sakurai D, Kanno Y, Hase H, Takahashi R, Totsuka Y, Semenza GL, Sitkovsky MV, Kobata T. Differentiation stage-specific requirement in hypoxia-inducible factor-1alpha-regulated glycolytic pathway during murine B cell development in bone marrow. J Immunol. 2010;184:154–63.
41. Jellusova J, Cato MH, Apgar JR, Ramezani-Rad P, Leung CR, Chen C, Richardson AD, Conner EM, Benschop RJ, Woodgett JR, Rickert RC. Gsk3 is a metabolic checkpoint regulator in B cells. Nat Immunol. 2017;18:303–12.
42. Man K, Miasari M, Shi W, Xin A, Henstridge DC, Preston S, Pellegrini M, Belz GT, Smyth GK, Febbraio MA, Nutt SL, Kallies A. The transcription factor IRF4 is essential for TCR affinity-mediated metabolic programming and clonal expansion of T cells. Nat Immunol. 2013;14:1155–65.
43. De Silva NS, Klein U. Dynamics of B cells in germinal centres. Nat Rev Immunol. 2015;15:137–48.
44. Lin WH, Adams WC, Nish SA, Chen YH, Yen B, Rothman NJ, Kratchmarov R, Okada T, Klein U, Reiner SL. Asymmetric PI3K signaling driving developmental and regenerative cell fate bifurcation. Cell Rep. 2015;13:2203–18.
45. Grumont RJ, Gerondakis S. Rel induces interferon regulatory factor 4 (IRF-4) expression in lymphocytes: modulation of interferon-regulated gene expression by rel/nuclear factor kappaB. J Exp Med. 2000;191:1281–92.
46. Heise N, De Silva NS, Silva K, Carette A, Simonetti G, Pasparakis M, Klein U. Germinal center B cell maintenance and differentiation are controlled by distinct NF-kappaB transcription factor subunits. J Exp Med. 2014;211:2103–18.
47. Basso K, Klein U, Niu H, Stolovitzky GA, Tu Y, Califano A, Cattoretti G, Dalla-Favera R. Tracking CD40 signaling during germinal center development. Blood. 2004;104:4088–96.
48. Recaldin T, Fear DJ. Transcription factors regulating B cell fate in the germinal centre. Clin Exp Immunol. 2016;183:65–75.
49. Gloury R, Zotos D, Zuidscherwoude M, Masson F, Liao Y, Hasbold J, Corcoran LM, Hodgkin PD, Belz GT, Shi W, Nutt SL, Tarlinton DM, Kallies A. Dynamic changes in Id3 and E-protein activity orchestrate germinal center and plasma cell development. J Exp Med. 2016;213:1095–111.
50. Wohner M, Tagoh H, Bilic I, Jaritz M, Poliakova DK, Fischer M, Busslinger M. Molecular functions of the transcription factors E2A and E2-2 in controlling germinal center B cell and plasma cell development. J Exp Med. 2016;213:1201–21.
51. Chan LN, Chen Z, Braas D, Lee JW, Xiao G, Geng H, Cosgun KN, Hurtz C, Shojaee S, Cazzaniga V, Schjerven H, Ernst T, Hochhaus A, Kornblau SM, Konopleva M, Pufall MA, Cazzaniga G, Liu GJ, Milne TA, Koeffler HP, Ross TS, Sanchez-Garcia I, Borkhardt A, Yamamoto KR, Dickins RA, Graeber TG, Muschen M. Metabolic gatekeeper function of B-lymphoid transcription factors. Nature. 2017;542:479–83.
52. Coronella JA, Telleman P, Kingsbury GA, Truong TD, Hays S, Junghans RP. Evidence for an antigen-driven humoral immune response in medullary ductal breast cancer. Cancer Res. 2001;61:7889–99.
53. Sautes-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer. 2019;19:307–25.
54. Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, Johansson I, Phung B, Harbst K, Vallon-Christersson J, van Schoiack A, Lovgren K, Warren S, Jirstrom K, Olsson H, Pietras K, Ingvar C, Isaksson K, Schadendorf D, Schmidt H, Bastholt L, Carneiro A, Wargo JA, Svane IM, Jonsson G. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature. 2020;577:561–5.
55. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, Yizhak K, Sade-Feldman M, Blando J, Han G, Gopalakrishnan V, Xi Y, Zhao H, Amaria RN, Tawbi HA, Cogdill AP, Liu W, LeBleu VS, Kugeratski FG, Patel S, Davies MA, Hwu P, Lee JE, Gershenwald JE, Lucci A, Arora R, Woodman S, Keung EZ, Gaudreau PO, Reuben A, Spencer CN, Burton EM, Haydu LE, Lazar AJ, Zapassodi R, Hudgens CW, Ledesma DA, Ong S, Bailey M, Warren S, Rao D, Krijgsman O, Rozeman EA, Peeper D, Blank CU, Schumacher TN, Butterfield LH, Zelazowska MA, McBride KM, Kalluri R, Allison J, Petitprez F, Fridman WH, Sautes-Fridman C, Hacohen N, Rezvani K, Sharma P, Tetzlaff MT, Wang L, Wargo JA. B cells and tertiary lymphoid structures promote immunotherapy response. Nature. 2020;577:549–55.
56. Petitprez F, de Reynies A, Keung EZ, Chen TW, Sun CM, Calderaro J, Jeng YM, Hsiao LP, Lacroix L, Bougouin A, Moreira M, Lacroix G, Natario I, Adam J, Lucchesi C, Laizet YH, Toulmonde M, Burgess MA, Bolejack V, Reinke D, Wani KM, Wang WL, Lazar AJ, Roland CL, Wargo JA, Italiano A, Sautes-Fridman C, Tawbi HA, Fridman WH. B cells are associated with survival and immunotherapy response in sarcoma. Nature. 2020;577:556–60.
57. DeFalco J, Harbell M, Manning-Bog A, Baia G, Scholz A, Millare B, Sumi M, Zhang D, Chu F, Dowd C, Zuno-Mitchell P, Kim D, Leung Y, Jiang S, Tang X,

Williamson KS, Chen X, Carroll SM, Espiritu Santo G, Haaser N, Nguyen N, Giladi E, Minor D, Tan YC, Sokolove JB, Steinman L, Serafini TA, Cavet G, Greenberg NM, Glanville J, Volkmuth W, Emerling DE, Robinson WH. Non-progressing cancer patients have persistent B cell responses expressing shared antibody paratopes that target public tumor antigens. Clin Immunol. 2018;187:37–45.

58. Haro MA, Dyevoich AM, Phipps JP, Haas KM. Activation of B-1 cells promotes tumor cell killing in the peritoneal cavity. Cancer Res. 2019;79:159–70.
59. Lu Y, Zhao Q, Liao JY, Song E, Xia Q, Pan J, Li Y, Li J, Zhou B, Ye Y, Di C, Yu S, Zeng Y, Su S. Complement signals determine opposite effects of B cells in chemotherapy-induced immunity. Cell. 2020;180:1081–1097.e1024.
60. Liu D, Xu H, Shih C, Wan Z, Ma X, Ma W, Luo D, Qi H. T-B-cell entanglement and ICOSL-driven feed-forward regulation of germinal centre reaction. Nature. 2015;517:214–8.
61. Shalapour S, Lin XJ, Bastian IN, Brain J, Burt AD, Aksenov AA, Vrbanac AF, Li W, Perkins A, Matsutani T, Zhong Z, Dhar D, Navas-Molina JA, Xu J, Loomba R, Downes M, Yu RT, Evans RM, Dorrestein PC, Knight R, Benner C, Anstee QM, Karin M. Inflammation-induced IgA+ cells dismantle anti-liver cancer immunity. Nature. 2017;551:340–5.
62. Shalapour S, Font-Burgada J, Di Caro G, Zhong Z, Sanchez-Lopez E, Dhar D, Willimsky G, Ammirante M, Strasner A, Hansel DE, Jamieson C, Kane CJ, Klatte T, Birner P, Kenner L, Karin M. Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. Nature. 2015;521:94–8.
63. Gu Y, Liu Y, Fu L, Zhai L, Zhu J, Han Y, Jiang Y, Zhang Y, Zhang P, Jiang Z, Zhang X, Cao X. Tumor-educated B cells selectively promote breast cancer lymph node metastasis by HSPA4-targeting IgG. Nat Med. 2019;25:312–22.
64. Iglesia MD, Parker JS, Hoadley KA, Serody JS, Perou CM, Vincent BG. Genomic analysis of immune cell infiltrates across 11 tumor types. J Natl Cancer Inst. 2016;108:djw144.
65. Sharonov GV, Serebrovskaya EO, Yuzhakova DV, Britanova OV, Chudakov DM. B cells, plasma cells and antibody repertoires in the tumour microenvironment. Nat Rev Immunol. 2020;20:294–307.
66. Shi JY, Gao Q, Wang ZC, Zhou J, Wang XY, Min ZH, Shi YH, Shi GM, Ding ZB, Ke AW, Dai Z, Qiu SJ, Song K, Fan J. Margin-infiltrating CD20(+) B cells display an atypical memory phenotype and correlate with favorable prognosis in hepatocellular carcinoma. Clin Cancer Res. 2013;19:5994–6005.
67. Nielsen JS, Sahota RA, Milne K, Kost SE, Nesslinger NJ, Watson PH, Nelson BH. CD20+ tumor-infiltrating lymphocytes have an atypical CD27-memory phenotype and together with CD8+ T cells promote favorable prognosis in ovarian cancer. Clin Cancer Res. 2012;18:3281–92.
68. Tsuda B, Miyamoto A, Yokoyama K, Ogiya R, Oshitanai R, Terao M, Morioka T, Niikura N, Okamura T, Miyako H, Saito Y, Suzuki Y, Kametani Y, Tokuda Y. B-cell populations are expanded in breast cancer patients compared with healthy controls. Breast Cancer. 2018;25:284–91.
69. Alban TJ, Alvarado AG, Sorensen MD, Bayik D, Volovetz J, Serbinowski E, Mulkearns-Hubert EE, Sinyuk M, Hale JS, Onzi GR, McGraw M, Huang P, Grabowski MM, Wathen CA, Ahluwalia MS, Radivoyevitch T, Kornblum HI, Kristensen BW, Vogelbaum MA, Lathia JD. Global immune fingerprinting in glioblastoma patient peripheral blood reveals immune-suppression signatures associated with prognosis. JCI Insight. 2018;3(21):e122264.
70. Balkwill FR, Capasso M, Hagemann T. The tumor microenvironment at a glance. J Cell Sci. 2012;125:5591–6.
71. Konig H, Pfisterer P, Corcoran LM, Wirth T. Identification of CD36 as the first gene dependent on the B-cell differentiation factor Oct-2. Genes Dev. 1995;9:1598–607.
72. Pasare C, and Medzhitov R. Control of B-cell responses by toll-like receptors. Nature. 2005; 438(7066):364–8.
73. Cho SH, Raybuck AL, Stengel K, Wei M, Beck TC, Volanakis E, Thomas JW, Hiebert S, Haase VH, Boothby MR. Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system. Nature. 2016;537:234–8.
74. Huang, Li T, Li X, Zhang L, Sun L, He X, Zhong X, Jia D, Song L, Semenza GL, Gao P, Zhang H. HIF-1-mediated suppression of acyl-CoA dehydrogenases and fatty acid oxidation is critical for cancer progression. Cell Rep. 2014;8:1930–42.
75. Gong J, Lin Y, Zhang H, Liu C, Cheng Z, Yang X, Zhang J, Xiao Y, Sang N, Qian X, Wang L, Cen X, Du X, Zhao Y. Reprogramming of lipid metabolism in cancer-associated fibroblasts potentiates migration of colorectal cancer cells. Cell Death Dis. 2020;11:267.
76. Seok S, Kim YC, Byun S, Choi S, Xiao Z, Iwamori N, Zhang Y, Wang C, Ma J, Ge K, Kemper B, Kemper JK. Fasting-induced JMJD3 histone demethylase epigenetically activates mitochondrial fatty acid beta-oxidation. J Clin Invest. 2018;128:3144–59.
77. Bibby JA, Purvis HA, Hayday T, Chandra A, Okkenhaug K, Wood M, Lachmann HJ, Kemper C, Cope AP, Perucha E. Cholesterol metabolism drives regulatory B cell function. bioRxiv. 2020; https://doi.org/10.1101/2020.01.03.893982.
78. Shimabukuro-Vornhagen A, Zoghi S, Liebig TM, Wennhold K, Chemitz J, Draube A, Kochanek M, Blaschke F, Pallasch C, Holtick U, Scheid C, Theurich S, Hallek M, von Bergwelt-Baildon MS. Inhibition of protein geranylgeranylation specifically interferes with CD40-dependent B cell activation, resulting in a reduced capacity to induce T cell immunity. J Immunol. 2014;193:5294–305.

79. Swanson KM, Hohl RJ. Anti-cancer therapy: targeting the mevalonate pathway. Curr Cancer Drug Targets. 2006;6:15–37.
80. Bathaie SZ, Ashrafi M, Azizian M, Tamanoi F. Mevalonate pathway and human cancers. Curr Mol Pharmacol. 2017;10:77–85.
81. Jiao Z, Cai H, Long Y, Sirka OK, Padmanaban V, Ewald AJ, Devreotes PN. Statin-induced GGPP depletion blocks macropinocytosis and starves cells with oncogenic defects. Proc Natl Acad Sci U S A. 2020;117:4158–68.
82. Longo J, Smirnov P, Li Z, Branchard E, van Leeuwen JE, Licht JD, Haibe-Kains B, Andrews DW, Keats JJ, Pugh TJ, Trudel S, Penn LZ. The mevalonate pathway is an actionable vulnerability of t(4;14)-positive multiple myeloma. Leukemia. 2020.
83. Shimabukuro-Vornhagen A, Zoghi S, Liebig TM, Wennhold K, Chemitz J, Draube A, Kochanek M, Blaschke F, Pallasch C, Holtick U, Scheid C, Theurich S, Hallek M, von Bergwelt-Baildon MS. Inhibition of protein geranylgeranylation specifically interferes with CD40-dependent B cell activation, resulting in a reduced capacity to induce T cell immunity. J Immunol. 2014;193(10):5294–305.
84. Kloudova A, Guengerich FP, Soucek P. The role of oxysterols in human cancer. Trends Endocrinol Metab. 2017;28:485–96.
85. Liu C, Yang XV, Wu J, Kuei C, Mani NS, Zhang L, Yu J, Sutton SW, Qin N, Banie H, Karlsson L, Sun S, Lovenberg TW. Oxysterols direct B-cell migration through EBI2. Nature. 2011;475:519–23.
86. Yi T, Wang X, Kelly LM, An J, Xu Y, Sailer AW, Gustafsson JA, Russell DW, Cyster JG. Oxysterol gradient generation by lymphoid stromal cells guides activated B cell movement during humoral responses. Immunity. 2012;37:535–48.
87. Cyster JG, Dang EV, Reboldi A, Yi T. 25-hydroxycholesterols in innate and adaptive immunity. Nat Rev Immunol. 2014;14:731–43.
88. Wyss A, Raselli T, Perkins N, Ruiz F, Schmelczer G, Klinke G, Moncsek A, Roth R, Spalinger MR, Hering L, Atrott K, Lang S, Frey-Wagner I, Mertens JC, Scharl M, Sailer AW, Pabst O, Hersberger M, Pot C, Rogler G, Misselwitz B. The EBI2-oxysterol axis promotes the development of intestinal lymphoid structures and colitis. Mucosal Immunol. 2019;12:733–45.
89. Kim M, Qie Y, Park J, Kim CH. Gut microbial metabolites fuel host antibody responses. Cell Host Microbe. 2016;20:202–14.
90. Sanchez HN, Moroney JB, Gan H, Shen T, Im JL, Li T, Taylor JR, Zan H, Casali P. B cell-intrinsic epigenetic modulation of antibody responses by dietary fiber-derived short-chain fatty acids. Nat Commun. 2020;11:60.
91. Abouleila Y, Onidani K, Ali A, Shoji H, Kawai T, Lim CT, Kumar V, Okaya S, Kato K, Hiyama E, Yanagida T, Masujima T, Shimizu Y, Honda K. Live single cell mass spectrometry reveals cancer-specific metabolic profiles of circulating tumor cells. Cancer Sci. 2019;110:697–706.

Lipid Metabolism in Tumor-Infiltrating T Cells

10

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Abstract

T cells recognize "foreign" antigens and induce durable humoral and cellular immune responses, which are indispensable for defending pathogens, as well as maintaining the integrity and homeostasis of tissues and organs. T cells are the major immune cell population in the tumor microenvironment which play a critical role in the antitumor immune response and cancer immune surveillance. Defective immune response of tumor-infiltrating T cells is the main cause of cancer immune evasion. The antitumor response of T cells is affected by multiple factors in the tumor microenvironment, including immunosuppressive cells, immune inhibitory cytokines, tumor-derived suppressive signals like PD-L1, immnuogenicity of tumor cells, as well as metabolic factors like hypoxia and nutrient deprivation. Abundant studies in past decades have proved the metabolic regulations of the immune response of T cells and the tumor-infiltrating T cells. In this chapter, we will discuss the regulations of the antitumor response of tumor-infiltrating T cells by lipid metabolism, which is one of the main components of metabolic regulation.

Keywords

Tumor-infiltrating T cell · Metabolism · Lipid
Tumor microenvironment · Fatty acid

10.1 Tumor-Infiltrating T Lymphocytes

The degree of T cell infiltration varies across tumor types. Tumors mainly fall into two categories, "hot" and "cold", which is based on the presence of immune cells in them. Hot tumors have a higher density of tumor-infiltrating lymphocytes (TILs), while cold tumors are defined by the absence of TILs [1, 2]. Moreover, TILs can be further divided into multiple subsets with distinctive functions. Yost et al. have collected 33,106 TILs from the tumor samples of 11 patients with advanced basal or squamous cell carcinoma. By using droplet-based 5′ single-cell RNA-sequencing and T cell receptor (TCR)-sequencing libraries, they successfully identified nine distinct T cell clusters in the specimens. The $CD4^+$ clusters included regulatory T cells (Treg) cells, follicular helper T (T_{fh}) cells, and T helper 17 cells (Th17) cells. $CD8^+$ clusters included naive T cells, memory T cells (T_{mem}), effector memory T cells, activated cells, chronically acti-

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vated/exhausted cells, and intermediate exhausted/activated cells [3]. Here, we will review the current understanding of lipid metabolism of the different subsets of tumor-infiltrating T cells.

10.2 Roles of Lipid Metabolism in Tumor-Infiltrating T Cells

10.2.1 $CD8^+$ T Cell

$CD8^+$ T cells are one of the most important subpopulations of tumor-infiltrating T cells, including cytotoxic effector T cells (T_{eff}) and memory T cells, which both have distinct programs of lipid metabolism. Hypoxia in the tumor microenvironment (TME) induces the metabolic switch from oxidative phosphorylation to glycolysis, which consumes more carbohydrates otherwise lipids. In contrast, the differentiation and function of memory $CD8^+$ T cells are mainly relying on fatty acid oxidation and oxidative phosphorylation (OXPHOS) in mitochondria to produce energy [4].

10.2.1.1 $CD8^+$ Effector T Cell

Upon tumor antigen engagement, naive T cells are activated and differentiate into effector cells. Quiescent T cells primarily metabolize pyruvate and fatty acids (FAs) via OXPHOS in the mitochondria to produce energy (ATP). Antigen recognition by the TCR and the ligation of co-stimulatory receptors like CD28 and ICOS activate downstream signal pathways, such as phosphatidylinositol-3-OH kinase (PI3K)-protein kinase B (Akt) which correlates with metabolic regulation [5, 6]. After T cell activation, phosphorylation of Akt increases glycolysis and elevates mammalian target of rapamycin (mTOR) signaling, which is central to protein translation regulation. Therefore, the PI3K-mTOR signaling pathway has been suggested to be a regulator of the metabolism of effector T cells, which in turn shuttles carbon derived from glucose into intermediates for the synthesis of lipid and protein [7–9].

As a key metabolism regulator, mTORC1 upregulates fatty acid synthesis (FAS) by regulating the activity of sterol regulatory element-binding proteins (SREBPs) [10]. SREBP1 and SREBP2 are master transcriptional regulators of genes involved in de novo lipid and sterol biosynthesis, which are essential for meeting the heightened lipid requirements during the transition from quiescent to activated state [11]. The inactive precursors of SREBPs reside in the endoplasmic reticulum (ER), where they interact with the sterol cleavage activating protein (SCAP). The SREBP/SCAP complex is processed by proteases in the Golgi to release the N-terminal of the SREBP protein. The activated SREBP then translocates to the nucleus to regulate the expression of genes, which encode the key enzymes of fatty acid and cholesterol biosynthesis, through binding to the promoter that contains sterol regulatory elements (SRE) and E box sequences [12]. Once released from the Golgi, the active form of SREBP1 is susceptible to proteasomal degradation [13]. A recent study found that overexpression of an activated version of Akt led to a rapamycin-sensitive increase in the processed form of SREBP1. Akt induces expression of a number of lipogenic genes, including ATP citrate lyase (ACLY) and fatty acid synthetase (FASN). ACLY converts cytosolic citrate into acetyl-CoA and oxaloacetate, supplying the essential metabolites for lipid biosynthesis. FASN catalyzes the condensation of acetyl-CoA and malonyl-CoA to generate long-chain fatty acids [12, 13].

$CD8^+$ T_{eff} cells also adopt lipid catabolism to preserve viability in the tumor microenvironment. For instance, $CD8^+$ tumor-infiltrating T cells enhance catabolism of FAs through peroxisome proliferator-activated receptor-α (PPAR-α) signaling. This metabolic switch can partially support the effector function of $CD8^+$ TILs in tumor microenvironment in mice model. Further studies use $^{13}C_{16}$–palmitate to study the change in metabolic pathways. Isotope labeling shows that many amino acids and metabolites in tricarboxylic acid cycle (TCA cycle) including acetyl-CoA are synthesized with FA-derived carbons. Tumor-infiltrating $CD8^+$ T cells are also charac-

terized by increased transcription of PPAR-α, which leads to subsequent FA uptake, triglyceride turnover, and peroxisomal and mitochondrial FA catabolism. The same trend is seen in the downstream molecules of PPAR-α, such as acetylcarnitine, palmitoylcarnitine and the ketone body 3-hydroxybutyrate [14]. Bezafibrate, an agonist of PPAR-gamma coactivator 1α (PGC-1α)/PPARs axis, could upregulate the expression of PGC-1α and attenuate tumor progression on hyporesponsive LLC xenograft models. Bezafibrate treatment could improve the survival and functional capacity of tumor-infiltrating cytotoxic T lymphocytes (CTLs). Also, the transcription of PGC-1α, a gene that regulates mitochondrial biogenesis, was found to be enhanced in tumor-infiltrating T cells. Moreover, the expression of genes related to fatty acid oxidation (FAO), like PGC-1α, carnitine palmitoyltransferase 1a (CPT1a) and LCAD, was significantly upregulated after treatment with bezafibrate within tumors. Taken together, these findings suggest that bezafibrate can activate PGC-1α/PPAR and regulate FAO of tumor-infiltrating CTLs to achieve better antitumor efficacy [15].

However, tumor microenvironment may induce the transition of metabolic program of the effector $CD8^+$ T cells, mainly the mitochondrial metabolism, to induce hyporesponsiveness of effector $CD8^+$ T cells to tumor antigens. The transferred antigen-specific effector $CD8^+$ T cells in the TME showed a progression loss of PGC1α, which is induced by chronic Akt signaling by persistent antigen stimulation in TME [16]. As PGC1α regulates mitochondrial replication, repression of this protein promotes the loss of mitochondrial mass and eventually mitochondrial function. Metabolic reprogramming of T cells through enforced PGC1α expression may rescue mitochondrial function and induce superior antitumor responses characterized by increased cytokine production and tumor control [17].

10.2.1.2 $CD8^+$ Memory T Cells

When encountering stimulatory antigens, the naïve or quiescent $CD8^+$ T cells are activated and undergo clonal expansion to boost the population of antigen-specific T cells. However, after pathogen or antigen clearance, most of the antigen-specific T cells undergo programmed cell death. Only a small fraction of them survive and become long-lived memory T cells [18]. The memory T cells can be divided into two main subtypes: the central memory T cells (T_{cm}) and the effector memory T cells (T_{em}). These cells can be further categorized by more distinctive surface markers. There are many novel populations of T_{mem} cells that were discovered over the years, like tissue-resident memory T (T_{rm}) cells and stem memory T cells (T_{scm}) [19, 20]. In a seminal study, van der Windt et al. have demonstrated that memory T cells engage in lipid metabolism to support survival, which correlates with the longevity of T_{mem} cells. In particular, fatty acid oxidation provides a protective metabolic advantage for the survival of memory T cells so that they can be activated and initiate an immune response more quickly [21, 22]. Unlike $CD8^+$ effector T cells or quiescent naive $CD8^+$ T cells, $CD8^+$ memory T cells maintained substantial spare respiratory capacity (SRC, a measure of mitochondrial reserve in a cell to produce energy) in their mitochondria, which is important for long-term cellular survival and function [23]. Enhanced SRC in $CD8^+$ memory T cells is associated with mitochondrial biogenesis and expression of CPT1a, a protein that is involved in the utilization of fatty acids in the mitochondria. It is suggested that FAO regulated SRC and $CD8^+$ memory T cell development [22, 24]. Also, it can't be ignored that at the early stage of T cell activation, the co-stimulatory receptor CD28 upregulates CPT1a to support the generation of T_{mem} through the remodeling of mitochondrial and development of spare respiratory capacity [25].

In terms of the source of FA for FAO, rather than acquiring extracellular FA directly, memory T cells use extracellular glucose to support FAO and OXPHOS. This phenomenon indicates that lipids need to be synthesized to satisfy the need for FAO. It is found that $CD8^+$ effector T cells acquire more long-chain FA (LCFA) from their surroundings than $CD8^+$ memory T cells. As O'Sullivan et al. found non-accumulation of lipid droplets in T_{mem} cells, it is speculated that

cellular FAs were converted to triacylglycerol (TAG) in ER. Then the TAG would directly undergo lysosomal hydrolase lysosome acid lipase (LAL)-mediated lipolysis to generate FA for FAO [26]. Under optimal conditions, lipolysis is not the necessary metabolic event for T cells. However, upon nutrient deprivation, lipolysis could provide a large amount of energy to T cells in the tumor microenvironment. Lipolytic machinery in T_{mem} cells differs from those in adipocytes, which involves critical enzymes like adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) [27]. More evidences have also displayed that the transcription factor forkhead homeobox type protein O1 (FoxO1) promotes T_{mem} differentiation by increasing lipolysis [26]. Processes of lipolysis or autophagy can degrade lipids not only to fuel OXPHOS but also for the generation of lipid signaling molecules, such as lipid ligands that activate the PPAR pathway [28, 29]. This futile metabolic cycling of FAS and FAO, which has been documented in muscle and adipose tissue, confers lymphocytes the advantage of extremely rapid activation [27].

The importance of fatty acid metabolism in $CD8^+$ memory T cells can also be found in mice that lack tumor necrosis factor receptor-associated factor 6 (TRAF6), which shows compromised CD8 T_{mem} generation but normal activation and expansion of CD8 T_{eff}. TRAF6 mainly stimulates AMP-activated protein kinase (AMPK) and inhibits mTOR signaling in $CD8^+$ T cells, thereby increasing fatty acid oxidation [28]. AMPK is a cellular energy sensor and controller of FAO; it inhibits acetyl-CoA carboxylase 2 (ACC2) by phosphorylation, thus promoting the oxidation of long-chain fatty acid. These studies suggested that FAO is instrumental in the generation of $CD8^+$ memory T cells. Without FAO, the differentiation of T_{mem} cells will be hindered [30]. However, Raud et al. noticed that at high concentration (40–200 μM), etomoxir, a widely used inhibitor of long-chain fatty acid oxidation by CPT1a, may have an off-target effect as affecting the abundance of TCA cycle intermediates [31]. Thus, it reminds us that attention should be paid in the concentration of chemicals used in metabolic research.

T_{mem} cells also obtain lipids to sustain FAO in the long term, though in a relatively smaller amount compared with $CD8^+$ T_{mem} cells [26]. Consistent with recent findings, IL-7 induces the expression of aquaporin 9 (AQP9), which transports glycerol into the cells. Elevated expression of AQP9 leads to glycerol uptake and lipogenesis, sustaining the level of TAG in memory $CD8^+$ T cells. Exogenous glycerol serves as potential fuel sources for the synthesis of triacylglycerides and ATP and for the activation of mTORC1 and expression of c-Myc [32].

The difference of mitochondria between T_{mem} and T_{eff} cells may also be the mechanism underlying the distinct lipid metabolism of T_{mem}. T_{eff} cells have morphologically distinctive mitochondria, which are scattered in the cytoplasm, while the mitochondria of T_{mem} cells maintain densely packed and transform into fused tubes. Through the study of Opa1 floxed mouse model, fusion protein Opa1 is found to be instrumental in T_{mem} generation after infection but not to T_{eff} cells [33]. Inducing mitochondrial fusion in T_{eff} cells makes it more similar to T_{mem} cells, improving their antitumor function. Also, CD8 T_{mem} cells have more mitochondrial mass. Therefore, when $CD8^+$ T_{mem} cells differentiate into secondary T_{eff} cells, they have higher levels of ATP than primary effector T cells and develop robust immune response [21]. It is also suggested that the difference in cristae morphology between T_{mem} and T_{eff} is linked to their metabolism adaption. Fusion of mitochondria in T_{mem} cells remodels the electron transport chain (ETC) complex to favor OXPHOS and FAO. In contrast, fission of mitochondria in T_{eff} cells leads to ETC inefficiency, increasing aerobic glycolysis [33, 34].

The tissue-resident $CD8^+$ memory T cells (T_{rm}) are a recently identified subpopulation of memory $CD8^+$ T cells that do not circulate in the blood and are primarily inhabited in nonlymphoid tissues, such as the skin and intestine. The infiltration of T_{rm} leads to better clinical outcomes in various types of human cancers, including lung cancer. T_{rm} cells appear to play a role in tumor-specific T cell response [35]. Elevated level of lipid in the external environment of T_{rm} cells greatly influences their metabolism. Several

metabolism-related surface molecules like low-density lipoprotein receptor (LDLR), ApoE, scavenger receptor CD36, and fatty acid binding proteins (FABP) 4 and 5 are highly expressed on intestinal T_{rm} cells to transfer lipids from the extracellular space to cytosol. FABPs regulate fatty acid influx and transfer free fatty acid (FFA) to mitochondria, providing the substrate for the FAO. Furthermore, FABP4 reduces cholesterol ester accumulation via inhibition of PPARγ pathways. Both FABP4 and FABP5 have a critical role in the maintenance, longevity and function of $CD8^+$ T_{rm} cells [36]. All the evidence suggest that T_{rm} have specialized lipid metabolism when compared with traditional T_{mem} [37–39].

10.2.2 $CD4^+$ T Cells

The proper differentiation of $CD4^+$ T cells into effector cells and suppressive cells provides a balance between protective immune response and excessive inflammation. $CD4^+$ T cells differentiate into effector T cells, i.e., Th1, Th2 and Th17, or inducible regulatory T cell (Treg), providing protection against a wide range of pathogens and "nonself" cells, including tumor cells [40].

The differentiation of $CD4^+$ T cells is finely tuned by a complex network including cytokine signaling, transcription factors, as well as metabolic programs. The differentiation of $CD4^+$ T cells to T_{eff} and Treg cells shows distinct metabolic programs. Th1, Th2 and Th17 cells are highly glycolytic, while Treg cells favor enhanced lipid oxidation rates [41]. Changes in metabolic program as enhanced glycolysis or lipid oxidation can affect the differentiation of $CD4^+$ T cells. In a recent study, Michalek et al. found the cytokine production of Th1, Th2 and Th17 is inhibited by promoting lipid oxidation with the addition of exogenous fatty acid during initial T cell activation. Instead, Treg cells have slightly increased expression of lineage-specific transcription factors Foxp3. Exogenous FA reduced the number of viable Th1, Th2 and Th17 cytokine production cells. The compromised function of $CD4^+$ T cells cannot be rescued by adding cytokines like IL-12, IL-4, or TGF-β, respectively. This might be explained by the different metabolic programs in $CD4^+$ T cell subsets; T_{eff} cells mainly utilize glucose as energy source, while Treg cells more favor lipid as energy source [41].

The cellular fatty acid level also controls the differentiation of Th17 and Treg cells. Cellular fatty acids are mainly from de novo synthesis and exogenous uptake. Inhibition of ACC1 not only blocks de novo fatty acid synthesis but also interferes with Th17 differentiation. Th17 cells rely on ACC1 for the production of phospholipids, a critical component of cellular membranes. Meanwhile Treg cells use exogenous fatty acids for phospholipid supply. Evidence suggests that in the tumor tissue, where most of the fatty acids are consumed by tumor cells, exogenous fatty acid deficiency impedes Treg response and promotes Th17 response [4, 7, 42].

AMPK/mTOR signaling axis have been previously demonstrated to regulate the metabolism and differentiation of $CD4^+$ T cells into T_{eff} or Treg cells. T cell activation increases glycolysis and reduces FAO with mTOR signaling [41, 43]. Inhibition of mTOR signaling results in elevated oxidation of fatty acid [44] and promotes Treg differentiation. In the presence of TGF-β, rapamycin resulted in an elevated level of Treg cells. Further study showed that etomoxir, a CPT1a inhibitor, reduced rapamycin induction of Treg cells. Upon mTOR inhibition, the differentiation of Treg subset requires lipid oxidation to support increased energy requirement [41]. As illustrated in mTOR-deficient mice, lack of mTOR severely impairs the differentiation of effector T cell subsets. Both natural and inducible Treg cells exhibited elevated levels of AMPK phosphorylation and activation compared with T_{eff} and naive $CD4^+$ T cells [45, 46]. Metformin is the activator of AMPK, the administration of which leads to increased FAO and increased Treg cells [30]. Together, co-treatment of $CD4^+$ T cells with rapamycin and metformin to inhibit mTOR and activate AMPK results in increased lipid oxidation [41].

Non-alcoholic fatty liver disease (NAFLD) is an important risk factor for predicting hepatocel-

lular carcinoma (HCC), which is characterized by the accumulation of lipid. It is found that increased fat deposition in the liver may induce selective apoptosis of $CD4^+$ T cells. Lipid-laden hepatocytes release linoleic acid (C18:2), which is taken up by $CD4^+$ T cells and upregulates CPT1a expression [47]. By feeding mice with a diet rich in linoleic acid, the same phenomenon can be seen in $CD4^+$ T cells inhabited in the liver. Additionally, it has been demonstrated that the level of PPAR-α mRNA increases in human hepatocellular carcinoma. Studies also show PPAR-α, together with PGC-1, directly upregulate the transcription of CPT1a. There is no difference in the uptake of linoleic acid between $CD4^+$ and $CD8^+$ T cells. Nevertheless, the upregulation of CPT1a increases the transportation of linoleic acid into mitochondria, disrupting electron transport chain. Leakage of electron increased reactive oxygen species (ROS) led to cell death of $CD4^+$ T cells. Taken together, release of linoleic acid leads to the loss of $CD4^+$ T cells that facilitate tumorigenesis. Therefore, inhibiting PPAR-α may reduce the expression of CPT1a and subsequent $CD4^+$ T cell apoptosis to prevent HCC induced by NAFLD [48, 49].

10.2.2.1 $CD4^+$ Regulatory T Cells

Accumulating evidence has revealed that regulatory T cell is an important part of TME in various types of cancers, such as skin, pancreas, breast and ovarian tumors in both humans and mice [50]. In contrast to the activated $CD4^+$ effector T cells, nutrient-restrictive TME does not seem to impair the functions of Treg cells. As previously reported, regulatory T cell favors FAO and has a heightened level of AMPK [24]. Consistent with previous findings, inhibition of fatty acid transport and oxidation, using sulfo-*N*-succinimidyl oleate (SSO) and etomoxir, results in a significant loss of Treg cells. Apart from the decrease of number, treatment with etomoxir and SSO also significantly suppresses the expression of markers of Treg cells, like Granzyme B, CD39 and NRP1 [51]. These results suggest that free FA may suppress immune response within tumors.

The increase in the production of FA by cancer cells and cancer-associated adipocytes makes lipids the available fuel for Treg cells in the TME. The differentiation and survival of Treg cells are supported by FA uptake and catabolism [7]. In a mouse tumor model, Pacella et al. stained tumor-infiltrating Treg cells with BODIPY, a fluorescent dye that enables the detection of lipids and other lipophilic compounds. It showed that increased intracellular lipid content accumulated in tumor-infiltrating Treg cells. It seems like the Treg cells rely on FAS, rather than uptake of fatty acid, to build up the lipid storage, which is similar to T_{mem}. Recent studies found that tumor-infiltrating Treg cells prefer to use glycolysis to fuel fatty acid synthesis. Gene set enrichment analysis showed that genes associated with glycolysis and lipid biosynthesis have heightened expression in tumor-infiltrating Treg cells extracted from liver cancer [52].

Other bioactive lipids, such as steroids, sphingolipids and fat-soluble vitamins, can also affect the function of Treg cells [50, 53–55]. Sphingosine 1-phosphate (S1P) is an important regulator of many biological processes. Both the number of tumor-infiltrating Treg cells and the expression of sphingosine 1-phosphate receptor 1 (S1P1, one of the five G protein-coupled receptors of S1P) were substantially enhanced in bladder cancer tissues. There is a strong and positive correlation between the expression of S1P1 and the number of tumor-infiltrating Treg cells. S1p1 induced the secretion of TGF-β and IL-10 and promoted the generation of cancer-associated inducible Treg (iTreg) and recruitment of natural regulatory T cells (nTreg). Survival analysis showed that increased levels of both S1P1 and Treg cells are linked to shorter overall survival and poor prognosis in bladder cancer patients [56, 57].

10.2.2.2 $CD4^+$ Th17 Cells

$CD4^+$ T helper 17 cells are a subset of the pro-inflammatory T cells which are characterized by RAR-related orphan receptor gamma (RORγ). RORγ is the master transcriptional regulator of Th17 cells. Th17 cells exhibit great diversity in the roles they play in the immune system, most prominently serving as a controversial double-edged sword in cancer. For one thing, Th17 cells are responsible for impairing tumor surveillance,

and inducing immunosuppression and tumor growth. For another, they can also mediate protective antitumor immunity through recruiting immune cells into tumors, which shows great advantage for cancer immunotherapy like adoptive cell transfer (ACT) [58].

As a regulator of lipid metabolism, CD5L is found to be the switch that controls the transition between pathogenic and nonpathogenic Th17 cells [59]. Pathogenic Th17 cells express more pro-inflammatory genes, while nonpathogenic Th17 cells mainly express immunosuppressive genes [60]. CD5L is primarily expressed in nonpathogenic Th17 cells. And the knockdown of CD5L skewed Th17 cells phenotype toward disease-inducing Th17 cells by regulating the lipidome of Th17 cells. As a result of CD5L deletion, the lipid profile of Th17 cells changes remarkably, increasing the level of cholesterol ester and the ratio of saturated fatty acid (SFA). CD5L restricts the synthesis of cholesterol, which has been linked to the production of endogenous RORγt ligand. Moreover, CD5L manipulated the binding of RORγ to the promoter of different genes by altering the composition of lipids in the cell. SFA increased RORγ binding to *Il17* and *Il10* gene locus, while polyunsaturated fatty acid promoted RORγ binding to *Il10* CNS-9 locus. Therefore, CD5L regulated the expression of IL-17, IL-23, and IL-10. Also, CD5L controlled the cholesterol and long-chain fatty acid biosynthetic pathways, subsequently affecting the transcriptional activity of RORγ and pathogenicity of Th17 cells [61].

In mice fed with a high-fat diet (HFD), Th17 cells showed significant upregulation of genes in the lipid metabolic pathways in gene ontology analysis. HFD induces the expression of genes related to FAS, including Acaca. ACC1 is encoded by Acaca, which is found to control the differentiation of Th17 cells. The differentiation of Th17 cells was significantly declined when ACC1 was inhibited by 5-(tetradecyloxy)-2-furoic acid. It was found that ACC1 modulated the specific binding of RORγt to control the functions of RORγt in Th17 cells [62].

Interestingly, liver X receptors (LXR) suppress Th17 differentiation. LXR ligands negatively regulate mouse [44] and human [46] Th17 differentiation. This is in part due to the LXR promoting the expression of sterol regulatory element binding protein 1 (SREBP1). SREBP1 is subsequently recruited to the E-box element of the *Il17* promoter. Aryl hydrocarbon receptor (AHR) also binds to the promoter of *Il17*, enhancing Th17 polarization [46]. SREBP1 interferes with the binding of AHR and *Il17*. It appears that LXR mediates the suppression of Th17 cells through the interference with AHR [63, 64].

The crosstalk between the gut microbiome and bile acid (BA) has emerged as a critical regulator of T cell function in the digestive tract. Primary bile acids are derivatives of cholesterol synthesized by the hepatocyte, and secondary bile acids are converted by microbes from primary bile acids. According to recent studies, gut microbiota has high prognostic value in predicting the risk and progression of gastrointestinal cancers [65, 66]. MDR1 (ABCB1) is widely recognized to be responsible for the drug resistance to chemotherapy. MDR1 is also widely expressed in intestinal T_{eff} cells. Bile acid could be toxic to T cells at high concentrations. In order to adapt to the environment of the intestine, T_{eff} cells inhabiting in the ileum upregulate Mdr1 to maintain homeostasis in the face of conjugated bile acids. T_{eff} cells lacking Mdr1 display mucosal dysfunction in the ileum and displayed increased TNF and IFN-γ expression [66].

Lithocholic acid (LCA) is a secondary bile acid formed in the intestine. In a screening of more than 30 kinds of bile acid metabolites, two types of lithocholic acid were found to affect the differentiation of Th17 and Treg cells. 3-OxoLCA inhibited the differentiation of Th17 cells, reducing the expression of IL-17a by interacting with RORγt ligand-binding domain. Additional isoalloLCA led to increased production of mitoROS to enhance the differentiation of Treg cells, through increasing Foxp3 expression [67].

10.2.2.3 CD4⁺ Effector Memory T Cells

Compared with the central memory T cells mentioned above, CD4⁺ effector memory T cells have limited ability to conduct FAS and FAO. In order to simulate the tumor microenvironment, research-

ers have created a special medium, which is chemically defined as serum-free. The activated $CD4^+$ T cells grown in optimal glucose have a large number of lipid droplets. When subsequently placed in low-glucose conditions, lipid droplets are consumed as fuel for $CD4^+$ T cells. However, things are a little different for T_{em} cells, which do not upregulate FAS in optimal glucose concentration and possess much fewer lipid droplets. Also, inhibition of fatty acid synthesis and uptake produced little or no change in T_{em} expansion [26]. It suggests that the expansion and function of T_{em} are largely independent of fatty acid metabolism. In low glucose, T_{em} cells could convert some of the glutamine imported from the medium into fatty acids. Using citrate labeled with four heavy carbon isotopes to examine how glutamine is utilized, it is discovered that a very low level of glutamine is routed to FAS in effector memory T cells [68].

Apart from the ability to expand, Ecker et al. noticed a strong correlation between the effector activity of T_{em} cells and fatty acid metabolism. Upon glucose deprivation, naive T cells and T_{cm} cells increased FAS, which resulted in a drop of IFN-γ secretion. If the FAS of naive T cells was inhibited, naive T cells dramatically upregulated IFN-γ. As mentioned above, the lipid metabolism of T_{em} cells remained largely unchanged when they were facing glucose starvation. At the same time, T_{em} cells maintained a relatively high level of IFN-γ, which further illustrated the limited ability of T_{em} cells to metabolize fatty acid [68].

10.2.3 Exhausted T Cells

T cells usually drift into exhausted status in the tumor, which is represented by a group of T cells with impaired function and sustained expression of inhibitory receptors [69, 70]. For instance, the expansion ability of $CD8^+$ T cells was severely impaired because of chronic infection [70]. The dysfunction of exhausted T cells is related to the co-expression of multiple inhibitory receptors, including cytotoxic T lymphocyte antigen 4 (CTLA-4), lymphocyte activation gene 3 (LAG-3), T cell immunoglobulin and mucin-containing gene 3 (Tim-3) and programmed death-1 (PD-1). A good example is that the $CD8^+$ TILs isolated from diet-induced obese (DIO) mice express more PD-1, Tim-3, and Lag3 [71]. The upregulation of these molecules also regulates the lipid metabolism of exhausted T cells (T_{ex}) in the tumor [72, 73].

PD-1 expression is one of the characteristics of T cell exhaustion; its signaling favored the oxidation of fatty acid for T cells while inhibiting the transport and utilization of amino acid and glucose in vivo. Similar to the long-lived $CD8^+$ T_{mem}, T cells with PD-1 ligation had a substantial mitochondrial reserve to produce more energy, as shown by higher level of spare respiratory capacity. To be more specific, PD-1 inhibited PI3K activity, thereby inducing the expression of CPT1a [74]. Moreover, the level of FASN was greatly increased since the stimulation of PD-1. Therefore, T cells have altered lipid metabolism after incubation with PD-1, which is characterized by elevated lipolysis and FAO [75].

Meanwhile, PD-1 can promote the differentiation of Treg cells, which suppress T_{eff} functions [5, 72, 76]. PGC-1α expression is repressed by PD-1 through PI3K-Akt and mTOR pathways on the initial stage of the infection of the *lymphocytic choriomeningitis virus* (LCMV), leading to the mitochondrial depolarization. PGC-1α expression was enhanced in the absence of PD-1, and retroviral expression of PGC-1 could partly rescue the mitochondrial function of T_{eff} [29].

Tnfrsf9 (4-1BB or CD137) is expressed after T cell activation, which stimulates cell differentiation and survival [77]. LAG-3 functions as an inhibitory receptor. In humans, tumor-infiltrating $CD8^+$ T cells separated from melanoma co-expressed 4-1BB and LAG-3 [78]. Activating 4-1BB with agonistic anti-4-1BB antibody demonstrated potent antitumor effects in murine tumor models [79]. The stimulation of 4-1BB increased the metabolism of glucose and fatty acids, which were important for cell proliferation. The signaling pathway liver kinase B1 (LKB1)-AMPK-ACC was activated by anti-4-1BB treatment, which supported the energy need of T cells [80].

10.2.4 γδ T Cells

γδ T cells are a unique subset of T cells, which are usually regarded as innate immune cells. γδ T cells have been reported to play a controversial role in different types of cancer [81]. In humans, the majority of γδ T cells express the Vγ9Vδ2$^+$ TCR [82]. γδ T cells can recognize and respond to the presentation of lipid antigens [83], cancer cell-derived antigens [84] and mevalonate metabolites in tumor cells [85, 86]. Non-peptide antigens like phosphoantigens (such as isopentenyl pyrophosphate, IPP), which are derivatives of the metabolites of bacterial isoprenoid biosynthesis or the mevalonate pathway, can selectively activate Vγ9Vδ2$^+$ T cells [87]. Vγ9Vδ2$^+$ T cells are also activated by accumulated phosphorylated mevalonate metabolites due to dysregulated mevalonate pathway in tumor cells [86].

LDL cholesterol has been identified as a risk factor for breast cancer proliferation and invasion [88]. Rodrigues et al. showed that upon activation, up to 30% of Vγ9Vδ2$^+$ T cells expressed LDLR to internalize LDL cholesterol. Although about 30% Vγ9Vδ2$^+$ T cells express LDLR upon activation in tumor microenvironment, the accumulation of cholesterol inhibits activation and antitumor function of Vγ9Vδ2$^+$ T cells, including the expression of IFN-γ. As shown in a tumor-bearing mouse model, LDL-cholesterol compromised the capacity of Vγ9Vδ2$^+$ T cells to control the growth of breast cancer [89].

27-hydroxycholesterol (27HC) is oxysterol, which serves as an endogenous estrogen receptor. 27HC has been found to be a predictive marker of breast cancer progression [90]. 27HC could promote metastasis in several models of mammary cancer. Inhibition of CYP27A1, a rate-limiting enzyme in 27-hydroxycholesterol biosynthesis, led to less metastasis by decreasing 27HC. In the presence of polymorphonuclear neutrophils (PMNs), 27HC stimulated the proliferation of γδ T cells. Therefore, more PMN and γδ T cells infiltrated in tumors, facilitating further recruitment and creating positive feedback. Also, PMN and γδ T cells suppressed the tumor-infiltrating CD8$^+$ T cells within tumors and metastatic lesions [85].

10.3 Cholesterol Metabolism of Tumor-Infiltrating T Cells

Cholesterol is a key component of the cell membrane and a precursor for steroid hormone [91, 92]. Cholesterol metabolism is mainly regulated by two transcription factors: one is SREBPs, which induce the expression of proteins that promote cholesterol uptake and synthesis, such as LDLR and hydroxymethylglutaryl coenzyme A reductase (HMGCR), and the other one is LXRs, which control the efflux of cholesterol. LXRs regulate the synthesis of ATP-binding cassette transporter ABCA1, ABCG1, and inducible degrader of LDLR (Idol). LXRs inhibit the LDLR pathway through transcriptional induction of Idol, which triggers the ubiquitination and subsequent degradation of LDLR [93].

It is widely known that the activation and expansion of T cells require increased uptake and de novo synthesis of cholesterol for membrane formation. Therefore, cholesterol efflux pathways can limit cell expansion [94]. Bensinger et al. reported that the activation of T cell induced the expression of oxysterol-metabolizing enzyme sulfotransferase family 2b member 1 (Sult2b1) to suppress LXR pathway [95]. Oxidized cholesterol derivatives (oxysterols) can activate LXRs [96], and Sult2b1 promoted the sulfation of oxysterols, thus reducing the available ligand of LXR. For further study, they used radioactive isotope to label the substrate to detect cell division. T cells with LXRβ knockdown displayed higher division speed compared with wild-type T cells. A similar phenomenon can be found in lymphocytes lacking LXRβ expression that showed increased homeostatic proliferation [64]. As discussed above, LXR depended on ABCG1 to regulate sterol trafficking. In order to suppress cell proliferation, LXRβ stimulated ABCG1 and subsequently reduced cholesterol level in T cells. Therefore, Sult2b1-LXR-ABCG1 axis plays an important role in the proliferation of T cells by manipulating cellular cholesterol metabolism.

As mentioned above, LDLR mediates cholesterol uptake of tumor-infiltrating T cells [97]. In the tumor microenvironment, T cells increased the capacity of cholesterol uptake. Surprisingly,

an elevated level of cholesterol in tumor-infiltrating CD8⁺ T cells was linked to the expression of inhibitory receptors, such as PD-1, 2B4, TIM-3, and LAG-3 [98]. Ma et al. treated CD8⁺ T cells with different concentrations of cholesterol and supernatant of tumor culture. Consistent with previous findings, cholesterol induced immune checkpoint expression in a dose-dependent manner. Addition of cholesterol increased ER stress and disrupted lipid metabolism in T cells. In response to that, T cells upregulated the transcription of XBP1, an ER stress sensor, and that of PD-1 and 2B4. XBP1 was also a transcription factor, binding to the promoter of *pdcd1* and *CD244*. Overexpression of XBP1 led to a higher level of PD-1, 2B4, TIM-3, and LAG-3. So reasonably, inhibiting XBP1 restored the antitumor activity of CD8⁺ T cells, so was reducing cholesterol [99].

CD8⁺ T cells that are skewed toward the phenotype of producing IL-9 (Tc9) have been recognized as a target for adoptive cancer immunotherapy [100]. The mechanism underlying the antitumor function of Tc9 cells may be the low concentration of cholesterol in Tc9 compared with Tc1. As already noted, Tc9 cells had considerably lower expression of genes involved in cholesterol synthesis, such as *Hmgcr*. However, Tc9 cells had an active expression of cholesterol efflux genes like *Abca1* and *Abcg1*. The expression of IL-9 was important for Tc9 cells to maintain the antitumor activity. Cholesterol in Tc9 cells significantly affected IL-9 expression and the antitumor function. Further studies revealed that all oxysterols, except for 22(*S*)-hydroxycholesterol, inhibited IL-9 expression by activation and sumoylation of LXR. LXR sumoylation inhibited the binding of p65, a transcriptional factor, to *Il9* promoter. In summary, cholesterol adversely affected the antitumor function of Tc9 cells through modulating IL-9 [101].

However, increasing the membrane cholesterol level could augment the antitumor function of CD8⁺ tumor-infiltrating T cells. ACAT1 and ACAT2 are key enzymes of cholesterol esterification. Inhibition of ACAT1 and ACAT2 or ACAT1 alone in T cells resulted in enhanced effector function, characterized by increased cytotoxicity. Further studies conducted in genetically engineered mice showed similar results in CD8⁺ but not CD4⁺ T cells, which may be owing to different metabolic programs. In the melanoma model, ACAT1-deficient CD8⁺ tumor-infiltrating T cells generated potent antitumor activity. This was due to the increase of the plasma membrane cholesterol level of CD8⁺ T cells, which causes enhanced TCR clustering and signaling as well as a more efficient formation of the immunological synapse [102, 103]. Therefore, the inhibition of ACAT1 strengthened the antitumor function of CD8⁺ T cells through elevated membrane cholesterol content [104].

10.4 Oxygen Deprivation in Tumor Affects Lipid Metabolism in Tumor-Infiltrating T Cells

Recent studies suggested that the nutrient-restrictive TME dampens lymphocyte's viability and regulates their differentiation. Hypoxic areas are common within solid tumors, as angiogenesis often lags behind the rapid expansion of tumors. The average oxygen level in tumors ranges from 0.5 to 5%, which is lower than that in most healthy organs. Worse still, lack of O_2 prevents T cells from producing energy through OXPHOS [8]. As a consequence, some T cells like Treg cells increase lipid metabolism.

Hypoxia-inducible factors (HIF-1 and HIF-2) are key transcriptional factors induced by hypoxic pressure. The expression of HIF-1 increased exponentially at low oxygen levels [105, 106]. HIF-1 and HIF-2 have been reported to mediate hypoxic responses, driving angiogenesis, glycolysis and tumor invasion [72].

Recently, some studies have demonstrated that HIF-1α served as a metabolic checkpoint to regulate the differentiation of CD4⁺ T cells. Although differentiated from the same precursor, naive CD4⁺ T cells, Th17 and Treg cells expressed distinct transcriptional regulators (RORγt versus Foxp3, respectively), leading to their opposing functions (pro-inflammatory versus anti-inflam-

matory). The precursor of these T cells co-expresses RORγt and Foxp3. Upon activation, molecular cues such as HIF transform these cells until one of them becomes the dominant transcriptional regulator. Foxp3 activates a large bank of genes that mediate the suppressive phenotype of Treg [51, 107]. RORγt regulates the development of Th17 cells, which is linked to many autoimmune disorders [108].

In a HIF-1α knockout mouse model, it is found that the transcription and translation of RORγt were upregulated in T cells to promote Th17 differentiation. In addition, HIF-1α inhibited the differentiation of Treg through degrading Foxp3 protein rather than Foxp3 mRNA [109]. Other studies have also proved that HIF-1α promoted the differentiation of Th17 cell through affecting their metabolism. Shi et al. found that deficiency in HIF-1α resulted in the diminished glycolytic activity. It was indicated that HIF-1α mediated the increase in glycolytic activity, promoting Th17 differentiation [110]. On the other hand, some studies suggest that HIF-1α may protect Treg cells from glucose deprivation in tumor, as HIF-1α promotes tumor-infiltrating Treg cells to utilize free fatty acid [24]. Treg cells will utilize lipid metabolism to thrive in glucose deprivation environments, suggesting the metabolic shift may be responsible for their survival within tumors. For example, under a hypoxic situation, the transcription of Foxp3 in naive $CD4^+$ T cells upregulated more than tenfold [111]. Miska et al. reveal that the suppressive function of Treg cells is maintained by the lipid uptake and oxidation program under hypoxia. $Hif1a^{-/-}$ Treg cells have a higher basal oxygen consumption rate (OCR), maximal OCR, and ATP-linked respiration than wild-type Treg cells after incubation in 1% oxygen. However, there were no significant differences in the composition of the metabolite of freshly sorted Treg cells from both types of mice. It suggested that the metabolic change in $Hif1a^{-/-}$ Treg cells was due to the hypoxic environment. Further studies showed that tumor-infiltrating Treg cells dramatically increased the uptake of fatty acid compared with glucose within the glioma environment. HIF-1α is known to transport intracellular free fatty acid into lipid droplets. Inhibition of lipid metabolism using etomoxir abolished immunosuppression in $Hif1a^{-/-}$ Treg cells. After the injection of $Hif1a^{-/-}$ and wild-type Treg cells in tumor-bearing mice, the authors found that the ability of $Hif1a^{-/-}$ Treg cells to infiltrate the tumor was ablated. And $Hif1a^{-/-}$ tumor-bearing mice had prolonged survival. In conclusion, this suggested that HIF-1α promotes lipid metabolism in Treg cells, which is significant for its suppressive capability in the tumor [51]. However, there are conflicting views on the impact of HIF on Treg cells. Dang et al. showed that HIF-1α induced the degradation of Foxp3 protein, inhibiting Treg differentiation, while knockout of HIF-1α restored the Foxp3 stability [109]. It has been well established that HIF-1α degrades in an ubiquitin-dependent manner via proline hydroxylation at amino acid positions 402 and 564 by prolyl hydroxylases (PHDs). Researchers co-transfected 293T cells with Foxp3 and a HIF-1α mutant in which prolines 402 (p402A) and 564 (p564A) were mutated to alanines. In this way, 293T cells expressed HIF-1α protein that was resistant to posttranslational modification and subsequent degradation. The expression of mutant HIF-1α was incompetent to induce Foxp3 degradation in 293T cells [107]. Therefore, the normal expression of HIF-1α in T cells mediated the suppression of the development of Treg through the degradation of Foxp3. Inhibition of protease increased Foxp3 protein stability, suggesting that HIF-1α mediates the proteasomal degradation of Foxp3 [112].

10.5 Tumor Cell-Derived Lipids

Tumor cells constantly interact with the surrounding environment in order to sustain their survival and growth. Some types of tumors can release large quantities of prostaglandin and other kinds of lipids into TME [113, 114]. These metabolites can greatly influence TME and induce the loss of function in TILs. For example, in a study including 22 breast cancer patients, high levels of unbound free fatty acids (FFAu) are found in cancer tissue. To be more precise, the concentration of FFAu increased to at least

25 nM in most of the samples and exceeded 100 nM in 11 patients. The rise of free fatty acids in plasma membrane decreases membrane fluidity, and disrupts lipid raft and immune synapse formation [114]. The IC_{50} for unbound oleate is 125 ± 30 nM, 200-fold greater than normal plasma levels, which is lower than that in some of the samples. In vitro studies showed that elevated levels of FFA are released from tumor cells within minutes of CTL attack [115, 116]. Another study showed that with the treatment of oleic acid or linoleic acid, proliferation of lymphocytes was enhanced at 25 mM, and inhibited at higher concentrations (75 and 100 mM). Both fatty acids promoted cell death at 200 mM concentration [117].

What's more, cancer cell is not the only source of the FFAu in tumor tissue. In order to promote their own growth, tumor cells function as metabolic parasites to extract energy from tumor stromal cells, such as fibroblasts and adipocytes [118, 119]. Cancer-associated adipocytes exhibited a constant loss of lipid content following the initial crosstalk with tumor cells. FFAs could be released from these adipocytes through lipolysis. Then FFAs were transferred to cancer cells and stored in the lipid droplets as a source of energy. The transfer of lipids from host cells can be seen in both prostate [120] and ovarian cancer [121, 122].

Considering all the negative effects of FFAu, it comes as no surprise that obesity also impairs the function of TILs. To investigate the effect of obesity, mice were divided into two groups. In the control group, mice were fed with a diet containing 10% fat, in contrast to a 60% fat diet in diet-induced obese (DIO) group. Tumor-infiltrating T cells in DIO mice had higher expression of PD-1, causing T cell dysfunction. Consistent with that, the expression of CPT1a was also upregulated in the $CD8^+$ T cells of DIO mice. CPT1a was upregulated in early exhausted T cells after infection, regulating fatty acid oxidation [29]. Similar results were observed with 4T1 breast carcinoma cells in DIO mice, demonstrating that the correlation between obesity and T cell dysfunction exists across strains and tumor types [71].

10.5.1 Prostaglandins (PG)

Tumor cell-derived lipid mediators such as prostaglandin also serve to build an immunosuppressive TME. Arachidonic acid, the precursor of prostaglandin (PG), is converted by cyclooxygenase (Cox) enzymes Cox-1 and Cox-2 to prostaglandins, prostacyclins, and thromboxanes. COX-2/PGE_2 can affect tumor progression through interaction with tumor-infiltrating Treg and Th17 cells.

There is a metabolic crosstalk between tumor cells and Treg through tumor-derived prostaglandins. High level of Cox-2 expression is correlated with tumorigenesis and tumor neovascularization in human prostate cancer [123, 124]. Cox-2 expression is enhanced in diverse tumor entities, such as bone, stomach, pancreatic, lung, breast, and colorectal cancers and ductal pancreatic adenocarcinomas (PDAC) [125–128]. Overexpression of Cox-2 in the tumor results in increased production of PGE_2, which in turn increased the suppressive ability of Treg [129]. Furthermore, tumor-derived COX-2/PGE_2 promoted tumor-infiltrating Treg cell activity by inducing the expression of Foxp3 [130]. The accumulation of Foxp3-positive Treg cells led to immune suppression in gastric tumors. Moreover, elevated Foxp3 expression in Treg cells was linked to the advanced stage of gastric cancer [131].

These tumor-infiltrating Treg also suppressed the antitumor function of CD8 αβ T cells, as shown by the decrease of viability in $CD8^+$ T cells [128]. Moreover, in response to the cytotoxic activity of γδ T cells, PDAC cells released PGE_2, which bounds to prostaglandin E_2 and E_4 receptors on the γδ T cells. PGE_2 inhibited γδ T cells by cyclic adenosine monophosphate (cAMP)-mediated protein kinase [132–135]. Through the PGE_2-cAMP pathway, PGE_2 also induced the expression of Tim-3, which is an inhibitory receptor of exhausted T cells [136]. Also, PGE_2 has been found to stimulate the differentiation of Treg from naive $CD4^+$ T cells [130]. Therefore, inhibiting the production of PGE_2 abrogated the suppression of tumor-infiltrating Treg and decreased the size of tumor.

Additionally, Treg cells are found to be effective producers of PGE_2 themselves [137, 138]. iTreg cells generated in PGE_2-secreting tumor also released PGE_2. These iTreg cells were strongly immunosuppressive and produced higher levels of PGE_2 and adenosine [139].

Th17 cells have been recognized to cause chronic inflammation in the tumor microenvironment. Qian et al. found that Th17 cells were increased in the tumor tissues of mammary gland tumor-bearing mice. IL-23 has two subunits, p19 and p40, which are important for the formation and maintenance of Th17 cells. Data indicated that tumor-secreted PGE_2 enhanced the expression of IL-23. PGE_2 is bound to the cAMP-response element in the p19 gene promoter, promoting p19 gene transcription and therefore the increase of Th17 cells [140].

10.5.2 Polyunsaturated Fatty Acids

Apart from prostaglandin, lipid-containing food like fish oil can also affect the lipid metabolism of TILs. Intake of fish oil, which is rich in marine ω-3 polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and docosapentaenoic acid (DPA), modulates T cell activity in the TME. PUFAs can alter the immune system through multiple ways, for example, by changing membrane lipid composition and signaling protein posttranslational lipidation like palmitoylation, myristoylation and prenylation. In addition, dietary DHA blunted the production of intracellular second messengers, like diacylglycerol and ceramide [141].

Omega-3 polyunsaturated fatty acid supplementation can modify the immune response and mammary carcinogenesis in murine models. In the control group, mice were fed on a diet with 11% of corn oil (CO) compared with the experimental diet containing 10% of menhaden fish oil (FO) and 1% of corn oil. FO significantly reduced the incidence and multiplicity of tumors in HER-2/neu mice. In comparison, FO-fed PyMT mice did not show any significant difference in the incidence and multiplicity of tumors. Similarly, lymphocytes in FO-fed HER-2/neu mice had greater ability to infiltrate the tumor, but not in FO-fed PyMT mice. The antitumor effect that fish oil possessed in HER-2/neu mice can be attributed by the increase in lymphocytes and tumor-infiltrating lymphocytes in FO-fed HER2/neu mice [142].

The positive effect of PUFA on immunity is further supported by human data. Two prospective cohort studies among 121,700 participants in the Nurses' Health Study (NHS) (1984–2010) and 51,529 participants in the Health Professionals Follow-Up Study (1986–2010) showed that a high dose of marine ω-3 polyunsaturated fatty acids was associated with a lower risk of CRC accompanied with high-level $Foxp3^+$ T cell infiltration. Intake of marine ω-3 polyunsaturated fatty acids downregulated the suppressive activity of tumor-infiltrating Treg cells [143].

Although EPA and DHA can reduce inflammation associated with obesity and diabetes, high dose of EPA and DHA can be deleterious possibly by generating a hyporesponsive host environment. Treatment of DHA and EPA at high concentration (100 μM) suppressed the proliferation and cytokine secretion of Th1 cells in a PPAR-dependent pattern. Pre-treatment of PPAR-gamma antagonist restored the function of Th1 cells [144].

10.6 Conclusion

The tumor microenvironment imposes metabolic hurdles, such as hypoxia and glucose deprivation, to the survival and development of tumor-infiltrating lymphocytes, causing metabolic reprogramming in them. In response to these challenges, T cells fine-tune their metabolism to restrict tumor progression. Lipid metabolism is of great importance to regulate the function and cell fate decisions of T cells (Table 10.1).

Quiescent cells like naive T cells utilize fatty acids and amino acids interchangeably for energy. Notably, upon antigen recognition and co-stimulation, naive T cells are activated and differentiate into effector T cells. It is clear that

Table 10.1 Impact of lipids in T cells

Lipid	Cell type	Impact	Molecular mechanism	References
Fatty acid	$CD4^+$ T cells	Inhibit Th1, Th2, Th17 cell differentiation	Decrease expression of lineage-specific transcription factors	[41]
Linoleic acid	$CD4^+$ T cells	Induce selective apoptosis of CD4+ T cells	Increase CPT1a expression, resulting in increased leakage of electron and reactive oxygen species	[47–49]
Conjugated bile acid	$CD4^+$ T cells	Maintain T cell homeostasis in the ileum	MDR1 alleviates oxidative stress	[66]
Cholesterol	$CD8^+$ T cells	Increase ER stress, induce T cell exhaustion	Increase XBP1 to upregulate expression of the inhibitory receptors PD-1 and 2B4	[99]
27-Hydroxycholesterol (27-HC)	$CD8^+$ T cells	Decrease the number of cytotoxic $CD8^+$ T cells in the tumor	Not fully illustrated	[85]
Fatty acid	Treg cells	Promote Treg differentiation	Increase expression of FoxP3	[41]
Sphingosine 1-phosphate	Treg cells	Promoted generation of cancer-associated inducible Treg and recruitment of natural Treg cells	Induce secretion of TGF-β and IL-10	[57]
5-(tetradecyloxy)-2-Furoic acid	Th17 cells	Inhibit Th17 cell differentiation	Impair the functions of RORγt through inhibiting ACC1	[62, 63]
3-OxoLCA	Th17 cells	Inhibit Th17 cell differentiation	Reduce IL-17a expression by interference with RORγt ligand-binding domain	[67]
IsoalloLCA	Th17 cells	Promote Th17 cell differentiation	Increase Foxp3 expression	[67]
27-Dihydroxycholesterol (27-OHC)	Th17 cells	Promote Th17 T cell differentiation	Increase IL-17 production dependent on RORγt	[63]
27-Hydroxycholesterol (27-HC)	γδ T cells	Promote γδ T cell proliferation in the presence of PMNs to enhance tumor metastasis	Not fully illustrated	[85]
22(*R*)-Hydroxycholesterol	Tc9 cells	Inhibit IL-9 production	Increase activation and sumoylation of LXR to repress IL-9 expression	[101]

each T cell subset utilizes a metabolic program that is adapted to its function. Within the TME, $CD8^+$ effector T cells significantly upregulate de novo fatty acid synthesis and uptake. With the contraction of the primary immune response, decreased glycolysis and increased lipid oxidation can drive the enrichment of long-lived $CD8^+$ memory cells [41].

$CD4^+$ Th1, Th2 and Th17 cells expressed high surface levels of the glucose transporter Glut1 and were highly glycolytic. The addition of lipids strongly inhibited the production of Th1, Th2 and Th17 cytokines. In comparison, Treg cells have activated AMPK signaling and rely on lipid oxidation as a main energy source [41].

In order to escape immunosurveillance, tumor cells and cancer-associated adipocytes can generate an immunosuppressive environment through hypoxia and lipid secretion. High levels of FFA, prostaglandin and oxygen deprivation in the TME will dampen the antitumor activity of T cells.

Considering the fact that hypoglycemia and hypoxia are common in the TME, lipid metabolism will play a more significant role in TILs. Extensive researches prove a synergistic antitumor effect by combination of targeting lipid metabolism with traditional immunotherapy. Inhibitors of lipid metabolism, like statin and avasimibe, show great potential to reboost the antitumor ability of TILs in animal models. However, further research and clinical trials are needed to clarify the precise mechanism of these drugs. In summary, we believe that targeting lipid metabolic pathways will be a promising therapy with the prospect for further application.

References

1. van der Woude LL, et al. Migrating into the tumor: a roadmap for T cells. Trends Cancer. 2017;3(11):797–808.
2. Palucka AK, Coussens LM. The basis of oncoimmunology. Cell. 2016;164(6):1233–47.
3. Yost KE, et al. Clonal replacement of tumor-specific T cells following PD-1 blockade. Nat Med. 2019;25(8):1251–9.
4. Chalmin F, et al. Regulation of T cell antitumor immune response by tumor induced metabolic stress. Cell Stress. 2018;3(1):9–18.
5. Patsoukis N, et al. Immunometabolic regulations mediated by coinhibitory receptors and their impact on T cell immune responses. Front Immunol. 2017;8:330.
6. Frauwirth KA, et al. The CD28 signaling pathway regulates glucose metabolism. Immunity. 2002;16(6):769–77.
7. Lochner M, Berod L, Sparwasser T. Fatty acid metabolism in the regulation of T cell function. Trends Immunol. 2015;36(2):81–91.
8. Zhang Y, Ertl HC. Starved and asphyxiated: how can CD8(+) T cells within a tumor microenvironment prevent tumor progression. Front Immunol. 2016;7:32.
9. Jiang S, Yan W. T-cell immunometabolism against cancer. Cancer Lett. 2016;382(2):255–8.
10. Raud B, et al. Fatty acid metabolism in CD8(+) T cell memory: challenging current concepts. Immunol Rev. 2018;283(1):213–31.
11. Kidani Y, et al. Sterol regulatory element-binding proteins are essential for the metabolic programming of effector T cells and adaptive immunity. Nat Immunol. 2013;14(5):489–99.
12. Porstmann T, et al. SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth. Cell Metab. 2008;8(3):224–36.
13. Duvel K, et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. Mol Cell. 2010;39(2):171–83.
14. Zhang Y, et al. Enhancing CD8(+) T cell fatty acid catabolism within a metabolically challenging tumor microenvironment increases the efficacy of melanoma immunotherapy. Cancer Cell. 2017;32(3):377–391.e9.
15. Wan H, et al. PGC-1alpha activator-induced fatty acid oxidation in tumor-infiltrating CTLs enhances effects of PD-1 blockade therapy in lung cancer. Tumori. 2019;106(1):55–63. https://doi.org/10.1177/0300891619868287.
16. Fernandez-Marcos PJ, Auwerx J. Regulation of PGC-1α, a nodal regulator of mitochondrial biogenesis. Am J Clin Nutr. 2011;93(4):884S–90S.
17. Scharping NE, et al. The tumor microenvironment represses T cell mitochondrial biogenesis to drive Intratumoral T cell metabolic insufficiency and dysfunction. Immunity. 2016;45(2):374–88.
18. Cui W, Kaech SM. Generation of effector CD8+ T cells and their conversion to memory T cells. Immunol Rev. 2010;236:151–66.
19. Enamorado M, et al. Enhanced anti-tumour immunity requires the interplay between resident and circulating memory CD8(+) T cells. Nat Commun. 2017;8:16073.
20. Steinert EM, et al. Quantifying memory CD8 T cells reveals regionalization of Immunosurveillance. Cell. 2015;161(4):737–49.
21. van der Windt GJ, et al. CD8 memory T cells have a bioenergetic advantage that underlies their rapid recall ability. Proc Natl Acad Sci U S A. 2013;110(35):14336–41.
22. van der Windt GJ, et al. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. Immunity. 2012;36(1):68–78.
23. Henson SM, et al. p38 signaling inhibits mTORC1-independent autophagy in senescent human CD8(+) T cells. J Clin Invest. 2014;124(9):4004–16.
24. Gupta S, Roy A, Dwarakanath BS. Metabolic cooperation and competition in the tumor microenvironment: implications for therapy. Front Oncol. 2017;7:68.
25. Klein Geltink RI, et al. Mitochondrial priming by CD28. Cell. 2017;171(2):385–397.e11.
26. O'Sullivan D, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. Immunity. 2014;41(1):75–88.
27. Zechner R, et al. FAT SIGNALS—lipases and lipolysis in lipid metabolism and signaling. Cell Metab. 2012;15(3):279–91.
28. Pearce EL, et al. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature. 2009;460(7251):103–7.
29. Bengsch B, et al. Bioenergetic insufficiencies due to metabolic alterations regulated by the inhibitory receptor PD-1 are an early driver of CD8(+) T cell exhaustion. Immunity. 2016;45(2):358–73.

30. Byersdorfer CA. The role of fatty acid oxidation in the metabolic reprograming of activated t-cells. Front Immunol. 2014;5:641.
31. Raud B, et al. Etomoxir actions on regulatory and memory T cells are independent of Cpt1a-mediated fatty acid oxidation. Cell Metab. 2018;28(3):504–515.e7.
32. Cui G, et al. IL-7-induced glycerol transport and TAG synthesis promotes memory CD8+ T cell longevity. Cell. 2015;161(4):750–61.
33. Patten DA, et al. OPA1-dependent cristae modulation is essential for cellular adaptation to metabolic demand. EMBO J. 2014;33(22):2676–91.
34. Buck MD, et al. Mitochondrial dynamics controls T cell fate through metabolic programming. Cell. 2016;166(1):63–76.
35. Mami-Chouaib F, et al. Resident memory T cells, critical components in tumor immunology. J Immunother Cancer. 2018;6(1):87.
36. Pan Y, et al. Survival of tissue-resident memory T cells requires exogenous lipid uptake and metabolism. Nature. 2017;543(7644):252–6.
37. Beura LK, et al. T cells in nonlymphoid tissues give rise to lymph-node-resident memory T cells. Immunity. 2018;48(2):327–338.e5.
38. Fahrer AM, et al. Attributes of gammadelta intraepithelial lymphocytes as suggested by their transcriptional profile. Proc Natl Acad Sci U S A. 2001;98(18):10261–6.
39. Konjar S, Veldhoen M. Dynamic metabolic state of tissue resident CD8 T cells. Front Immunol. 2019;10:1683.
40. Perucha E, et al. The cholesterol biosynthesis pathway regulates IL-10 expression in human Th1 cells. Nat Commun. 2019;10(1):498.
41. Michalek RD, et al. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory CD4+ T cell subsets. J Immunol. 2011;186(6):3299–303.
42. Berod L, et al. De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. Nat Med. 2014;20(11):1327–33.
43. Wieman HL, Wofford JA, Rathmell JC. Cytokine stimulation promotes glucose uptake via phosphatidylinositol-3 kinase/Akt regulation of Glut1 activity and trafficking. Mol Biol Cell. 2007;18(4):1437–46.
44. Brown NF, et al. The mammalian target of rapamycin regulates lipid metabolism in primary cultures of rat hepatocytes. Metabolism. 2007;56(11):1500–7.
45. O'Sullivan D, Pearce EL. Fatty acid synthesis tips the TH17-Treg cell balance. Nat Med. 2014;20(11):1235–6.
46. Pollizzi KN, Powell JD. Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. Nat Rev Immunol. 2014;14(7):435–46.
47. Ma C, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. Nature. 2016;531(7593):253–7.
48. Brown ZJ, et al. Carnitine palmitoyltransferase gene upregulation by linoleic acid induces CD4(+) T cell apoptosis promoting HCC development. Cell Death Dis. 2018;9(6):620.
49. Sugiura A, Rathmell JC. Metabolic barriers to T cell function in tumors. J Immunol. 2018;200(2):400–7.
50. Wang H, Franco F, Ho PC. Metabolic regulation of Tregs in cancer: opportunities for immunotherapy. Trends Cancer. 2017;3(8):583–92.
51. Miska J, et al. HIF-1alpha is a metabolic switch between glycolytic-driven migration and oxidative phosphorylation-driven immunosuppression of Tregs in glioblastoma. Cell Rep. 2019;27(1):226–237.e4.
52. Pacella I, et al. Fatty acid metabolism complements glycolysis in the selective regulatory T cell expansion during tumor growth. Proc Natl Acad Sci U S A. 2018;115(28):E6546–55.
53. Campbell DJ, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. Nat Rev Immunol. 2011;11(2):119–30.
54. Liu G, et al. The receptor S1P1 overrides regulatory T cell-mediated immune suppression through Akt-mTOR. Nat Immunol. 2009;10(7):769–77.
55. Jeffery LE, et al. 1,25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. J Immunol. 2009;183(9):5458–67.
56. Blaho VA, et al. HDL-bound sphingosine-1-phosphate restrains lymphopoiesis and neuroinflammation. Nature. 2015;523(7560):342–6.
57. Liu YN, et al. Sphingosine 1 phosphate receptor-1 (S1P1) promotes tumor-associated regulatory T cell expansion: leading to poor survival in bladder cancer. Cell Death Dis. 2019;10(2):50.
58. Bailey SR, et al. Th17 cells in cancer: the ultimate identity crisis. Front Immunol. 2014;5:276.
59. Kurokawa J, et al. Macrophage-derived AIM is endocytosed into adipocytes and decreases lipid droplets via inhibition of fatty acid synthase activity. Cell Metab. 2010;11(6):479–92.
60. Wu X, Tian J, Wang S. Insight into non-pathogenic Th17 cells in autoimmune diseases. Front Immunol. 2018;9:1112.
61. Wang C, et al. CD5L/AIM regulates lipid biosynthesis and restrains Th17 cell pathogenicity. Cell. 2015;163(6):1413–27.
62. Endo Y, et al. Obesity drives Th17 cell differentiation by inducing the lipid metabolic kinase, ACC1. Cell Rep. 2015;12(6):1042–55.
63. Soroosh P, et al. Oxysterols are agonist ligands of RORgammat and drive Th17 cell differentiation. Proc Natl Acad Sci U S A. 2014;111(33):12163–8.
64. Fessler MB. The intracellular cholesterol landscape: dynamic integrator of the immune response. Trends Immunol. 2016;37(12):819–30.
65. Jia W, Xie G, Jia W. Bile acid-microbiota crosstalk in gastrointestinal inflammation and carcinogenesis. Nat Rev Gastroenterol Hepatol. 2018;15(2):111–28.

66. Cao W, et al. The xenobiotic transporter Mdr1 enforces T cell homeostasis in the presence of intestinal bile acids. Immunity. 2017;47(6):1182–1196. e10.
67. Hang S, et al. Bile acid metabolites control TH17 and Treg cell differentiation. Nature. 2019;576:143.
68. Ecker C, et al. Differential reliance on lipid metabolism as a salvage pathway underlies functional differences of T cell subsets in poor nutrient environments. Cell Rep. 2018;23(3):741–55.
69. Speiser DE, et al. T cell differentiation in chronic infection and cancer: functional adaptation or exhaustion? Nat Rev Immunol. 2014;14(11):768–74.
70. Man K, et al. Transcription factor IRF4 promotes CD8(+) T cell exhaustion and limits the development of memory-like T cells during chronic infection. Immunity. 2017;47(6):1129–1141.e5.
71. Wang Z, et al. Paradoxical effects of obesity on T cell function during tumor progression and PD-1 checkpoint blockade. Nat Med. 2019;25(1):141–51.
72. Xia A, et al. T cell dysfunction in cancer immunity and immunotherapy. Front Immunol. 2019;10:1719.
73. Wherry EJ. T cell exhaustion. Nat Immunol. 2011;12(6):492–9.
74. Deberardinis RJ, Lum JJ, Thompson CB. Phosphatidylinositol 3-kinase-dependent modulation of carnitine palmitoyltransferase 1A expression regulates lipid metabolism during hematopoietic cell growth. J Biol Chem. 2006;281(49):37372–80.
75. Patsoukis N, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat Commun. 2015;6:6692.
76. Francisco LM, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med. 2009;206(13):3015–29.
77. Eun SY, et al. 4-1BB ligand signaling to T cells limits T cell activation. J Immunol. 2015;194(1):134–41.
78. Gros A, et al. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. J Clin Invest. 2014;124(5):2246–59.
79. Chester C, Ambulkar S, Kohrt HE. 4-1BB agonism: adding the accelerator to cancer immunotherapy. Cancer Immunol Immunother. 2016;65(10):1243–8.
80. Choi BK, et al. 4-1BB signaling activates glucose and fatty acid metabolism to enhance CD8(+) T cell proliferation. Cell Mol Immunol. 2017;14(9):748–57.
81. Li L, et al. Microenvironmental oxygen pressure orchestrates an anti- and pro-tumoral gammadelta T cell equilibrium via tumor-derived exosomes. Oncogene. 2019;38(15):2830–43.
82. Muto M, et al. Myeloid molecular characteristics of human gammadelta T cells support their acquisition of tumor antigen-presenting capacity. Cancer Immunol Immunother. 2015;64(8):941–9.
83. Cheng HY, et al. Increased cholesterol content in gammadelta (gammadelta) T lymphocytes differentially regulates their activation. PLoS One. 2013;8(5):e63746.
84. Peng G, et al. Tumor-infiltrating gammadelta T cells suppress T and dendritic cell function via mechanisms controlled by a unique toll-like receptor signaling pathway. Immunity. 2007;27(2):334–48.
85. Baek AE, et al. The cholesterol metabolite 27 hydroxycholesterol facilitates breast cancer metastasis through its actions on immune cells. Nat Commun. 2017;8(1):864.
86. Gober HJ, et al. Human T cell receptor gammadelta cells recognize endogenous mevalonate metabolites in tumor cells. J Exp Med. 2003;197(2):163–8.
87. Thurnher M, Gruenbacher G. T lymphocyte regulation by mevalonate metabolism. Sci Signal. 2015;8(370):re4.
88. dos Santos CR, et al. LDL-cholesterol signaling induces breast cancer proliferation and invasion. Lipids Health Dis. 2014;13:16.
89. Rodrigues NV, et al. Low-density lipoprotein uptake inhibits the activation and antitumor functions of human Vgamma9Vdelta2 T cells. Cancer Immunol Res. 2018;6(4):448–57.
90. Nelson ER, et al. 27-hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. Science. 2013;342(6162):1094–8.
91. Dessi S, et al. Cholesterol content in tumor tissues is inversely associated with high-density lipoprotein cholesterol in serum in patients with gastrointestinal cancer. Cancer. 1994;73(2):253–8.
92. Simons K, Ikonen E. How cells handle cholesterol. Science. 2000;290(5497):1721–6.
93. Zelcer N, et al. LXR regulates cholesterol uptake through idol-dependent ubiquitination of the LDL receptor. Science. 2009;325(5936):100–4.
94. Glass CK, Saijo K. Immunology: oxysterols hold T cells in check. Nature. 2008;455(7209):40–1.
95. Bensinger SJ, et al. LXR signaling couples sterol metabolism to proliferation in the acquired immune response. Cell. 2008;134(1):97–111.
96. Janowski BA, et al. An oxysterol signalling pathway mediated by the nuclear receptor LXR alpha. Nature. 1996;383(6602):728–31.
97. Luo J, Yang H, Song B-L. Mechanisms and regulation of cholesterol homeostasis. Nat Rev Mol Cell Biol. 2019;21(4):225–45.
98. Murai T. Cholesterol lowering: role in cancer prevention and treatment. Biol Chem. 2015;396(1):1–11.
99. Ma X, et al. Cholesterol induces CD8(+) T cell exhaustion in the tumor microenvironment. Cell Metab. 2019;30(1):143–156.e5.
100. Lu Y, et al. Tumor-specific IL-9–producing CD8+Tc9 cells are superior effector than type-I cytotoxic Tc1 cells for adoptive immunotherapy of cancers. Proc Natl Acad Sci. 2014;111(6):2265–70.
101. Ma X, et al. Cholesterol negatively regulates IL-9-producing CD8(+) T cell differentiation and antitumor activity. J Exp Med. 2018;215(6):1555–69.
102. Molnár E, et al. Cholesterol and sphingomyelin drive ligand-independent T-cell antigen receptor nanoclustering. J Biol Chem. 2012;287(51):42664–74.

103. Zech T, et al. Accumulation of raft lipids in T-cell plasma membrane domains engaged in TCR signalling. EMBO J. 2009;28(5):466–76.
104. Yang W, et al. Potentiating the antitumour response of CD8(+) T cells by modulating cholesterol metabolism. Nature. 2016;531(7596):651–5.
105. Nakamura H, et al. TCR engagement increases hypoxia-inducible factor-1 alpha protein synthesis via rapamycin-sensitive pathway under hypoxic conditions in human peripheral T cells. J Immunol. 2005;174(12):7592–9.
106. Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell. 2012;148(3):399–408.
107. Semenza GL. Hypoxia-inducible factor 1 (HIF-1) pathway. Sci STKE. 2007;2007(407):cm8.
108. Zhang Y, et al. ROR nuclear receptors: structures, related diseases, and drug discovery. Acta Pharmacol Sin. 2015;36(1):71–87.
109. Dang EV, et al. Control of T(H)17/T(reg) balance by hypoxia-inducible factor 1. Cell. 2011;146(5):772–84.
110. Shi LZ, et al. HIF1alpha-dependent glycolytic pathway orchestrates a metabolic checkpoint for the differentiation of TH17 and Treg cells. J Exp Med. 2011;208(7):1367–76.
111. Clambey ET, et al. Hypoxia-inducible factor-1 alpha-dependent induction of FoxP3 drives regulatory T-cell abundance and function during inflammatory hypoxia of the mucosa. Proc Natl Acad Sci U S A. 2012;109(41):E2784–93.
112. Hsiao HW, et al. Deltex1 antagonizes HIF-1alpha and sustains the stability of regulatory T cells in vivo. Nat Commun. 2015;6:6353.
113. Muenst S, et al. The immune system and cancer evasion strategies: therapeutic concepts. J Intern Med. 2016;279(6):541–62.
114. Cohen IJ, Blasberg R. Impact of the tumor microenvironment on tumor-infiltrating lymphocytes: focus on breast cancer. Breast Cancer (Auckl). 2017;11:1178223417731565.
115. Kleinfeld AM, Okada C. Free fatty acid release from human breast cancer tissue inhibits cytotoxic T-lymphocyte-mediated killing. J Lipid Res. 2005;46(9):1983–90.
116. Richieri GV, Kleinfeld AM. Free fatty acids are produced in and secreted from target cells very early in cytotoxic T lymphocyte-mediated killing. J Immunol. 1991;147(8):2809–15.
117. Gorjao R, et al. Regulation of human lymphocyte proliferation by fatty acids. Cell Biochem Funct. 2007;25(3):305–15.
118. Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Power surge: supporting cells "fuel" cancer cell mitochondria. Cell Metab. 2012;15(1):4–5.
119. Martinez-Outschoorn UE, et al. Energy transfer in "parasitic" cancer metabolism: mitochondria are the powerhouse and Achilles' heel of tumor cells. Cell Cycle. 2011;10(24):4208–16.
120. Gazi E, et al. Direct evidence of lipid translocation between adipocytes and prostate cancer cells with imaging FTIR microspectroscopy. J Lipid Res. 2007;48(8):1846–56.
121. Nieman KM, et al. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. Nat Med. 2011;17(11):1498–503.
122. Wang YY, et al. Mammary adipocytes stimulate breast cancer invasion through metabolic remodeling of tumor cells. JCI Insight. 2017;2(4):e87489.
123. Howe LR. Inflammation and breast cancer. Cyclooxygenase/prostaglandin signaling and breast cancer. Breast Cancer Res. 2007;9(4):210.
124. Miller AM, Pisa P. Tumor escape mechanisms in prostate cancer. Cancer Immunol Immunother. 2007;56(1):81–7.
125. Miao J, et al. Prostaglandin E2 and PD-1 mediated inhibition of antitumor CTL responses in the human tumor microenvironment. Oncotarget. 2017;8(52):89802–10.
126. Alzabin S, et al. Hematopoietic progenitor kinase 1 is a critical component of prostaglandin E2-mediated suppression of the anti-tumor immune response. Cancer Immunol Immunother. 2010;59(3):419–29.
127. Krysan K, et al. COX-2-dependent stabilization of survivin in non-small cell lung cancer. FASEB J. 2004;18(1):206–8.
128. Karavitis J, et al. Regulation of COX2 expression in mouse mammary tumor cells controls bone metastasis and PGE2-induction of regulatory T cell migration. PLoS One. 2012;7(9):e46342.
129. Bergmann C, et al. Expansion of human T regulatory type 1 cells in the microenvironment of cyclooxygenase 2 overexpressing head and neck squamous cell carcinoma. Cancer Res. 2007;67(18):8865–73.
130. Sharma S, et al. Tumor cyclooxygenase-2/prostaglandin E2-dependent promotion of FOXP3 expression and CD4+ CD25+ T regulatory cell activities in lung cancer. Cancer Res. 2005;65(12):5211–20.
131. Yuan XL, et al. Elevated expression of Foxp3 in tumor-infiltrating Treg cells suppresses T-cell proliferation and contributes to gastric cancer progression in a COX-2-dependent manner. Clin Immunol. 2010;134(3):277–88.
132. Gonnermann D, et al. Resistance of cyclooxygenase-2 expressing pancreatic ductal adenocarcinoma cells against gammadelta T cell cytotoxicity. Onco Targets Ther. 2015;4(3):e988460.
133. Wong JL, et al. Synergistic COX2 induction by IFNgamma and TNFalpha self-limits Type-1 immunity in the human tumor microenvironment. Cancer Immunol Res. 2016;4(4):303–11.
134. Gupta RA, Dubois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer. 2001;1(1):11–21.
135. Dannenberg AJ, Subbaramaiah K. Targeting cyclooxygenase-2 in human neoplasia: rationale and promise. Cancer Cell. 2003;4(6):431–6.
136. Yun SJ, et al. Regulation of TIM-3 expression in a human T cell line by tumor-conditioned media and

cyclic AMP-dependent signaling. Mol Immunol. 2019;105:224–32.
137. Mandapathil M, et al. Adenosine and prostaglandin E2 cooperate in the suppression of immune responses mediated by adaptive regulatory T cells. J Biol Chem. 2010;285(36):27571–80.
138. Mandapathil M, Whiteside TL. Targeting human inducible regulatory T cells (Tr1) in patients with cancer: blocking of adenosine-prostaglandin E(2) cooperation. Expert Opin Biol Ther. 2011;11(9):1203–14.
139. Whiteside TL. Regulatory T cell subsets in human cancer: are they regulating for or against tumor progression? Cancer Immunol Immunother. 2014;63(1):67–72.
140. Qian X, et al. Increased Th17 cells in the tumor microenvironment is mediated by IL-23 via tumor-secreted prostaglandin E2. J Immunol. 2013;190(11):5894–902.
141. Chapkin RS, et al. Immunomodulatory effects of (n-3) fatty acids: putative link to inflammation and colon cancer. J Nutr. 2007;137(1 Suppl):200s–4s.
142. Turbitt WJ, et al. Fish oil enhances T cell function and tumor infiltration and is correlated with a cancer prevention effect in HER-2/neu but not PyMT transgenic mice. Nutr Cancer. 2015;67(6):965–75.
143. Song M, et al. Marine omega-3 polyunsaturated fatty acid intake and risk of colorectal cancer characterized by tumor-infiltrating T cells. JAMA Oncol. 2016;2(9):1197–206.
144. Jaudszus A, et al. Evaluation of suppressive and pro-resolving effects of EPA and DHA in human primary monocytes and T-helper cells. J Lipid Res. 2013;54(4):923–35.

Lipid Metabolism and Tumor Antigen Presentation

11

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Abstract

Tumors always evade immune surveillance and block T cell activation in a poorly immunogenic and immunosuppressive environment. Cancer cells and immune cells exhibit metabolic reprogramming in the tumor microenvironment (TME), which intimately links immune cell function and edits tumor immunology. In addition to glucose metabolism, amino acid and lipid metabolism also provide the materials for biological processes crucial in cancer biology and pathology. Furthermore, lipid metabolism is synergistically or negatively involved in the interactions between tumors and the microenvironment and contributes to the regulation of immune cells. Antigen processing and presentation as the initiation of adaptive immune response play a critical role in antitumor immunity. Therefore, a relationship exists between antigen-presenting cells and lipid metabolism in TME. This chapter introduces the updated understandings of lipid metabolism of tumor antigen-presenting cells and describes new directions in the manipulation of immune responses for cancer treatment.

Keywords

Lipid metabolism · Antigen presentation
Immune response · Tumor microenvironment

11.1 Introduction

Among the most important biological components, lipids participate in many key biological functions, including maintaining steady-state levels of membrane biosynthesis, serving as energy storage sources, and playing pivotal roles as inflammatory mediators in immunity and cancer [1]. Cellular lipid metabolism importantly facilitates the functions of immune cells [2]. Increasing evidence suggests that tumor metabolism, including lipid metabolism, inhibits the antitumor response. Over the past decade, studies have demonstrated the importance of the immune system in affecting the outcome of cancer. Tumor antigen processing and presentation play vital roles in the antitumor immune response, and several studies reported that antigen-presenting cells, especially dendritic cells (DCs), are influenced by lipid metabolism, resulting in tumor progression. In this chapter, we summarize the current reports and recent advances in lipid metabolism and tumor antigen presentation.

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11.2 Antigen Presentation

Antigen processing and presentation refer to the ability of an antigen-presenting cell (APC) to process and present antigenic peptides to antigen-specific T cells, which is a complex biological process with many molecular contributors and involves phagocytosis, antigen processing, peptide loading, localization of major histocompatibility complex (MHC) molecules to the cell surface, and T cell binding. Antigen processing can be depicted in a simplistic manner as the degradation of large antigens into smaller fragments, which are compatible with binding to antigen-presenting molecules [3]. In addition, the MHC/peptide complex together with costimulatory molecules and secretion of pro-inflammatory cytokines induce an appropriate immune response via interactions with T cells [4]. Antigenic peptides present antigens to T cells in two ways: on the one hand, they present endogenous antigens to $CD8^+$ T cells through endogenous pathways of MHC Class I (MHC-I); on the other hand, they bind to MHC Class II (MHC-II) molecules through exogenous pathways and present them to $CD4^+$ T cells (Fig. 11.1).

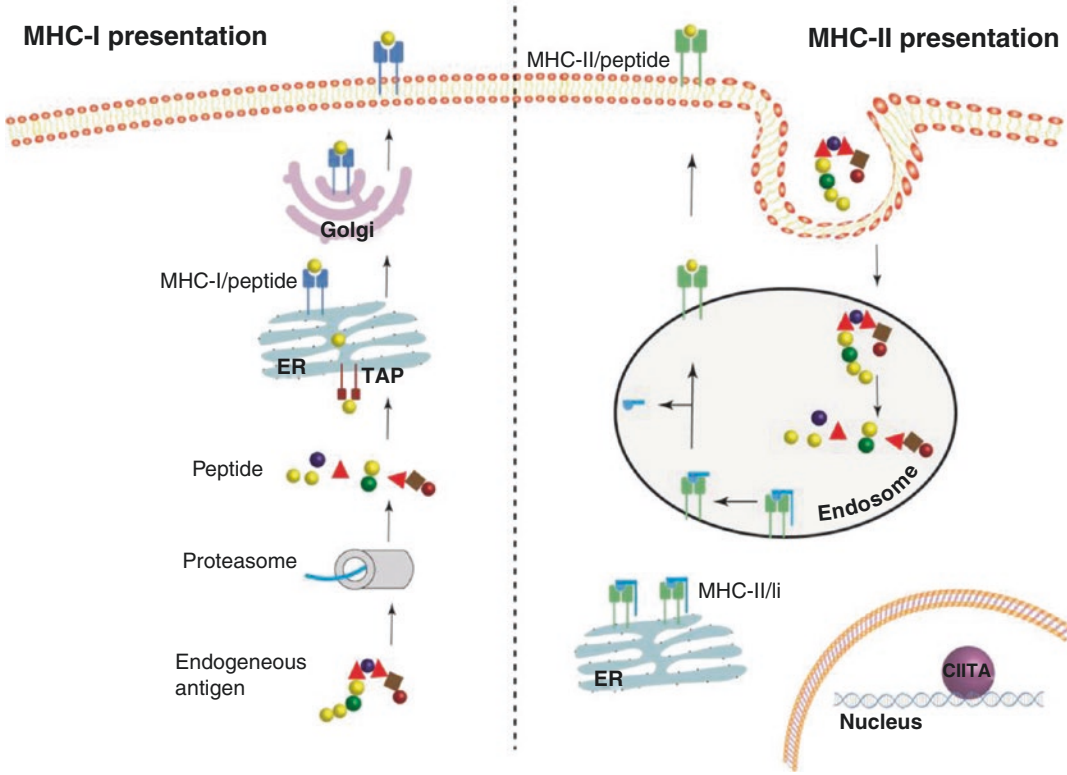

Fig. 11.1 Classical antigen presentation. (1) Classical MHC class I (MHC-I) presentation: endogenous antigen is degraded into peptides by proteasomes in the cytosol, transporter-associated with antigen processing (TAP) translocates the peptides into the endoplasmic reticulum (ER) and combines the peptides with MHC-I, and then the MHC-I/peptide complex is transported to the antigen-presenting cell (APC) membrane through the Golgi complex. (2) Classical MHC class II (MHC-II) presentation: exogenous antigens are internalized by professional APC, degraded into peptides in endosomes, and bound to MHC-II provided by ER. Then, MHC class II/peptide complexes are delivered to the cell surface and interact with the T cell. In addition, MHC-II molecule expression is promoted by the MHC class II transactivator (CIITA), and invariant chain (li) is proteolyzed by cathepsin and replaced by peptide

11.2.1 MHC Class I Presentation

MHC-I presentation refers to that in the cytosol, endogenous antigens are degraded into small molecular antigenic peptides by proteasomes, and the treated peptides are translocated by endoplasmic reticulum (ER), after modified by aminopeptidases, peptides bind to newly synthesized MHC-I molecules to form an antigen–peptide–MHC-I molecular complex, which is recognized by $CD8^+$ T lymphocytes on the cell surface. Endogenous antigens, including tumor antigens, are processed in this manner. If self-peptides are produced by tumor cells, they trigger an antitumor response. In contrast, if self-peptides are produced in normal host cells, it can lead to autoimmunity or tolerance [5].

MHC-I molecules are expressed in most nucleated cell types. Peptides binding to MHC-I molecules are produced through a two-step proteolytic mechanism: they are produced by cytoplasmic proteases, and then modified by aminopeptidase. These peptides are joined with MHC-I molecules in the ER. The degradation of most intracellular proteins and endogenous antigens in cells is accomplished by proteasome. After cytoplasmic protein is dissolved, following cytosolic proteolysis, antigenic peptides are recruited into the ER and translocated into the endoplasmic reticulum lumen via the transporter associated with antigen processing (TAP), which is composed of TAP1 and TAP2 [6]. The proteins ERp57, tapasin and the calnexin–calreticulin system certainly compose of peptide-loading complex (PLC) with TAP. Peptides loaded onto MHC-I molecules are only 8- to 11-amino acid residues long, while peptides can be much longer after the TAP translocation.. Finally, high-affinity peptide/MHC-I complexes are transported to the cell membrane via the Golgi body, triggering an antigen-specific $CD8^+$ T cell response.

11.2.2 MHC Class II Presentation

Unlike the MHC-I molecules expressed in most nuclear cell types, MHC-II molecules are constitutively expressed in a small number of immune cells, such as DCs, macrophages, and B lymphocytes. MHC-II molecules can also be expressed in restricted types of endothelial and epithelial–mesenchymal cells under inflammatory conditions. Transcriptional control of the MHC-II locus depends on the activity of the MHC-II transactivator (CIITA) [7]. In addition, interferon γ (IFN-γ) is the main cytokine that drives the synthesis of CIITA and induces the expression of MHC-II. Other important immunological molecular stimuli, such as Toll-like receptor (TLR) or transforming growth factor β (TGFβ) signaling, also contribute to this process. APCs take up antigens through phagocytosis, micro- or macropinocytosis, and endocytosis using Fc receptors, integrins, C-type lectin receptors, apoptotic cell receptors, and scavenger receptors. After uptake, exogenous antigens are internalized into phagosomes or endosomes [8, 9], and then, APCs process antigens into peptides that bind to MHC-II molecules to form the peptide/MHC-II complex. Invariant chain (Ii) protein, HLA-DM, HLA-DO, and other proteases are involved in this process. Peptide/MHC-II molecular complexes are very stable and can continuously present antigens, increasing the chance of matching with $CD4^+$ T lymphocytes. Finally, peptide/MHC-II complexes are transported to the plasma membrane and trigger T cell-mediated immune responses [5].

11.2.3 Cross-Presentation

Antigen cross-presentation combines both MHC-I and MHC-II pathways, and this process has received considerable attention during the last 20 years. Antigenic peptides cross-present antigens in two ways: on the one hand, they bind endogenous antigens to $CD8^+$ T cells through endogenous pathways of MHC-I; on the other hand, they bind to MHC-II molecules through exogenous pathways and present them to $CD4^+$ T cells [10, 11]. DCs are the most efficient cross-presenting cell type; however, different subtypes of DC cells have different cross-presentation abilities [12]. Two major pathways of antigen

cross-presentation have been described: the cytosolic pathway and the vacuolar pathway [9]. Proteins that are endocytosed or phagocytosed enter the cytosol. In the cytosolic pathway, antigens are transferred to the cytoplasm, processed in the proteasome, and then loaded onto the newly formed MHC class I molecules. This process may involve the participation of the ER machinery. Similar to direct presentation, this approach relies on TAP. In contrast, the vacuolar pathway is TAP independent, and exogenous proteins are degraded into peptides by lysosomal proteases within the phagolysosome or endosome in this pathway. These peptides are then loaded onto MHC-I molecules that recycle through the endocytic compartments by peptide exchange. The vacuolar pathway is less defined but is thought to occur in the endocytic compartments because antigens are resistant to proteasome inhibitors but sensitive to lysosomal proteolysis inhibitors. In addition, this pathway depends on cathepsin [13]. The use of each pathway may depend on the type of antigen and the mechanism of its uptake. Both of these two antigen presentation pathways are important in the process of cross-presentation, and existing evidence suggests that cytoplasmic pathways also play an important role [5].

11.2.4 Nonclassical MHC Presentation

The recognition of lipids and glycolipids is restricted by a family of MHC-like molecules called CD1 that have evolved from MHC by acquiring a very hydrophobic groove capable of accommodating the acyl chains of a large number of lipids. Lipid antigen is captured by the four types of human CD1 antigen-presenting molecules: CD1a, CD1b, CD1c, and CD1. At the cell surface, CD1a and CD1c readily capture exogenous lipids, whereas CD1b and CD1d do this to a lesser degree [14]. Lipid antigen binding to CD1 can stimulate natural killer T (NKT) cells. NKT cells are very important lymphocytes in both rodents and humans as these cells exhibit the unique property of recruiting natural killer (NK) cells, CD4 and CD8 T cells and B cells at the site of initial insult, coordinating the early events of DC maturation.

11.3 Antigen-Presenting Cells

APCs are cells that can intake and process antigens and present their information to T cells. Common APCs include DCs, mononuclear/macrophage cells, and B lymphocytes that express MHC-II molecules. These cells are also called professional APCs. Nonprofessional APCs include endothelial cells, fibroblasts, epithelial cells, mesothelial cells, and eosinophilic granulocytes, which also express MHC-II molecules and costimulating molecules under the stimuli of inflammatory or cytokines. Nonprofessional APCs exhibit weaker phagocytosis, processing, and presentation of antigen information abilities compared with professional APCs. In addition, all nucleated cells that express MHC-I can process endogenous antigens, including virus-related antigens and tumor antigens. These cells then present antigens to $CD8^+$ T cells called target cells. Thus, most cells are capable of acting as APCs to $CD8^+$ T cells, but only professional APCs can present antigens to $CD4^+$ T cells. DCs exhibit the strongest antigen-presenting function among APCs at present. DCs stimulate the activation and proliferation of naive T cells, which play an important role in adaptive immune responses.

11.3.1 Dendritic Cells

Dendritic cells play a pivotal role in the mediation of innate immune responses and maintenance of adaptive immune responses. DCs are divided into immature (imDCs) and mature (mDC). imDCs exhibit a high capability of phagocytosis but low expression of MHC molecules. Moreover, imDCs lack costimulatory molecules (CD40, CD80, CD86) and cannot effectively activate T cells. In contrast, mDCs exhibit high expression of MHC molecules and costimulatory molecules and activate T cells.

However, these cells do not effectively phagocytize antigens. DCs are particularly adept at initiating T cell responses, inducing T cell polarization, and presenting exogenous and endogenous antigens on either MHC-I or MHC-II [15].

DC maturation is critical for T cell expansion and differentiation, allowing T cells to become activated by making contact at the immunological synapse. DCs also activate naive and memory B cells through their ability to stimulate $CD4^+$ T cells. DCs accumulate in blood and lymphoid tissues; however, these cells are found throughout the body, i.e., skin Langerhans cells and intestinal DCs. DCs originate in bone marrow from macrophage and DC precursors (MDP), which give rise to monocytes and common DC precursors (CDP). CDP can differentiate into two major categories: classical DCs (cDCs) and plasmacytoid DC (pDCs). cDCs express CD11c and CD11b markers. Furthermore, cDCs are classified into two major subpopulations: cDC1 and cDC2 [16]. Some cDC1 cells that reside in lymphoid tissues express CD8α, whereas others not in lymphoid tissues express CD103 [17]. cDC1 focuses on binding the internalized antigen to MHC-I and presenting it to $CD8^+$ T cells in a process known as cross-presentation. $CD103^+$ DCs produce large amounts of IL-12 and play a crucial role in the antigen cross-presentation and the initial initiation of $CD8^+$ T cells. The migration of $CD103^+$ DCs from tumor environment to draining lymph nodes is regulated by CCR7, and initially prime naive $CD8^+$ T cells are started by DC in lymph nodes [18]. Among migratory DCs, $CD103^+$ cells are considered to be the main subset of cross-presenting antigens from peripheral tissues, such as skin, lung, and intestine. cDC2 cells are the main subtype of APC. Unlike DC1 cells, cDC2 express CD11b and reside in lymphoid tissues, and present endogenous internalized exogenous antigens to $CD4^+$ T cells, which is the first step of acquired immunity. The other major subset of DCs is pDCs, which specialize in the production of large amounts of type I interferon (IFN) in response to pathogen recognition and participate in antiviral immune responses. However, these cells also secrete IL-12, IL-6, tumor necrosis factor-alpha (TNF-α), and other pro-inflammatory cytokines. As APCs, pDCs also present antigens to T cells but less efficiently than cDCs. DCs can also process lipid antigens and present them on the CD1d molecule to activate NKT cells [19].

11.3.2 Macrophages

Macrophages are versatile innate immunocytes that contribute to diverse processes, express dozens of receptors, produce dozens of enzymes, and secrete hundreds of bioactive products. Thus, these cells play an important role in the body's defense and immune response. Macrophages exhibit a strong ability to intake antigens and express a variety of surface molecules related to antigen uptake, including the Fc receptor, complement receptor, mannose receptor, scavenger receptor, and TLR. Similar to DCs, macrophages also express costimulatory molecules and MHC-I/II molecules and process exogenous antigens to activated T cells. In addition, T cells secrete IFNγ, which positively activates and promotes macrophage function. Thus, macrophages also enhance self-function by presenting antigens. Numerous macrophages are located in the liver and are known as Kupffer cells. These cells suppress T cell activation induced by DCs. TLR2 and TLR4 ligation activates human Kupffer cells by inducing IL-10 synthesis. Moreover, both reactive oxygen species and TLR3 ligation increased the expression of MHC class II and promoted the APC function of these cells. Kupffer cells can switch their immunological roles via two scenarios. These cells can switch from inactivators to activators of NK cells and from tolerance-inducing APCs to immunogenic APCs [20]. Macrophage can be induced to the M1 and M2 phenotypes according to the surrounding microenvironment. Conventional M1 macrophages promote immune responses and mainly participate in cellular immunity, whereas M2 macrophages participate in humoral immunity, which is closely related to immunosuppressive ability.

11.3.3 Other APCs

B lymphocytes play an essential role in humoral immunity. As a professional APC, B cells present specific antigens to promote immunity but induce tolerance when presenting nonspecific antigens. In the presence of DCs or activated macrophages, the role of B cells in presenting nonspecific antigens is negligible. B cells that develop in the bone marrow express MHC class II molecules and this expression is maintained throughout B cell differentiation and maturation. The function of MHC-II in bone marrow-derived B cells differs from that of mature B cells given the reduced expression of CD40, CD80, and CD86 as well as minimal MHC class II-associated invariant chain peptide (CLIP) on their HLA-DR molecules, HLA-DO is lacking, which inhibits DM function and attenuates its peptide-loading activity [21]. Exogenous protein gains access to B cells through fluid-phase pinocytosis or B cell receptor (BCR)-mediated endocytosis. BCR-mediated presentation of specific antigen is far more efficient than presentation in pinocytotic antigens and subsequent T cell activation.

Endothelial cells are recognized as nonprofessional APCs and include vascular endothelial cells (VECs) and lymphatic endothelial cells (LECs). Liver sinusoidal endothelial cells (LSECs) are a typical type of VECs that express various scavenger receptors, C-type lectin receptors, and lipoprotein receptor-related protein-1 for strong endocytic ability. LSECs are not only able to present exogenous antigens on MHC-II but also on MHC-I through cross-presentation, including antigens from virus-infected hepatocytes and apoptotic tumor cells. Amazingly, LSECs cross-present soluble antigens even more efficiently than DCs [22]. Prostaglandin E2 (PGE_2) and IL-10 downregulate the expression of MHC class II, CD80 and CD86, compromising antigen-specific and costimulatory signals [20]. LECs also exhibit a strong endocytic ability and present exogenous antigen to T cells on both MHC-I/II molecules. Moreover, LECs are potent immunoregulators and inhibit DC-mediated antigen presentation.

Tumor cells are regarded as target cells that express MHC class I molecules; process mutated autoantigens, i.e., tumor antigens; and present antigenic information to $CD8^+$ T cells in the form of antigenic peptide/MHC-1 molecular complex. Researchers recently reported that some types of tumors express MHC-II molecules, and upregulation of MHC-II expression prolongs the survival time of tumor patients [23].

11.4 Tumor Antigen Presentation

Tumor antigens originate from endogenous self-antigens, which are poorly immunogenic and subject to changes during tumor progression. In the early stage of cancer development, the immune system generates tumor antigen-specific $CD8^+$ T cells; therefore, tumor cells must clearly use additional approaches to escape immune recognition. Several requirements must be met for antigen presentation to efficiently stimulate anti-tumor T cell responses: (a) the appropriate type of DC effectively recognize and capture tumor antigens; (b) antigens were processed into antigenic peptides and expressed on the surface of DCs; and (c) fully enhance the expression of DC costimulation molecules to ensure the effective activation of T cells [24]. As a tumor grows, tumor cells attempt to become “invisible” to the immune system by modifying the MHC-I antigen loading and presentation pathway. Thus, when cancer progresses, MHC-I expression is downregulated or lost. In general, tumors exhibit broad dysregulation of antigen presentation, especially B cell malignancies. However, malignant cells can affect the antigen presentation function of DCs through various mechanisms, on the one hand, disabling the generation of tumor-associated antigen-specific T cells, and on the other hand, increasing the tolerance of immune cells to tumors [25].

Thus, tumor antigens derived from apoptotic cells are captured by immature DCs, and antigen presentation by these cells likely results in immune tolerance [26]. Immune escape of tumor cells is mainly to block the process of tumor anti-

gen presentation. Antigen presentation has two important processes in antitumor immunity. First, APC activates naive T cells. Second, activated cytotoxic effector T cells recognize target antigens that bind to MHC-I. Second, activated cytotoxic effector T cells recognize target antigens that bind to MHC-I [27]. The dominant paradigm of tumor immunology dictates that the efficient cytotoxic T lymphocytes (CTL) initiation requires the uptake of tumor antigens by DCs in the peripheral tumor area. These cells then migrate to draining lymph nodes and present the antigens to $CD8^+$ T cells in the context of MHC-I [23, 28]. DCs have access to a large amount of tumor antigens via numerous mechanisms, such as phagocytosis/endocytosis of cell-associated or soluble antigens bound to heat shock proteins, gap junction transfer through the capture of exosomes, or "cross-dressing" [29, 30].

Moreover, tumor antigen is cross-presented by professional APCs, such as DCs, via the MHC-I pathway. Thus, understanding and exploiting cross-presentation is becoming a very important topic in cancer immunotherapy because it affects a variety of key issues, including the development of more efficacious vaccines [31]. The selective pressure of $CD8^+$ T cells on tumor cells themselves and immunoediting by malignant cells help to limit T cells' attack of tumor cells [32, 33]. Tumors also inhibit the function of the proteasome, thereby reducing the quantity and quality of antigenic peptides for binding to MHC-I [34]. Disruption of MHC-I function in tumor cells is a common method by which tumors prevent T cell recognition, but we are not aware of dysregulation of MHC-I expression in DCs in the tumor microenvironment [27]. In 2003, Nowak et al. found that induction of apoptosis in tumor cells increased the cross-presentation of tumor antigens and the activation of specific $CD8^+$ T cells, thereby inhibiting tumor growth [35]. Importantly, Sec22b-dependent antigen cross-presentation is important in the treatment of anti-programmed death 1(PD-1). Another study also illustrated that both cross-presented DC subsets, migratory $CD103^+$ DCs, and resident $CD8^+$ DCs are necessary for the effectiveness of anti-PD-1 therapy and radiotherapy for tumors [36]. In addition, tumor antigens are occasionally cross-expressed with MHC-II, which is controlled by the APC-specific regulator of transcription CIITA. In summary, cross-presentation seems to play a critical role both in inducing antitumor $CD8^+$ cytotoxicity and in regulating the outcome of anti-immune checkpoint therapies.

11.4.1 Tumor Antigen Presentation by Dendritic Cells

DCs are professional APCs that can endocytose cell debris or dead tumor cells and transport cancer-associated antigens to the draining lymph node [37]. These cells then present tumor antigens to T lymphocytes and express high levels of costimulatory or coinhibitory molecules that determine immune activation or immunosuppression [38, 39]. Moreover, DCs consistently activate cancer-specific T helper cells and CTL and mediate the early stage of the antitumor response. In general, DCs remain in a dormant immature state and gradually mature after capturing, recognizing, and internalizing specific tumor antigens in peripheral tissues. DCs express a series of pattern-recognition receptors, including TLRs, that allow them to recognize microbial products or inflammatory stimuli and respond quickly. After encountering tumor antigens, DCs are activated via a process that involves enhanced capturing and processing of antigens for the stable presentation of antigen-derived peptides in the context of MHC-I/II and induction of the expression of genes encoding chemokine receptors, cytokines, and costimulatory molecules. Internalized antigen is processed, loaded onto MHC-I/II molecules, and then presented to $CD8^+$ and $CD4^+$ T cells, respectively [40]. Antigen presentation in MHC class I molecules is important for the induction of $CD8^+$ cytotoxic effector lymphocytes, which are essential for clearing tumor cells. Collectively, these changes enable DCs to promote local inflammation and traffic to T cell zones of secondary lymphoid organs, where they prime T cell responses [41]. Different subsets of

DCs are equipped to induce different types of T cell responses. In addition, the location and ability to capture tumor antigens also regulate DCs processing of antigens and subsequent T cell responses. Cross-presentation of antigens is a unique feature of DCs that is very important for antitumor immunity. DCs produce biologically active IL-12 p70, inducing remarkable anticancer immunity by potentiating the activity of NK cells.

The role of pDCs in cancer is thought to be tolerogenic, and high tumor infiltration by pDC is associated with poor prognosis [42]. During the presentation of tumor cell antigens, pDCs, which are unable to internalize cell membrane fragments by phagocytosis, can efficiently acquire membrane patches and associated molecules from cancer cells of different histotypes. The transfer of membrane patches to pDCs occurs in a very short time and requires cell-to-cell contact. Membrane transfer also included intact human leukocyte antigen (HLA) complexes such that tumor-specific $CD8^+$ T cells efficiently recognized the antigens acquire by pDCs [43]. Defects in DC function have been well documented in tumor-bearing patients or mice with advanced disease. These defects manifest in the expansion of immature DCs, which are unable to properly present antigen, and the generation of cells with immune-suppressive activity, including regulatory DCs and myeloid-derived suppressor cells (MDSCs) [44].

In general, mature DCs are considered immune-stimulatory, whereas immature DCs are considered suppressive and tolerogenic. Moreover, increased imDCs, decreased mDC, and DCs with impaired functions are observed in the cancer microenvironment [45]. Tumor cells can secrete IL-10 and IL-6 to impair DC maturation by downregulating both MHC-II and lymph node-homing receptor CCR7 expression and activating signal transducers and activators of transcription 3 (STAT3). Immature and paralyzed tumor-infiltrating DCs (TIDCs) suppress both innate and adaptive immune responses through a variety of mechanisms [46]. TIDCs showed reduced expression of costimulatory molecules and reduced antigen cross-presentation ability [47] and increased expression of related molecules and receptors regulating immunosuppression [48]. TIDCs are characterized by high expression of IL-10 and low IL-12 secretion and induce $FoxP3^+$ Treg differentiation from naive $CD4^+$ T cells [23]. In melanoma, TIDC frequency tends to be increased in the peritumoral area, and these cells exhibit a more mature phenotype. In contrast, more immature TIDCs are found within the tumors [49]. DCs' functional plasticity is complicated in the tumor microenvironment (TME), which makes it difficult to generalize its role in TME. TIDCs as a group exhibited poor response to TLR stimulation in terms of antigen presentation capability. Data showed that the TIDCs coexpress PD-1 and programmed death 1 ligand (PD-L1). Murine DCs expressed low levels of PD-1 in the early stage of tumor growth; however, as the disease progresses, almost all TIDCs eventually have high levels of PD-1, which is induced by the transcription factor STAT3. In vitro, blocking PD-1 signaling in TIDCs enhances the production of immune-stimulatory cytokines, increases the activation of NF-κB in DCs, improves the expression of costimulatory molecules, and improves the ability of these DCs to activate T cells [48, 50]. TIDCs can also inhibit tumor immunity by upregulating the expression of T cell Ig and mucin domain 3 (TIM-3).TIM-3 is an inhibitory marker of Th1-type T cells. Various factors present in both murine and human tumors induce upregulation of TIM-3 in DCs. The immune-activating potential of TIDCs is a balance between multiple inhibitory and activating molecules. Besides PD-L1, DCs also have other mechanisms to block T cell activation. Liu et al. [51] reported that PGE_2 and TGF-β which murine lung tumor cells released transformed immune-activating DCs into immune-suppressive DCs. Tumor-derived PGE2 induces indoleamine 2,3-dioxygenase (IDO), which is expressed in TIDCs and plays an important role in mediating the suppression of adaptive immune responses [52]. TIDCs also suppress adaptive immune responses indirectly by induction of Treg [53].

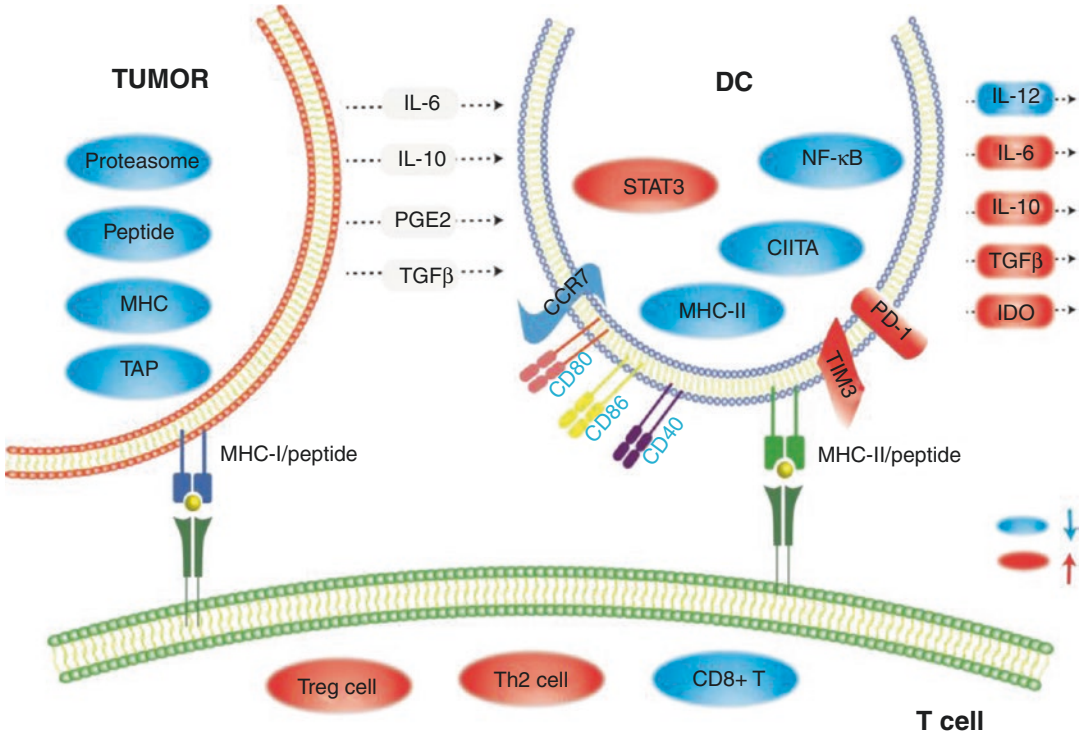

Fig. 11.2 Tumor antigen presentation by DCs. Tumor cells with MHC-I mutations, inhibition proteasome function, and downregulation of TAP lead to reduced expression of MHC-I/peptide. In addition, tumor cells secrete IL-6, IL-10, transforming growth factor β (TGFβ) and prostaglandin E2 (PGE2), thus inhibiting the production of IL-12 and decreasing expression of peptide/MHC-II, costimulatory molecules on DCs. In addition, programmed cell death protein 1 (PD-1), T cell Ig, and mucin domain 3 (TIM-3) are upregulated on the DC surface. Furthermore, activation of transcription factor activators of transcription 3 (STAT3) and attenuated NF-κB signaling suppress DC maturation and promote DC dysfunction. Additional defects in MHC-II expression by downregulation of CIITA are noted. Here, tumor-infiltrating DCs suppress $CD8^+$ T cell activation, skewing $CD4^+$ T cell differentiation toward the Th2 phenotype and increase Treg cells

TIDCs interact with other immune cells, including NK cells and B cells. The impact of factors on tumor antigen presentation by DCs is summarized in Fig. 11.2.

11.4.2 Tumor Antigen Presentation by Macrophages

Numerous types of immune cells are found in advanced tumors, and macrophages are the most abundant. Macrophages are extremely versatile and adopt different activation states or phenotypes in response to signals under different circumstances. Macrophages exhibit both anti- and pro-tumor functions by regulating tumor growth, angiogenesis, invasion, and metastasis [54]. Macrophages are innate immune cells in tissues and their main function is phagocytosis, functioning as the host's first line of defense against pathogens [55]. Macrophages can redirect antigens away from cDCs, reduce the presence of danger signal molecules or damage-associated molecular patterns (DAMPs) by clearance of apoptotic cells and debris and suppress their own activation in response to apoptotic cell phagocytosis [56].

As mentioned above, macrophages are classified into pro-inflammatory M1 and anti-inflammatory M2 cell types. M1 type cells are induced by IFNγ and lipopolysaccharide (LPS); express high levels of MHC molecules, espe-

cially MHC-II; and play crucial roles in pathogen clearance and tumor antigen presentation. M2 type cells are induced by IL-4 and IL-10 and express moderate levels of MHC molecules and IL-12, but these cells produce abundant anti-inflammatory cytokine to promote immunosuppression, tumor infiltration, and metastasis [57]. Unlike DCs, macrophages are generally tissue-resident cells, serving as the first cell to recognize and phagocytose antigens, including tumor cells in the host, and then present them to T cells.

Tumor-associated macrophages (TAM) are a unique group of macrophages, and most TAMs are M2 type. TAMs are important regulators of tumorigenesis that are either tissue-resident cells or derived from peripheral reservoirs, such as the bone marrow and spleen [58]. Depletion of TAMs markedly decreased tumor growth in mice, illuminating the importance of these cells for tumor progression [59]. TAMs lack costimulatory signals, such as CD80/CD86 coreceptors which are the second signals required for T cell activation. In the absence of a costimulatory signal, T cells can be expanded to unresponsive or anergic cells to induce immunotolerance [60]. Furthermore, TAMs and tumor cells secrete immunosuppressive cytokines, such as IL-10, TGFβ1, and PGE2, resulting in downregulation of MHC class II molecules in macrophages not only in the TME but also in the distant spleen and peritoneum, where TAMs exhibit dysfunctional antigen presentation [61].

Recent studies identified a set of macrophages with a unique distribution in secondary lymphoid organs called CD169$^+$ macrophages. These macrophages were identified as lymph node-resident APCs that dominate the early activation of CD8$^+$ T cells. The CD169 molecule is highly expressed by macrophages found in the subcapsular sinus and the medulla of lymph nodes and marginal zone in the spleen [62]. CD169$^+$ macrophages in regional lymph nodes promote CD8$^+$ T cell-mediated antitumor immunity and are associated with a better prognosis for colorectal and endometrial carcinoma patients. The density of CD169$^+$ macrophages exhibits a positive correlation with the number of CD8$^+$ cytotoxic T cells infiltrating tumor tissues [63, 64]. These cells can activate invariant natural killer T (iNKT) cells and CD8$^+$ T cells via two different mechanisms: directly present antigen to CD8$^+$ T cells or indirectly transfer antigens to DCs in the spleen [65]. Asano, K [66] reported that CD169$^+$ macrophages phagocytose dead tumor cells transported via lymphatic flow and subsequently cross-present tumor antigens to CD8$^+$ T cells. Moreover, CD169$^+$ macrophages capture exosomes and mediate the immune response to exosomal antigen [67]. Via this function, CD169$^+$ macrophages control the dissemination of tumor-derived extracellular vesicles and reduce their pro-tumorigenic potential [68]. Therefore, targeting tumor-related macrophages for cancer treatment may represent an attractive approach to prevent tumor progression.

11.4.3 Tumor Antigen Presentation by Other APCs

Most tumor cells are able to present self-antigens to CD8$^+$ T cells through the MHC-I pathway. In fact, tumor cells lose or downregulate their MHC class I molecules and other costimulatory signals to prevent antigen presentation [69]. Studies have demonstrated that high levels of MHC-I activate adaptive responses, the complete lack of MHC-I leads to cytotoxic NK cell-mediated tumor clearance, and low MHC-I leads to tumor progression [70–72]. In addition, tumor cells lack costimulatory signals to present antigens; express high levels of inhibitory ligands, such as PD-L1; and clear tumor-specific T lymphocytes. Interestingly, some types of tumors express MHC-II, and MHC-II expression is associated with tumor regression, increased cytotoxic T cells, and increased overall patient survival [73, 74].

B lymphocytes are considered professional APCs and express a specialized B cell receptor (BCR). B lymphocytes also express MHC-I and II as well as costimulatory molecules that allow them to sense and process antigens from a variety of sources. In cancer, the presence of B cells or tumor-specific antibodies is associated with

tumor progression. In primary tumors, like lung cancer, breast cancer, and colorectal cancer, the accumulation of tumor-specific antibodies is related to poor prognosis and late-stage disease [75–77]. Although B cells have a dual role in tumor immunity through their cellular and humoral responses, there is a defect in the B cells that presents tumor antigens to $CD4^+$ T cells. On one hand, this presentation leads to the inhibition of cytotoxic T lymphocyte activity. On the other hand, it leads to a tumor humoral immune response to B cells. For example, in diffuse large B cell lymphoma, the antigen presentation of B cells is dysregulated because MHC-II expression is reduced by downregulation of CIITA and mutations within the MHC-II locus itself. In addition, in Hodgkin's B cell lymphoma, HLA-DM fails to remove CLIP from the MHC-II peptide and influence the expression of MHC-II/peptide compounds [27].

Endothelial cells, including vascular endothelial cells (VECs) and lymphatic endothelial cells (LECs), exhibit the ability for antigen presentation to regulate immunotolerance in cancer. Treatment with monoclonal antibodies against vascular endothelial growth factors (VEGF) and VEGF-receptors (VEGFR) restores tumor immunity. Liver sinusoidal vascular endothelial cells (LSEC) process tumor antigens from apoptotic cells and cross-present them to $CD8^+$ T cells, inducing tumor immunotolerance [78]. Moreover, VECs express immunosuppressive molecules and inhibitory ligands, such as B7-H3, and PD-L1/PD-L2, to inhibit antitumor immunity [79–81]. LECs are an important component of the structure of primary and secondary lymphoid tissues, where the maturation and activation of immune cells occurs, and these cells play a critical role in tumor escape and metastasis. LECs can cross-present tumor antigens to $CD8^+$ T cells in draining lymph nodes and cause CTL inhibition and deletion. This process is dependent on the secretion of VEGF-C by tumor cells [82]. Other cells, such as neutrophils, mast cells, and eosinophils, also participate in antitumor immunity, but the role of these cells in antigen presentation remains controversial.

11.5 Lipid Metabolism in Tumor APCs

The role of lipid metabolism in the regulation of immune cells has aroused general concerns. Several lines of evidence have demonstrated the importance of tumor immune metabolic reprogramming. Lipids are critical in malignant tumors as they are necessary not only for providing the membrane constituents of proliferating cells but also for energetic, biophysical, and signaling pathways that drive tumorigenesis [83, 84]. Lipid depletion in $CD8^+$ T cells dramatically inhibits cell proliferation and signal transduction, which partly explains the lower number of $CD8^+$ T cells in cancer tissues compared with adjacent tissues [85]. Evidence suggests that alterations in tumor lipid metabolism, including metabolite abundance and accumulation of lipid metabolic products, lead to local immunosuppression in the TME [86]. Unlike normal cells, cancer cells take up fatty acid (FA) from the microenvironment and exhibit a high de novo lipid synthesis rate, suggesting FA accumulation in tumor cells. Many studies have focused on the effects of lipid reprogramming on the tumor immune response, but a few have reported the effects on antigen presentation function.

11.5.1 Lipid Metabolism in DCs

Pathological impairment of DC function is considered a cause of decreased tumor immunity in cancer patients. To avoid the immune response, the maturation or differentiation of DCs is suppressed in several tumors. For example, DCs from hepatocellular carcinoma (HCC) patients exhibit an impaired ability to trigger immune responses, thus promoting immunosuppression [46]. One study confirmed that both NF-κB and STAT3 signaling pathways were simultaneously repressed by cancer sera, suggesting that attenuated NF-κB and STAT3 signaling could be a leading cause of DC dysfunction in cancer [87].

The adverse effect of dietary lipid intake on DC functions has been confirmed by many stud-

ies. Lipid accumulation in DCs was observed in many tumors, such as lung cancer, renal cell carcinoma, colon carcinomas, and thymic lymphomas. Several studies showed that lipid accumulation in DCs in cancer patients might suppress DC function, which subsequently reduces antitumor immunity [88, 89] as well as the expression of costimulatory molecules and DC-related cytokines. Arai, R et al. showed that lung cancer patients had significantly fewer DCs than healthy individuals, especially the number of myeloid DCs (mDcs), and patients with higher-stage cancers had a significantly reduced number of mDCs. In addition, DCs from stage IV lung cancer patients exhibit increased lipid content and reduced T cell proliferation compared with early-stage patients, and further tests revealed higher levels of triglycerides (TAG) in mDCs but not in pDCs [90]. Gardner, Gardner J. K. et al. showed that mesothelioma tumors and their secreted factors promote DC lipid accumulation, reduce DC numbers, in particular cross-presenting $CD8\alpha^{+}CD4^{-}$DCs [91]. TAGs are the main lipid components that accumulate in DCs. Some studies reported no changes in the level of phospholipids and cholesteryl-esters, whereas others observed minimal increases in cholesterol in these DCs. Gao, F et al. reported that lipoprotein lipase (LPL), fatty acid-binding protein (FABP), and the level of triacylglycerol (TAG) in serum increased in mouse thymic lymphomas, contributing to lipid accumulation in DCs. LPL increases the uptake of lipids, and FABP plays roles in fatty acid uptake, transport, and metabolism. The mechanisms of lipid accumulation are unclear. The expression of macrophage scavenger receptor (Msr-1) is increased in DCs with high lipid accumulation, and this protein can increase lipid uptake, specifically TAG, which reduces the ability of DCs to process tumor antigens and stimulate T cell proliferation [88, 92]. In breast cancer, Nadine M. Lerret [92] showed that a single dose of irradiation leads to the downregulation of Msr-1 on DCs within the tumor and reduces lipid uptake of tumor-resident DCs, potentially enabling the DCs to present tumor antigen more efficiently and contribute to tumor clearance. However, in lung cancer patients, increased expression of Msr-1 on the surface of peripheral blood DCs was not observed. Zapata-Gonzalez et al. reported that fatty acids regulate the activity of human-derived DCs mainly via peroxisome proliferator-activated receptor-γ (PPAR-γ) [93]. PPAR-γ primarily acts as a positive transcriptional regulator in human developing DCs by controlling genes involved in lipid metabolism, such as ABCG1, ANGPTL4, CPT1A, and CD36 [94]. PPARγ is highly upregulated during monocyte-derived DC differentiation, and PPARγ-instructed DCs exhibit enhanced phagocytic activity and a modified cytokine-production profile. These cells exhibit increased NKT cell activating capacity. Lipid/fatty acid metabolism-related categories were overrepresented among the genes upregulated by PPARγ ligand. Everts, B. et al. found that during glycolysis, TLR can drive the generation of citrate, which increases the de novo synthesis of fatty acids in DC cells, and the expansion of ER and Golgi promotes the activation of DC, impacting their antigen-presenting ability [95]. This model of glycolysis supports the de novo synthesis of fatty acids by generating nicotinamide adenine dinucleotide 2′-phosphate reduced tetrasodium salt (NADPH) through the pentose phosphate pathway (PPP) and by providing the carbons to supplement intermediates of the tricarboxylic acid (TCA) cycle that are extracted from the mitochondrial TCA cycle in the form of citrate or isocitrate for the synthesis of fatty acids. Notably, the immunogenicity of DCs with high lipid content was diminished when fatty acid synthesis (FAS) in these cells was inhibited. These results support the notion that the de novo synthesis of fatty acid is an integral component of DC activation and is required for the acquisition of an immunogenic phenotype. In pathogen-infected disease, dyslipidemia inhibited TLR-induced production of pro-inflammatory cytokines, including IL-12, IL-6, and TNFα, as well as upregulation of costimulatory molecules by $CD8\alpha^{-}$ DCs. In addition, oxidized low-density lipoprotein (oxLDL) was the key active component responsible for this effect because it directly uncouples TLR-mediated signaling on $CD8\alpha^{-}$ myeloid DCs and inhibits NF-κB nuclear translo-

cation. Previous studies demonstrated that different types of fatty acids have different effects on TLR. Saturated fatty acids activate TLRs, while $n-3$ polyunsaturated fatty acids inhibit TLR activation. TLRs provide critical signals to induce innate immune responses in DCs that are subsequently linked to adaptive immune responses.

Recently, lipid droplets (LDs) in tumor-associated dendritic cells (TADCs) have attracted considerable attention. In addition, the accumulation of lipids in DCs manifests in the formation of large LDs. The presence and size of LDs are defined by the accumulation of fatty acid precursors and their esterification into TAGs and cholesterol esters, which are the major constituents of the hydrophobic core of LDs. In some cancers, TADCs express scavenging receptors, such as Msr-1, which facilitates lipid uptake and accumulation. These findings support the role of these cells in immunogenic immune responses and cross-presentation [88, 96]. Researchers report that tumor-derived factors trigger lipid peroxidation in TADCs, and this process is mediated by X-box binding protein 1 (XBP1). XBP1 activation subsequently induces the lipid biosynthetic program, which results in the accumulation of LDs and blunted antigen presentation, leading to tumor progression [97]. Furthermore, the oxidized lipids in TADCs also affect cross-presentation, demonstrating that the accumulation of oxidized polyunsaturated FAs, cholesterol esters, and TAG impaired cross-presentation without altering the presentation of endogenous antigens. However, the accumulation of non-oxidized lipids does not affect cross-presentation, suggesting that oxidized lipids block cross-presentation by reducing the expression of peptide–MHC class I complexes on the cell surface. In addition, the storage of lipids and the accumulation of modified lipids altered DC function [98].

The above information demonstrates that factors influencing lipid accumulation include advanced age, serum triglyceride levels, and cancer stage. The expression of scavenger receptor B is potentiated during lipid accumulation in mouse bone marrow and spleen-derived DCs. Given that Msr-1 expression is not altered in patients, the receptors mediating DC lipid accumulation may differ between mice and humans. The mechanism of lipid deposition in DCs is related to the PPAR and NF pathways.

11.5.2 Lipid Metabolism in Macrophages

Macrophages undergo changes in their lipid profile in the tumor setting. TAMs undergo changes in lipid metabolism, including enhanced FA biosynthesis, uptake, and storage. TAMs are the predominant M2 phenotype to inhibit CTL antitumor responses in solid cancers [99]. Increased expression of multiple genes involved in lipid metabolism and lipid signaling is noted in distinct populations of macrophages. FAS enzymes are upregulated in M2-polarized macrophages, and the de novo synthesized fatty acids are at least partially used for feedback into fatty acid oxidation (FAO) [100, 101]. In addition, enhanced FAS is required for the augmentation of phagocytosis in monocytes. M1 macrophage inducers LPS and IFN-γ suppress fatty acid intake and oxidation, while M2 macrophages are likely to increase FAO. These processes may be driven by the activation of signal sensors, such as transcriptional activator 6 and PPARγ coactivator-1 beta (PGC-1β), in response to IL-4 treatment. The uptake of lipids, especially TAG, is also critical for FAO and M2 activation [100]. Other studies showed that lipid loading of macrophages is associated with increased tumoricidal and inflammatory capacity. Increasing intracellular lipid levels is associated with an increased cytotoxic activity of murine peritoneal macrophages, particularly in those that were artificially enriched with polyunsaturated FAs in contrast with those enriched in cholesterol [102]. In contrast, one study showed that monoacylglycerol lipase (MGLL) deficiency and increased cofactor of adipose triglyceride lipase abhydrolase domain containing 5 (ABHD5) expression in TAMs contribute to lipid accumulation and promote tumor progression in colorectal cancer [103]. Several studies indicated that TAMs exhibit alterations in

arachidonic acid metabolism. Arachidonic acid metabolism mediates the switch of macrophage phenotypes. For example, PGE2 released by tumor cells can transform TAM from M1 to M2, resulting in immune system evasion [104]. PGE_2, a cyclo-oxygenase (COX)-derived eicosanoid, is increased by M1 stimulation, while IL-4 induces the upregulation of 15-lipoxygenase (15-LOX) in macrophages. IL-10, IL-4, and TGFβ induce adenosine 5′-monophosphate-activated protein kinase (AMPK) activation and drive TAMs to an immunosuppressive M2 phenotype. In particular, increased COX2 expression and PGE_2 production were observed in macrophages infiltrating tumor-bearing lungs compared with the macrophages from naive lungs.

Saturated free fatty acids induce pro-inflammatory activation via TLR4, NF-κB, NLRP3, and JNK pathways in lipid metabolism. The mechanisms of lipid metabolism in TAMs are unclear, but the underlying mechanism involves peroxisome proliferator-activated receptors (PPARs), live X receptors (LXRs), and signal transducer and activator of transcription (STAT) [105, 106]. PPARγ mediates M2 macrophage polarization to promote tumor progression and metastasis. Caspase-1 inactivates medium-chain acyl-CoA dehydrogenase (MCAD) by cleaving PPARγ and induces lipid accumulation in TAMs [107]. MGLL in TAMs functions as a tumor suppressor, and its deficiency is the major contributor to lipid accumulation in TAMs. Moreover, CB2 cannabinoid signaling is an oncogenic factor in tumor cells. Xiang, W. et al. [103] reported that MGLL deficiency via CB2/TLR4 contributed to lipid accumulation, macrophage activation, $CD8^+$ T cell inhibition, and tumor progression in inoculated and genetic cancer models. In contrast, TAMs highly express epidermal fatty acid-binding protein (E-FABP), which promotes the formation of lipid droplets and IFN-β production, thereby inhibiting tumor progression by enhancing the recruitment of tumoricidal effector cells, especially NK cells.

Recent studies on intracellular metabolism in macrophages provide new insights into the functions of these critical controllers of innate and adaptive immunity [108, 109]. Complex changes in mitochondrial metabolism have been characterized in mouse macrophages. M1 type macrophages exhibit decreased respiration and a broken Krebs cycle, leading to accumulation of succinate and citrate, which act as signals to alter immune function. In M2 type macrophages, the Krebs cycle and oxidative phosphorylation are intact, and FAO is utilized. In addition, activated macrophages transform mitochondria from ATP synthesis to reactive oxygen species (ROS) production to promote a pro-inflammatory state. The lipid reprogramming of dendritic cells and macrophages in the tumor microenvironment are presented in Fig. 11.3.

11.5.3 Lipid Metabolism in Other APCs

Obesity may damage B cell function. In human and mouse obesity models, B cell responses are impaired, and essential fatty acid status influences humoral immunity potentially through specialized pro-resolving lipid mediators. This mechanism effectively increased murine Ab levels upon influenza infection [110]. The accumulation of fat influences B lymphopoiesis in bone marrow and further studies showed that adipocytes promote the accumulation of MDSC and subsequently inhibit B lymphopoiesis. Using cytokine array analysis, researchers found that IL-1 produced by MDSCs negatively regulates B lymphopoiesis [111]. Predictably, B cells also affect lipid metabolism. The absence of B cells causes a lack of IgA and impaired Gata4-dependent functions, which is a key player in intestinal gene regulation and function. This shift in intestinal function leads to lipid malabsorption and decreased deposition of body fat [112].

Endothelial cells (ECs) are an important part of new blood vessels in tumor progression. Many metabolic pathways, including FA metabolism, contribute to the altered behavior of tumor endothelial cells. ECs use FAs for DNA synthesis and cellular replication, and ECs express the enzymes required for FA synthesis, including ATP citrate lyase (ACLY), acetyl-CoA carboxylase (ACC), and fatty acid synthase (FASN). Vascular endo-

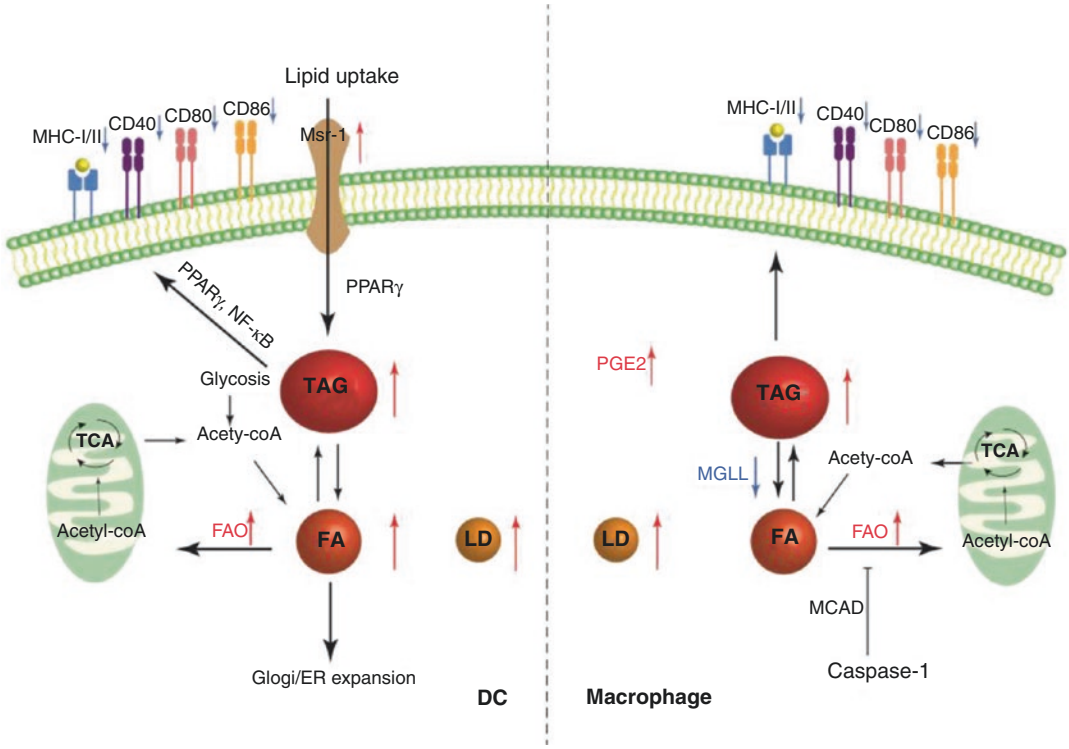

Fig. 11.3 Lipid reprogramming of dendritic cells and macrophages in the tumor microenvironment. (1) Lipid accumulation, primarily in the form of triglycerides (TAGs) and lipid droplets (LD), in DC contributes to its dysfunction in cancer. The expression of macrophage scavenger receptor (Msr-1) in DCs increases lipid uptake, resulting in the expression of costimulatory molecules and reduced MHC class I/II expression. The mechanisms of lipid accumulation involve the PPAR-γ or NF-κB pathways. In general, the metabolic switch from anabolic metabolism to catabolic metabolisms is consistent with DC function modulation from immunogenicity to tolerogenicity. Furthermore, fatty acid oxidation (FAO) renders DC tolerogenic, and increased generation of citrate by the tricarboxylic acid (TCA) cycle promotes fatty acid synthesis (FAS) and the expansion of ER and Golgi networks required for DC activation. (2) Tumor-associated macrophages (TAM) with upregulated FAS enzymes and increased FAO result in immune system evasion. Both monoacylglycerol lipase (MGLL) deficiency and medium-chain acyl-CoA dehydrogenase (MCAD) inactivation by caspase-1 through cleavage of PPARγ contribute to lipid accumulation in TAMs. In addition, PEG2 can transform TAMs from M1 to M2 and lead to the downregulation of MHC molecules

thelium expresses many FA transporter genes, including FAT/CD36 and FABP4, and influences EC proliferation, migration, and sprouting [113]. Carnitine palmitoyltransferase 1a (CPT1a) is a rate-controlling enzyme of FAO that imports fatty acids into the mitochondria, and CPT1a-controlled FAO stimulates EC proliferation as well as lymphatic ECs. Additionally, the transcription factor PROX1 binds to the CPT1a promoter and increases CPT1a gene expression, ultimately stimulating FAO [114].

11.6 Concluding Statements

Immune escape plays a fatal role in tumor progression and is one of the main reasons for dysfunctional tumor antigen presentation. In the TME, the disruption of MHC function as well as high expressions of inhibitory molecules, such as PD-1/PD-L1, IL-10, TGFβ, and PGE2 and downregulation of costimulatory molecules contribute to deficient antigen presentation function. Cancer and immune cell metabolism are instrumental in

tumor initiation, progression, and metastasis. Both MHC-I and cross-presentation processing pathways are involved in the antitumor immune response. Within the cancer microenvironment, there are complex mechanisms that suppress the actions of antitumor immune effectors, and lipid metabolism plays a crucial role in shaping immune cell differentiation and function. Here, we review that lipid metabolic disorders are related to immune suppression in APCs. APCs upregulate FAS and lipid uptake. These features result in lipid accumulation that impairs their function in the TME and ultimately promote tumor progression. As the most powerful APCs, DCs play an important role in tumor antigen presentation. DCs' function is impaired in tumors via the accumulation of lipids, especially TAG, which contributes to an increased number of imDCs and impaired antigen-presenting function and T lymphocyte activation. Furthermore, the lipid loading of DCs caused by increased expression of Msr-1 and PPAR-γ may regulate lipid accumulation via mechanisms involving NF-κB and AMPK. Similar to DCs, macrophage enhanced FA biosynthesis and lipid uptake and storage in the tumor microenvironment. Macrophages are more likely to switch to the M2 phenotype, and these changes may also be mediated by PPARs, LXRs, and STAT.

Above all, lipid metabolism disorders in APCs are associated with suppression of antigen presentation and reduced T cell activity in advancing tumors. The mechanisms of APC dysfunction remain unclear, and more studies are needed to explore these outstanding questions.

References

1. Shimano H, Sato R. SREBP-regulated lipid metabolism: convergent physiology—divergent pathophysiology. Nat Rev Endocrinol. 2017;13(12):710–30. https://doi.org/10.1038/nrendo.2017.91.
2. Loftus RM, Finlay DK. Immunometabolism: cellular metabolism turns immune regulator. J Biol Chem. 2016;291(1):1–10. https://doi.org/10.1074/jbc.R115.693903.
3. De Libero G. Editorial overview: antigen processing. Curr Opin Immunol. 2018;52:iv–v. https://doi.org/10.1016/j.coi.2018.05.016.
4. Lees JR. Targeting antigen presentation in autoimmunity. Cell Immunol. 2019;339:4–9. https://doi.org/10.1016/j.cellimm.2018.12.006.
5. Kotsias F, Cebrian I, Alloatti A. Antigen processing and presentation. Int Rev Cell Mol Biol. 2019;348:69–121. https://doi.org/10.1016/bs.ircmb.2019.07.005.
6. Jensen PE. Recent advances in antigen processing and presentation. Nat Immunol. 2007;8(10):1041–8. https://doi.org/10.1038/ni1516.
7. van den Elsen PJ. Expression regulation of major histocompatibility complex class I and class II encoding genes. Front Immunol. 2011;2:48. https://doi.org/10.3389/fimmu.2011.00048.
8. Burgdorf S, Kurts C. Endocytosis mechanisms and the cell biology of antigen presentation. Curr Opin Immunol. 2008;20(1):89–95. https://doi.org/10.1016/j.coi.2007.12.002.
9. Joffre OP, Segura E, Savina A, Amigorena S. Cross-presentation by dendritic cells. Nat Rev Immunol. 2012;12(8):557–69. https://doi.org/10.1038/nri3254.
10. Bevan MJ. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. J Exp Med. 1976;143(5):1283–8. https://doi.org/10.1084/jem.143.5.1283.
11. Bevan MJ. Minor H antigens introduced on H-2 different stimulating cells cross-react at the cytotoxic T cell level during in vivo priming. J Immunol. 1976;117(6):2233–8.
12. Sanchez-Paulete AR, Teijeira A, Cueto FJ, Garasa S, Perez-Gracia JL, Sanchez-Arraez A, Sancho D, Melero I. Antigen cross-presentation and T-cell cross-priming in cancer immunology and immunotherapy. Ann Oncol. 2017;28(suppl_12):xii44–55. https://doi.org/10.1093/annonc/mdx237.
13. Sabado RL, Balan S, Bhardwaj N. Dendritic cell-based immunotherapy. Cell Res. 2017;27(1):74–95. https://doi.org/10.1038/cr.2016.157.
14. Moody DB, Cotton RN. Four pathways of CD1 antigen presentation to T cells. Curr Opin Immunol. 2017;46:127–33. https://doi.org/10.1016/j.coi.2017.07.013.
15. Mildner A, Jung S. Development and function of dendritic cell subsets. Immunity. 2014;40(5):642–56. https://doi.org/10.1016/j.immuni.2014.04.016.
16. Guilliams M, Ginhoux F, Jakubzick C, Naik SH, Onai N, Schraml BU, Segura E, Tussiwand R, Yona S. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nat Rev Immunol. 2014;14(8):571–8. https://doi.org/10.1038/nri3712.
17. Patente TA, Pinho MP, Oliveira AA, Evangelista GCM, Bergami-Santos PC, Barbuto JAM. Human dendritic cells: their heterogeneity and clinical application potential in cancer immunotherapy. Front Immunol. 2018;9:3176. https://doi.org/10.3389/fimmu.2018.03176.

18. Roberts EW, Broz ML, Binnewies M, Headley MB, Nelson AE, Wolf DM, Kaisho T, Bogunovic D, Bhardwaj N, Krummel MF. Critical role for CD103(+)/CD141(+) dendritic cells bearing CCR7 for tumor antigen trafficking and priming of T cell immunity in melanoma. Cancer Cell. 2016;30(2):324–36. https://doi.org/10.1016/j.ccell.2016.06.003.
19. Houston SA, Cerovic V, Thomson C, Brewer J, Mowat AM, Milling S. The lymph nodes draining the small intestine and colon are anatomically separate and immunologically distinct. Mucosal Immunol. 2016;9(2):468–78. https://doi.org/10.1038/mi.2015.77.
20. Crispe IN. Liver antigen-presenting cells. J Hepatol. 2011;54(2):357–65. https://doi.org/10.1016/j.jhep.2010.10.005.
21. Chen X, Jensen PE. The role of B lymphocytes as antigen-presenting cells. Arch Immunol Ther Exp. 2008;56(2):77–83. https://doi.org/10.1007/s00005-008-0014-5.
22. Mehrfeld C, Zenner S, Kornek M, Lukacs-Kornek V. The contribution of non-professional antigen-presenting cells to immunity and tolerance in the liver. Front Immunol. 2018;9:635. https://doi.org/10.3389/fimmu.2018.00635.
23. Cerezo-Wallis D, Soengas MS. Understanding tumor-antigen presentation in the new era of cancer immunotherapy. Curr Pharm Des. 2016;22(41):6234–50. https://doi.org/10.2174/1381612822666160826111041.
24. Schnurr M, Chen Q, Shin A, Chen W, Toy T, Jenderek C, Green S, Miloradovic L, Drane D, Davis ID, Villadangos J, Shortman K, Maraskovsky E, Cebon J. Tumor antigen processing and presentation depend critically on dendritic cell type and the mode of antigen delivery. Blood. 2005;105(6):2465–72. https://doi.org/10.1182/blood-2004-08-3105.
25. Veglia F, Gabrilovich DI. Dendritic cells in cancer: the role revisited. Curr Opin Immunol. 2017;45:43–51. https://doi.org/10.1016/j.coi.2017.01.002.
26. Kim R, Emi M, Tanabe K, Arihiro K. Potential functional role of plasmacytoid dendritic cells in cancer immunity. Immunology. 2007;121(2):149–57. https://doi.org/10.1111/j.1365-2567.2007.02579.x.
27. Bandola-Simon J, Roche PA. Dysfunction of antigen processing and presentation by dendritic cells in cancer. Mol Immunol. 2019;113:31–7. https://doi.org/10.1016/j.molimm.2018.03.025.
28. Alvarez D, Vollmann EH, von Andrian UH. Mechanisms and consequences of dendritic cell migration. Immunity. 2008;29(3):325–42. https://doi.org/10.1016/j.immuni.2008.08.006.
29. Wakim LM, Bevan MJ. Cross-dressed dendritic cells drive memory CD8+ T-cell activation after viral infection. Nature. 2011;471(7340):629–32. https://doi.org/10.1038/nature09863.
30. Dolan BP, Gibbs KD Jr, Ostrand-Rosenberg S. Dendritic cells cross-dressed with peptide MHC class I complexes prime CD8+ T cells. J Immunol. 2006;177(9):6018–24. https://doi.org/10.4049/jimmunol.177.9.6018.
31. Bol KF, Schreibelt G, Gerritsen WR, de Vries IJ, Figdor CG. Dendritic cell-based immunotherapy: state of the art and beyond. Clin Cancer Res. 2016;22(8):1897–906. https://doi.org/10.1158/1078-0432.ccr-15-1399.
32. Garrido F, Romero I, Aptsiauri N, Garcia-Lora AM. Generation of MHC class I diversity in primary tumors and selection of the malignant phenotype. Int J Cancer. 2016;138(2):271–80. https://doi.org/10.1002/ijc.29375.
33. Carretero R, Romero JM, Ruiz-Cabello F, Maleno I, Rodriguez F, Camacho FM, Real LM, Garrido F, Cabrera T. Analysis of HLA class I expression in progressing and regressing metastatic melanoma lesions after immunotherapy. Immunogenetics. 2008;60(8):439–47. https://doi.org/10.1007/s00251-008-0303-5.
34. Seliger B, Maeurer MJ, Ferrone S. Antigen-processing machinery breakdown and tumor growth. Immunol Today. 2000;21(9):455–64. https://doi.org/10.1016/s0167-5699(00)01692-3.
35. Nowak AK, Lake RA, Marzo AL, Scott B, Heath WR, Collins EJ, Frelinger JA, Robinson BW. Induction of tumor cell apoptosis in vivo increases tumor antigen cross-presentation, cross-priming rather than cross-tolerizing host tumor-specific CD8 T cells. J Immunol. 2003;170(10):4905–13. https://doi.org/10.4049/jimmunol.170.10.4905.
36. Sanchez-Paulete AR, Cueto FJ, Martinez-Lopez M, Labiano S, Morales-Kastresana A, Rodriguez-Ruiz ME, Jure-Kunkel M, Azpilikueta A, Aznar MA, Quetglas JI, Sancho D, Melero I. Cancer immunotherapy with immunomodulatory anti-CD137 and anti-PD-1 monoclonal antibodies requires BATF3-dependent dendritic cells. Cancer Discov. 2016;6(1):71–9. https://doi.org/10.1158/2159-8290.CD-15-0510.
37. Gardner A, Ruffell B. Dendritic cells and cancer immunity. Trends Immunol. 2016;37(12):855–65. https://doi.org/10.1016/j.it.2016.09.006.
38. Reizis B. Plasmacytoid dendritic cells: development, regulation, and function. Immunity. 2019;50(1):37–50. https://doi.org/10.1016/j.immuni.2018.12.027.
39. Palucka K, Banchereau J. Cancer immunotherapy via dendritic cells. Nat Rev Cancer. 2012;12(4):265–77. https://doi.org/10.1038/nrc3258.
40. Boks MA, Bruijns SCM, Ambrosini M, Kalay H, van Bloois L, Storm G, Gruijl T, van Kooyk Y. In situ delivery of tumor antigen- and adjuvant-loaded liposomes boosts antigen-specific T-cell responses by human dermal dendritic cells. J Invest Dermatol. 2015;135(11):2697–704. https://doi.org/10.1038/jid.2015.226.
41. Palucka AK, Coussens LM. The basis of oncoimmunology. Cell. 2016;164(6):1233–47. https://doi.org/10.1016/j.cell.2016.01.049.
42. Faget J, Bendriss-Vermare N, Gobert M, Durand I, Olive D, Biota C, Bachelot T, Treilleux I, Goddard-

Leon S, Lavergne E, Chabaud S, Blay JY, Caux C, Menetrier-Caux C. ICOS-ligand expression on plasmacytoid dendritic cells supports breast cancer progression by promoting the accumulation of immunosuppressive CD4+ T cells. Cancer Res. 2012;72(23):6130–41. https://doi.org/10.1158/0008-5472.CAN-12-2409.

43. Bonaccorsi I, Morandi B, Antsiferova O, Costa G, Oliveri D, Conte R, Pezzino G, Vermiglio G, Anastasi GP, Navarra G, Munz C, Di Carlo E, Mingari MC, Ferlazzo G. Membrane transfer from tumor cells overcomes deficient phagocytic ability of plasmacytoid dendritic cells for the acquisition and presentation of tumor antigens. J Immunol. 2014;192(2):824–32. https://doi.org/10.4049/jimmunol.1301039.
44. Gabrilovich DI, Ostrand-Rosenberg S, Bronte V. Coordinated regulation of myeloid cells by tumours. Nat Rev Immunol. 2012;12(4):253–68. https://doi.org/10.1038/nri3175.
45. Dudek AM, Martin S, Garg AD, Agostinis P. Immature, semi-mature, and fully mature dendritic cells: toward a DC-cancer cells Interface that augments anticancer immunity. Front Immunol. 2013;4:438. https://doi.org/10.3389/fimmu.2013.00438.
46. Tran Janco JM, Lamichhane P, Karyampudi L, Knutson KL. Tumor-infiltrating dendritic cells in cancer pathogenesis. J Immunol. 2015;194(7):2985–91. https://doi.org/10.4049/jimmunol.1403134.
47. Harimoto H, Shimizu M, Nakagawa Y, Nakatsuka K, Wakabayashi A, Sakamoto C, Takahashi H. Inactivation of tumor-specific CD8(+) CTLs by tumor-infiltrating tolerogenic dendritic cells. Immunol Cell Biol. 2013;91(9):545–55. https://doi.org/10.1038/icb.2013.38.
48. Krempski J, Karyampudi L, Behrens MD, Erskine CL, Hartmann L, Dong H, Goode EL, Kalli KR, Knutson KL. Tumor-infiltrating programmed death receptor-1+ dendritic cells mediate immune suppression in ovarian cancer. J Immunol. 2011;186(12):6905–13. https://doi.org/10.4049/jimmunol.1100274.
49. Klarquist JS, Janssen EM. Melanoma-infiltrating dendritic cells: limitations and opportunities of mouse models. Onco Targets Ther. 2012;1(9):1584–93. https://doi.org/10.4161/onci.22660.
50. Karyampudi L, Lamichhane P, Scheid AD, Kalli KR, Shreeder B, Krempski JW, Behrens MD, Knutson KL. Accumulation of memory precursor CD8 T cells in regressing tumors following combination therapy with vaccine and anti-PD-1 antibody. Cancer Res. 2014;74(11):2974–85. https://doi.org/10.1158/0008-5472.CAN-13-2564.
51. Liu Q, Zhang C, Sun A, Zheng Y, Wang L, Cao X. Tumor-educated CD11bhighIalow regulatory dendritic cells suppress T cell response through arginase I. J Immunol. 2009;182(10):6207–16. https://doi.org/10.4049/jimmunol.0803926.
52. Hargadon KM. Tumor-altered dendritic cell function: implications for anti-tumor immunity. Front Immunol. 2013;4:192. https://doi.org/10.3389/fimmu.2013.00192.
53. Chen W, Liang X, Peterson AJ, Munn DH, Blazar BR. The indoleamine 2,3-dioxygenase pathway is essential for human plasmacytoid dendritic cell-induced adaptive T regulatory cell generation. J Immunol. 2008;181(8):5396–404. https://doi.org/10.4049/jimmunol.181.8.5396.
54. Franklin RA, Liao W, Sarkar A, Kim MV, Bivona MR, Liu K, Pamer EG, Li MO. The cellular and molecular origin of tumor-associated macrophages. Science. 2014;344(6186):921–5. https://doi.org/10.1126/science.1252510.
55. Noy R, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. Immunity. 2014;41(1):49–61. https://doi.org/10.1016/j.immuni.2014.06.010.
56. DeNardo DG, Ruffell B. Macrophages as regulators of tumour immunity and immunotherapy. Nat Rev Immunol. 2019;19(6):369–82. https://doi.org/10.1038/s41577-019-0127-6.
57. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol. 2004;25(12):677–86. https://doi.org/10.1016/j.it.2004.09.015.
58. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. Nat Med. 2013;19(11):1423–37. https://doi.org/10.1038/nm.3394.
59. Dehne N, Mora J, Namgaladze D, Weigert A, Brune B. Cancer cell and macrophage cross-talk in the tumor microenvironment. Curr Opin Pharmacol. 2017;35:12–9. https://doi.org/10.1016/j.coph.2017.04.007.
60. Tang X, Mo C, Wang Y, Wei D, Xiao H. Anti-tumour strategies aiming to target tumour-associated macrophages. Immunology. 2013;138(2):93–104. https://doi.org/10.1111/imm.12023.
61. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res. 2006;66(2):605–12. https://doi.org/10.1158/0008-5472.CAN-05-4005.
62. Chavez-Galan L, Olleros ML, Vesin D, Garcia I. Much more than M1 and M2 macrophages, there are also CD169(+) and TCR(+) macrophages. Front Immunol. 2015;6:263. https://doi.org/10.3389/fimmu.2015.00263.
63. Ohnishi K, Komohara Y, Saito Y, Miyamoto Y, Watanabe M, Baba H, Takeya M. CD169-positive macrophages in regional lymph nodes are associated with a favorable prognosis in patients with colorectal carcinoma. Cancer Sci. 2013;104(9):1237–44. https://doi.org/10.1111/cas.12212.
64. Ohnishi K, Yamaguchi M, Erdenebaatar C, Saito F, Tashiro H, Katabuchi H, Takeya M, Komohara Y. Prognostic significance of CD169-positive lymph node sinus macrophages in patients with endome-

trial carcinoma. Cancer Sci. 2016;107(6):846–52. https://doi.org/10.1111/cas.12929.
65. Martinez-Pomares L, Gordon S. CD169+ macrophages at the crossroads of antigen presentation. Trends Immunol. 2012;33(2):66–70. https://doi.org/10.1016/j.it.2011.11.001.
66. Asano K, Nabeyama A, Miyake Y, Qiu CH, Kurita A, Tomura M, Kanagawa O, Fujii S, Tanaka M. CD169-positive macrophages dominate antitumor immunity by crosspresenting dead cell-associated antigens. Immunity. 2011;34(1):85–95. https://doi.org/10.1016/j.immuni.2010.12.011.
67. Saunderson SC, Dunn AC, Crocker PR, McLellan AD. CD169 mediates the capture of exosomes in spleen and lymph node. Blood. 2014;123(2):208–16. https://doi.org/10.1182/blood-2013-03-489732.
68. Pucci F, Garris C, Lai CP, Newton A, Pfirschke C, Engblom C, Alvarez D, Sprachman M, Evavold C, Magnuson A, von Andrian UH, Glatz K, Breakefield XO, Mempel TR, Weissleder R, Pittet MJ. SCS macrophages suppress melanoma by restricting tumor-derived vesicle-B cell interactions. Science. 2016;352(6282):242–6. https://doi.org/10.1126/science.aaf1328.
69. Hicklin DJ, Marincola FM, Ferrone S. HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. Mol Med Today. 1999;5(4):178–86. https://doi.org/10.1016/s1357-4310(99)01451-3.
70. Bijen CB, Bantema-Joppe EJ, de Jong RA, Leffers N, Mourits MJ, Eggink HF, van der Zee AG, Hollema H, de Bock GH, Nijman HW. The prognostic role of classical and nonclassical MHC class I expression in endometrial cancer. Int J Cancer. 2010;126(6):1417–27. https://doi.org/10.1002/ijc.24852.
71. Bubenik J. MHC class I down regulation, tumour escape from immune surveillance and design of therapeutic strategies. Folia Biol. 2005;51(1):1–2.
72. Madjd Z, Spendlove I, Pinder SE, Ellis IO, Durrant LG. Total loss of MHC class I is an independent indicator of good prognosis in breast cancer. Int J Cancer. 2005;117(2):248–55. https://doi.org/10.1002/ijc.21163.
73. Brady MS, Eckels DD, Ree SY, Schultheiss KE, Lee JS. MHC class II-mediated antigen presentation by melanoma cells. J Immunother Emphasis Tumor Immunol. 1996;19(6):387–97. https://doi.org/10.1097/00002371-199611000-00001.
74. Bernsen MR, Hakansson L, Gustafsson B, Krysander L, Rettrup B, Ruiter D, Hakansson A. On the biological relevance of MHC class II and B7 expression by tumour cells in melanoma metastases. Br J Cancer. 2003;88(3):424–31. https://doi.org/10.1038/sj.bjc.6600703.
75. Schmidt M, Bohm D, von Torne C, Steiner E, Puhl A, Pilch H, Lehr HA, Hengstler JG, Kolbl H, Gehrmann M. The humoral immune system has a key prognostic impact in node-negative breast cancer. Cancer Res. 2008;68(13):5405–13. https://doi.org/10.1158/0008-5472.CAN-07-5206.
76. Tureci O, Mack U, Luxemburger U, Heinen H, Krummenauer F, Sester M, Sester U, Sybrecht GW, Sahin U. Humoral immune responses of lung cancer patients against tumor antigen NY-ESO-1. Cancer Lett. 2006;236(1):64–71. https://doi.org/10.1016/j.canlet.2005.05.008.
77. Lechpammer M, Lukac J, Lechpammer S, Kovacevic D, Loda M, Kusic Z. Humoral immune response to p53 correlates with clinical course in colorectal cancer patients during adjuvant chemotherapy. Int J Color Dis. 2004;19(2):114–20. https://doi.org/10.1007/s00384-003-0553-5.
78. Berg M, Wingender G, Djandji D, Hegenbarth S, Momburg F, Hammerling G, Limmer A, Knolle P. Cross-presentation of antigens from apoptotic tumor cells by liver sinusoidal endothelial cells leads to tumor-specific CD8+ T cell tolerance. Eur J Immunol. 2006;36(11):2960–70. https://doi.org/10.1002/eji.200636033.
79. Li X, Shao C, Shi Y, Han W. Lessons learned from the blockade of immune checkpoints in cancer immunotherapy. J Hematol Oncol. 2018;11(1):31. https://doi.org/10.1186/s13045-018-0578-4.
80. Rodig N, Ryan T, Allen JA, Pang H, Grabie N, Chernova T, Greenfield EA, Liang SC, Sharpe AH, Lichtman AH, Freeman GJ. Endothelial expression of PD-L1 and PD-L2 down-regulates CD8+ T cell activation and cytolysis. Eur J Immunol. 2003;33(11):3117–26. https://doi.org/10.1002/eji.200324270.
81. Eppihimer MJ, Gunn J, Freeman GJ, Greenfield EA, Chernova T, Erickson J, Leonard JP. Expression and regulation of the PD-L1 immunoinhibitory molecule on microvascular endothelial cells. Microcirculation. 2002;9(2):133–45. https://doi.org/10.1038/sj/mn/7800123.
82. Lund AW, Duraes FV, Hirosue S, Raghavan VR, Nembrini C, Thomas SN, Issa A, Hugues S, Swartz MA. VEGF-C promotes immune tolerance in B16 melanomas and cross-presentation of tumor antigen by lymph node lymphatics. Cell Rep. 2012;1(3):191–9. https://doi.org/10.1016/j.celrep.2012.01.005.
83. Nomura DK, Cravatt BF. Lipid metabolism in cancer. Biochim Biophys Acta. 2013;1831(10):1497–8. https://doi.org/10.1016/j.bbalip.2013.08.001.
84. Nakagawa H, Hayata Y, Kawamura S, Yamada T, Fujiwara N, Koike K. Lipid metabolic reprogramming in hepatocellular carcinoma. Cancers (Basel). 2018;10(11):447. https://doi.org/10.3390/cancers10110447.
85. Hu B, Lin JZ, Yang XB, Sang XT. Aberrant lipid metabolism in hepatocellular carcinoma cells as well as immune microenvironment: a review. Cell Prolif. 2020;53(3):e12772. https://doi.org/10.1111/cpr.12772.
86. Hao Y, Li D, Xu Y, Ouyang J, Wang Y, Zhang Y, Li B, Xie L, Qin G. Investigation of lipid metabolism dysregulation and the effects on immune microenvironments in pan-cancer using multiple omics data.

BMC Bioinformatics. 2019;20(Suppl 7):195. https://doi.org/10.1186/s12859-019-2734-4.
87. Li R, Fang F, Jiang M, Wang C, Ma J, Kang W, Zhang Q, Miao Y, Wang D, Guo Y, Zhang L, Guo Y, Zhao H, Yang TZ, Xiao W. STAT3 and NF-kappaB are simultaneously suppressed in dendritic cells in lung cancer. Sci Rep. 2017;7:45395. https://doi.org/10.1038/srep45395.
88. Herber DL, Cao W, Nefedova Y, Novitskiy SV, Nagaraj S, Tyurin VA, Corzo A, Cho HI, Celis E, Lennox B, Knight SC, Padhya T, McCaffrey TV, McCaffrey JC, Antonia S, Fishman M, Ferris RL, Kagan VE, Gabrilovich DI. Lipid accumulation and dendritic cell dysfunction in cancer. Nat Med. 2010;16(8):880–6. https://doi.org/10.1038/nm.2172.
89. Gao F, Liu C, Guo J, Sun W, Xian L, Bai D, Liu H, Cheng Y, Li B, Cui J, Zhang C, Cai J. Radiation-driven lipid accumulation and dendritic cell dysfunction in cancer. Sci Rep. 2015;5:9613. https://doi.org/10.1038/srep09613.
90. Arai R, Soda S, Okutomi T, Morita H, Ohmi F, Funakoshi T, Takemasa A, Ishii Y. Lipid accumulation in peripheral blood dendritic cells and anti-cancer immunity in patients with lung cancer. J Immunol Res. 2018;2018:5708239. https://doi.org/10.1155/2018/5708239.
91. Gardner JK, Mamotte CD, Patel P, Yeoh TL, Jackaman C, Nelson DJ. Mesothelioma tumor cells modulate dendritic cell lipid content, phenotype and function. PLoS One. 2015;10(4):e0123563. https://doi.org/10.1371/journal.pone.0123563.
92. Lerret NM, Rogozinska M, Jaramillo A, Marzo AL. Adoptive transfer of Mammaglobin-A epitope specific CD8 T cells combined with a single low dose of total body irradiation eradicates breast tumors. PLoS One. 2012;7(7):e41240. https://doi.org/10.1371/journal.pone.0041240.
93. Zapata-Gonzalez F, Rueda F, Petriz J, Domingo P, Villarroya F, Diaz-Delfin J, de Madariaga MA, Domingo JC. Human dendritic cell activities are modulated by the omega-3 fatty acid, docosahexaenoic acid, mainly through PPAR(gamma):RXR heterodimers: comparison with other polyunsaturated fatty acids. J Leukoc Biol. 2008;84(4):1172–82. https://doi.org/10.1189/jlb.1007688.
94. Szatmari I, Torocsik D, Agostini M, Nagy T, Gurnell M, Barta E, Chatterjee K, Nagy L. PPARgamma regulates the function of human dendritic cells primarily by altering lipid metabolism. Blood. 2007;110(9):3271–80. https://doi.org/10.1182/blood-2007-06-096222.
95. Everts B, Amiel E, Huang SC, Smith AM, Chang CH, Lam WY, Redmann V, Freitas TC, Blagih J, van der Windt GJ, Artyomov MN, Jones RG, Pearce EL, Pearce EJ. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKvarepsilon supports the anabolic demands of dendritic cell activation. Nat Immunol. 2014;15(4):323–32. https://doi.org/10.1038/ni.2833.
96. den Brok MH, Bull C, Wassink M, de Graaf AM, Wagenaars JA, Minderman M, Thakur M, Amigorena S, Rijke EO, Schrier CC, Adema GJ. Saponin-based adjuvants induce cross-presentation in dendritic cells by intracellular lipid body formation. Nat Commun. 2016;7:13324. https://doi.org/10.1038/ncomms13324.
97. Hossain F, Al-Khami AA, Wyczechowska D, Hernandez C, Zheng L, Reiss K, Valle LD, Trillo-Tinoco J, Maj T, Zou W, Rodriguez PC, Ochoa AC. Inhibition of fatty acid oxidation modulates immunosuppressive functions of myeloid-derived suppressor cells and enhances cancer therapies. Cancer Immunol Res. 2015;3(11):1236–47. https://doi.org/10.1158/2326-6066.CIR-15-0036.
98. Ramakrishnan R, Tyurin VA, Veglia F, Condamine T, Amoscato A, Mohammadyani D, Johnson JJ, Zhang LM, Klein-Seetharaman J, Celis E, Kagan VE, Gabrilovich DI. Oxidized lipids block antigen cross-presentation by dendritic cells in cancer. J Immunol. 2014;192(6):2920–31. https://doi.org/10.4049/jimmunol.1302801.
99. Yeung OW, Lo CM, Ling CC, Qi X, Geng W, Li CX, Ng KT, Forbes SJ, Guan XY, Poon RT, Fan ST, Man K. Alternatively activated (M2) macrophages promote tumour growth and invasiveness in hepatocellular carcinoma. J Hepatol. 2015;62(3):607–16. https://doi.org/10.1016/j.jhep.2014.10.029.
100. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, Beatty W, Love-Gregory L, Lam WY, O'Neill CM, Yan C, Du H, Abumrad NA, Urban JF Jr, Artyomov MN, Pearce EL, Pearce EJ. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. Nat Immunol. 2014;15(9):846–55. https://doi.org/10.1038/ni.2956.
101. Huang SC, Smith AM, Everts B, Colonna M, Pearce EL, Schilling JD, Pearce EJ. Metabolic reprogramming mediated by the mTORC2-IRF4 signaling Axis is essential for macrophage alternative activation. Immunity. 2016;45(4):817–30. https://doi.org/10.1016/j.immuni.2016.09.016.
102. Netea-Maier RT, Smit JWA, Netea MG. Metabolic changes in tumor cells and tumor-associated macrophages: a mutual relationship. Cancer Lett. 2018;413:102–9. https://doi.org/10.1016/j.canlet.2017.10.037.
103. Xiang W, Shi R, Kang X, Zhang X, Chen P, Zhang L, Hou A, Wang R, Zhao Y, Zhao K, Liu Y, Ma Y, Luo H, Shang S, Zhang J, He F, Yu S, Gan L, Shi C, Li Y, Yang W, Liang H, Miao H. Monoacylglycerol lipase regulates cannabinoid receptor 2-dependent macrophage activation and cancer progression. Nat Commun. 2018;9(1):2574. https://doi.org/10.1038/s41467-018-04999-8.
104. Yu XH, Ren XH, Liang XH, Tang YL. Roles of fatty acid metabolism in tumourigenesis: beyond providing nutrition (review). Mol Med Rep. 2018;18(6):5307–16. https://doi.org/10.3892/mmr.2018.9577.

105. Traversari C, Sozzani S, Steffensen KR, Russo V. LXR-dependent and -independent effects of oxysterols on immunity and tumor growth. Eur J Immunol. 2014;44(7):1896–903. https://doi.org/10.1002/eji.201344292.
106. Park H, Lee J, Park T, Lee S, Yi W. Enhancement of photo-current conversion efficiency in a CdS/CdSe quantum-dot-sensitized solar cell incorporated with single-walled carbon nanotubes. J Nanosci Nanotechnol. 2015;15(2):1614–7. https://doi.org/10.1166/jnn.2015.9319.
107. Niu Z, Shi Q, Zhang W, Shu Y, Yang N, Chen B, Wang Q, Zhao X, Chen J, Cheng N, Feng X, Hua Z, Ji J, Shen P. Caspase-1 cleaves PPARgamma for potentiating the pro-tumor action of TAMs. Nat Commun. 2017;8(1):766. https://doi.org/10.1038/s41467-017-00523-6.
108. Mills EL, Kelly B, Logan A, Costa ASH, Varma M, Bryant CE, Tourlomousis P, Dabritz JHM, Gottlieb E, Latorre I, Corr SC, McManus G, Ryan D, Jacobs HT, Szibor M, Xavier RJ, Braun T, Frezza C, Murphy MP, O'Neill LA. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell. 2016;167(2):457–470.e413. https://doi.org/10.1016/j.cell.2016.08.064.
109. Mills EL, O'Neill LA. Reprogramming mitochondrial metabolism in macrophages as an anti-inflammatory signal. Eur J Immunol. 2016;46(1):13–21. https://doi.org/10.1002/eji.201445427.
110. Kosaraju R, Guesdon W, Crouch MJ, Teague HL, Sullivan EM, Karlsson EA, Schultz-Cherry S, Gowdy K, Bridges LC, Reese LR, Neufer PD, Armstrong M, Reisdorph N, Milner JJ, Beck M, Shaikh SR. B cell activity is impaired in human and mouse obesity and is responsive to an essential fatty acid upon murine influenza infection. J Immunol. 2017;198(12):4738–52. https://doi.org/10.4049/jimmunol.1601031.
111. Kennedy DE, Witte PL, Knight KL. Bone marrow fat and the decline of B lymphopoiesis in rabbits. Dev Comp Immunol. 2016;58:30–9. https://doi.org/10.1016/j.dci.2015.11.003.
112. Shulzhenko N, Morgun A, Hsiao W, Battle M, Yao M, Gavrilova O, Orandle M, Mayer L, Macpherson AJ, McCoy KD, Fraser-Liggett C, Matzinger P. Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. Nat Med. 2011;17(12):1585–93. https://doi.org/10.1038/nm.2505.
113. Harjes U, Kalucka J, Carmeliet P. Targeting fatty acid metabolism in cancer and endothelial cells. Crit Rev Oncol Hematol. 2016;97:15–21. https://doi.org/10.1016/j.critrevonc.2015.10.011.
114. Rohlenova K, Veys K, Miranda-Santos I, De Bock K, Carmeliet P. Endothelial cell metabolism in health and disease. Trends Cell Biol. 2018;28(3):224–36. https://doi.org/10.1016/j.tcb.2017.10.010.

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

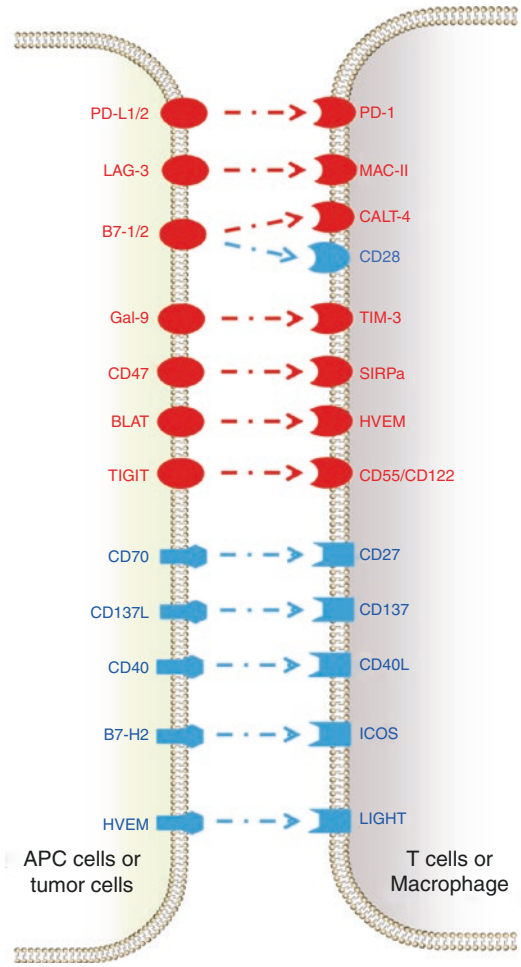

Fig. 12.1 Classification of inhibitory and stimulatory immune checkpoints in APCs, tumor cells, T cells, and macrophages. Red indicates inhibitory immune checkpoints; blue denotes stimulatory immune checkpoints

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

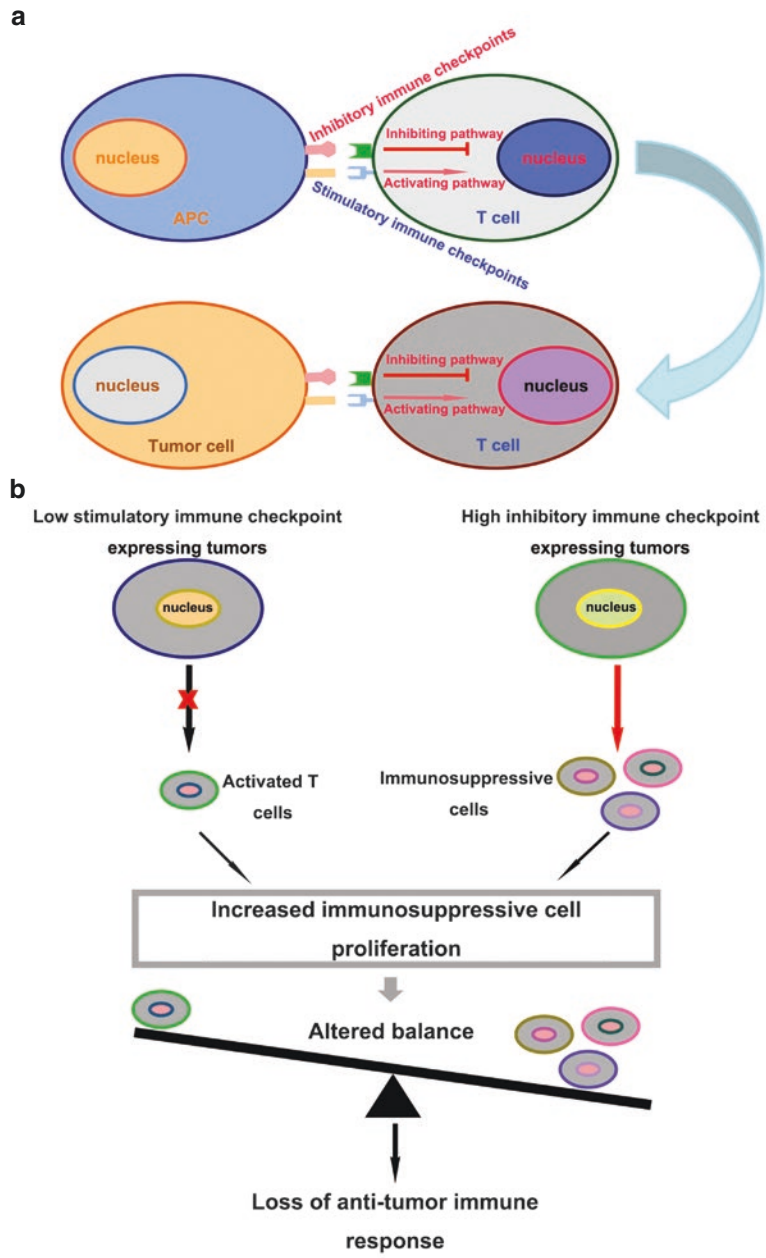

Fig. 12.2 (**a**) The functions of inhibitory and stimulatory immune checkpoints in APC cells and tumor cells. The stimulatory immune checkpoints in APC cells and tumor cells could induce the activation of T cells, whereas the inhibitory immune checkpoints suppress the process. (**b**) The differential 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.

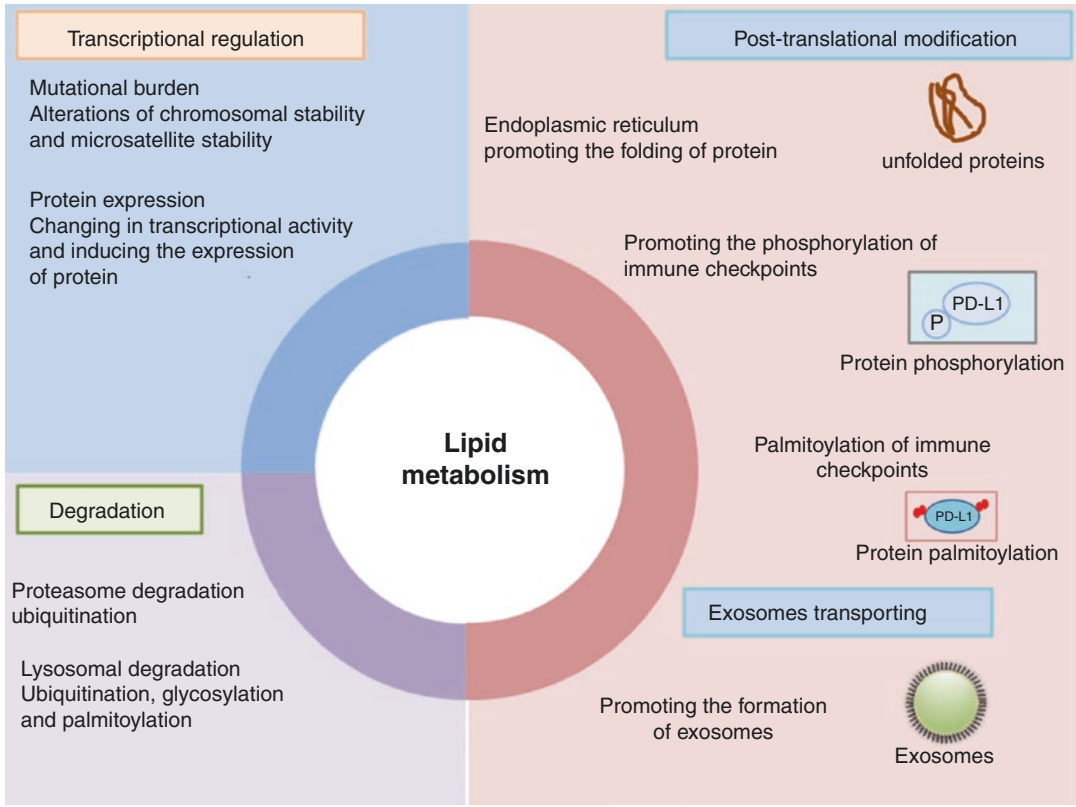

Fig. 12.3 The effect of lipid metabolism on the regulation of immune checkpoints. First, various metabolites produced by lipid metabolism can regulate gene expression of the immune checkpoints, such as affecting the stability of the genomic sets, acting on the promoter activity of the immune checkpoints, 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

References

1. Poulose N, Amoroso F, Steele RE, Singh R, Ong CW, Mills IG. Genetics of lipid metabolism in prostate cancer. Nat Genet. 2018;50(2):169–71.
2. Alves-Bezerra M, Cohen DE. Triglyceride metabolism in the liver. Compr Physiol. 2017;8(1):1–8.
3. Gago G, Diacovich L, Gramajo H. Lipid metabolism and its implication in mycobacteria-host interaction. Curr Opin Microbiol. 2018;41:36–42.
4. Mao K, Baptista AP, Tamoutounour S, Zhuang L, Bouladoux N, Martins AJ, et al. Innate and adaptive lymphocytes sequentially shape the gut microbiota and lipid metabolism. Nature. 2018;554(7691):255–9.
5. Scott CL, Zheng F, De Baetselier P, Martens L, Saeys Y, De Prijck S, et al. Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. Nat Commun. 2016;7:10321.
6. Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. Nat Immunol. 2012;13(11):1118–28.
7. Denoeud J, Moser M. Role of CD27/CD70 pathway of activation in immunity and tolerance. J Leukoc Biol. 2011;89(2):195–203.
8. Funes SC, Manrique de Lara A, Altamirano-Lagos MJ, Mackern-Oberti JP, Escobar-Vera J, Kalergis AM. Immune checkpoints and the regulation of tolerogenicity in dendritic cells: implications for autoimmunity and immunotherapy. Autoimmun Rev. 2019;18(4):359–68.
9. Remmerie A, Scott CL. Macrophages and lipid metabolism. Cell Immunol. 2018;330:27–42.

10. Phillips MC. Molecular mechanisms of cellular cholesterol efflux. J Biol Chem. 2014;289(35):24020–9.
11. Wang S, Yang X. Eleutheroside E decreases oxidative stress and NF-kappaB activation and reprograms the metabolic response against hypoxia-reoxygenation injury in H9c2 cells. Int Immunopharmacol. 2020;84:106513.
12. O'Neill LA, Kishton RJ, Rathmell J. A guide to immunometabolism for immunologists. Nat Rev Immunol. 2016;16(9):553–65.
13. Mehta MM, Weinberg SE, Chandel NS. Mitochondrial control of immunity: beyond ATP. Nat Rev Immunol. 2017;17(10):608–20.
14. Chen W, Wu Y, Lu Q, Wang S, Xing D. Endogenous ApoA-I expression in macrophages: a potential target for protection against atherosclerosis. Clin Chim Acta. 2020;505:55–9.
15. Farkkila A, Gulhan DC, Casado J, Jacobson CA, Nguyen H, Kochupurakkal B, et al. Author correction: immunogenomic profiling determines responses to combined PARP and PD-1 inhibition in ovarian cancer. Nat Commun. 2020;11(1):2543.
16. Luo J, Rizvi H, Egger JV, Preeshagul IR, Wolchok JD, Hellmann MD. Impact of PD-1 blockade on severity of COVID-19 in patients with lung cancers. Cancer Discov. 2020;10:1121.
17. Kassardjian A, Shintaku PI, Moatamed NA. Expression of immune checkpoint regulators, cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed death-ligand 1 (PD-L1), in female breast carcinomas. PLoS One. 2018;13(4):e0195958.
18. Coutzac C, Pernot S, Chaput N, Zaanan A. Immunotherapy in advanced gastric cancer, is it the future? Crit Rev Oncol Hematol. 2019;133:25–32.
19. Shu CA, Gainor JF, Awad MM, Chiuzan C, Grigg CM, Pabani A, et al. Neoadjuvant atezolizumab and chemotherapy in patients with resectable non-small-cell lung cancer: an open-label, multicentre, single-arm, phase 2 trial. Lancet Oncol. 2020;21:786.
20. Pakkala S, Owonikoko TK. Immune checkpoint inhibitors in small cell lung cancer. J Thorac Dis. 2018;10(Suppl 3):S460–S7.
21. Kieffer Y, Hocine HR, Gentric G, Pelon F, Bernard C, Bourachot B, et al. Single-cell analysis reveals fibroblast clusters linked to immunotherapy resistance in cancer. Cancer Discov. 2020;10(9):1330–51.
22. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science. 2018;359(6382):1350–5.
23. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. Blood. 2018;131(1):58–67.
24. Salvi S, Fontana V, Boccardo S, Merlo DF, Margallo E, Laurent S, et al. Evaluation of CTLA-4 expression and relevance as a novel prognostic factor in patients with non-small cell lung cancer. Cancer Immunol Immunother. 2012;61(9):1463–72.
25. Paulsen EE, Kilvaer TK, Rakaee M, Richardsen E, Hald SM, Andersen S, et al. CTLA-4 expression in the non-small cell lung cancer patient tumor microenvironment: diverging prognostic impact in primary tumors and lymph node metastases. Cancer Immunol Immunother. 2017;66(11):1449–61.
26. McIntire JJ, Umetsu SE, Akbari O, Potter M, Kuchroo VK, Barsh GS, et al. Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. Nat Immunol. 2001;2(12):1109–16.
27. Das M, Zhu C, Kuchroo VK. Tim-3 and its role in regulating anti-tumor immunity. Immunol Rev. 2017;276(1):97–111.
28. Saleh R, Toor SM, Taha RZ, Al-Ali D, Sasidharan Nair V, Elkord E. DNA methylation in the promoters of PD-L1, MMP9, ARG1, galectin-9, TIM-3, VISTA and TGF-beta genes in HLA-DR(−) myeloid cells, compared with HLA-DR(+) antigen-presenting cells. Epigenetics. 2020;15:1–14.
29. Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. Immunity. 2016;44(5):989–1004.
30. Piancone F, Saresella M, Marventano I, La Rosa F, Caputo D, Mendozzi L, et al. A deficit of CEACAM-1-expressing T lymphocytes supports inflammation in primary progressive multiple sclerosis. J Immunol. 2019;203(1):76–83.
31. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365(8):725–33.
32. Chester C, Sanmamed MF, Wang J, Melero I. Immunotherapy targeting 4-1BB: mechanistic rationale, clinical results, and future strategies. Blood. 2018;131(1):49–57.
33. Philipson BI, O'Connor RS, May MJ, June CH, Albelda SM, Milone MC. 4-1BB costimulation promotes CAR T cell survival through noncanonical NF-kappaB signaling. Sci Signal. 2020;13(625):eaay8248.
34. Sugiura D, Maruhashi T, Okazaki IM, Shimizu K, Maeda TK, Takemoto T, et al. Restriction of PD-1 function by cis-PD-L1/CD80 interactions is required for optimal T cell responses. Science. 2019;364(6440):558–66.
35. Zappasodi R, Sirard C, Li Y, Budhu S, Abu-Akeel M, Liu C, et al. Rational design of anti-GITR-based combination immunotherapy. Nat Med. 2019;25(5):759–66.
36. Shevach EM, Stephens GL. The GITR-GITRL interaction: co-stimulation or contrasuppression of regulatory activity? Nat Rev Immunol. 2006;6(8):613–8.
37. Calmels B, Paul S, Futin N, Ledoux C, Stoeckel F, Acres B. Bypassing tumor-associated immune suppression with recombinant adenovirus constructs expressing membrane bound or secreted GITR-L. Cancer Gene Ther. 2005;12(2):198–205.
38. Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, Burger MC, et al. Defining CD8+ T cells that

provide the proliferative burst after PD-1 therapy. Nature. 2016;537(7620):417–21.
39. Preite S, Cannons JL, Radtke AJ, Vujkovic-Cvijin I, Gomez-Rodriguez J, Volpi S, et al. Hyperactivated PI3Kdelta promotes self and commensal reactivity at the expense of optimal humoral immunity. Nat Immunol. 2018;19(9):986–1000.
40. Deng R, Hurtz C, Song Q, Yue C, Xiao G, Yu H, et al. Extrafollicular CD4(+) T-B interactions are sufficient for inducing autoimmune-like chronic graft-versus-host disease. Nat Commun. 2017;8(1):978.
41. Delaunay M, Caron P, Sibaud V, Godillot C, Collot S, Milia J, et al. Toxicity of immune checkpoints inhibitors. Rev Mal Respir. 2018;35(10):1028–38.
42. Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. N Engl J Med. 2018;378(2):158–68.
43. Syn NL, Teng MWL, Mok TSK, Soo RA. De-novo and acquired resistance to immune checkpoint targeting. Lancet Oncol. 2017;18(12):e731–e41.
44. Dyck L, Mills KHG. Immune checkpoints and their inhibition in cancer and infectious diseases. Eur J Immunol. 2017;47(5):765–79.
45. Topalian SL, Taube JM, Anders RA, Pardoll DM. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. Nat Rev Cancer. 2016;16(5):275–87.
46. Marcucci F, Rumio C, Corti A. Tumor cell-associated immune checkpoint molecules—drivers of malignancy and stemness. Biochim Biophys Acta Rev Cancer. 2017;1868(2):571–83.
47. Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, Iglesias M, et al. TGFbeta drives immune evasion in genetically reconstituted colon cancer metastasis. Nature. 2018;554(7693):538–43.
48. Kikushige Y, Miyamoto T, Yuda J, Jabbarzadeh-Tabrizi S, Shima T, Takayanagi S, et al. A TIM-3/Gal-9 autocrine stimulatory loop drives self-renewal of human myeloid leukemia stem cells and leukemic progression. Cell Stem Cell. 2015;17(3):341–52.
49. Wang Y, Wang H, Zhao Q, Xia Y, Hu X, Guo J. PD-L1 induces epithelial-to-mesenchymal transition via activating SREBP-1c in renal cell carcinoma. Med Oncol. 2015;32(8):212.
50. Saleh R, Taha RZ, Sasidharan Nair V, Alajez NM, Elkord E. PD-L1 blockade by atezolizumab downregulates signaling pathways associated with tumor growth, metastasis, and hypoxia in human triple negative breast cancer. Cancers (Basel). 2019;11(8):1050.
51. Gao L, Guo Q, Li X, Yang X, Ni H, Wang T, et al. MiR-873/PD-L1 axis regulates the stemness of breast cancer cells. EBioMedicine. 2019;41:395–407.
52. Ge H, Mu L, Jin L, Yang C, Chang YE, Long Y, et al. Tumor associated CD70 expression is involved in promoting tumor migration and macrophage infiltration in GBM. Int J Cancer. 2017;141(7):1434–44.
53. Nakaima Y, Watanabe K, Koyama T, Miura O, Fukuda T. CD137 is induced by the CD40 signal on chronic lymphocytic leukemia B cells and transduces the survival signal via NF-kappaB activation. PLoS One. 2013;8(5):e64425.
54. Palma C, Binaschi M, Bigioni M, Maggi CA, Goso C. CD137 and CD137 ligand constitutively coexpressed on human T and B leukemia cells signal proliferation and survival. Int J Cancer. 2004;108(3):390–8.
55. Li M, Yang Y, Wei J, Cun X, Lu Z, Qiu Y, et al. Enhanced chemo-immunotherapy against melanoma by inhibition of cholesterol esterification in CD8(+) T cells. Nanomedicine. 2018;14(8):2541–50.
56. Sansregret L, Vanhaesebroeck B, Swanton C. Determinants and clinical implications of chromosomal instability in cancer. Nat Rev Clin Oncol. 2018;15(3):139–50.
57. Menezo Y, Lichtblau I, Elder K. New insights into human pre-implantation metabolism in vivo and in vitro. J Assist Reprod Genet. 2013;30(3):293–303.
58. Zhao M, Bu Y, Feng J, Zhang H, Chen Y, Yang G, et al. SPIN1 triggers abnormal lipid metabolism and enhances tumor growth in liver cancer. Cancer Lett. 2020;470:54–63.
59. Ahmad F, Patrick S, Sheikh T, Sharma V, Pathak P, Malgulwar PB, et al. Telomerase reverse transcriptase (TERT)—enhancer of zeste homolog 2 (EZH2) network regulates lipid metabolism and DNA damage responses in glioblastoma. J Neurochem. 2017;143(6):671–83.
60. Ogino S, Kawasaki T, Ogawa A, Kirkner GJ, Loda M, Fuchs CS. Fatty acid synthase overexpression in colorectal cancer is associated with microsatellite instability, independent of CpG island methylator phenotype. Hum Pathol. 2007;38(6):842–9.
61. Rao CV, Sanghera S, Zhang Y, Biddick L, Reddy A, Lightfoot S, et al. Systemic chromosome instability resulted in colonic transcriptomic changes in metabolic, proliferation, and stem cell regulators in Sgo1−/+ mice. Cancer Res. 2016;76(3):630–42.
62. Sato Y, Tanaka S, Kinoshita M, Takemura S, Shinkawa H, Kokudo T, et al. Immunosuppressive tumor microenvironment in occupational cholangiocarcinoma: supportive evidence for the efficacy of immune checkpoint inhibitor therapy. J Hepatobiliary Pancreat Sci. 2020;27:860.
63. Choi E, Chang MS, Byeon SJ, Jin H, Jung KC, Kim H, et al. Prognostic perspectives of PD-L1 combined with tumor-infiltrating lymphocytes, Epstein-Barr virus, and microsatellite instability in gastric carcinomas. Diagn Pathol. 2020;15(1):69.
64. Leussink S, Aranda-Pardos I. N AG. Lipid metabolism as a mechanism of immunomodulation in macrophages: the role of liver X receptors. Curr Opin Pharmacol. 2020;53:18–26.
65. Jahangiri A, Dadmanesh M, Ghorban K. STAT3 inhibition reduced PD-L1 expression and enhanced antitumor immune responses. J Cell Physiol. 2020;235:9457.
66. Wang T, Fahrmann JF, Lee H, Li YJ, Tripathi SC, Yue C, et al. JAK/STAT3-regulated fatty acid beta-

oxidation is critical for breast cancer stem cell self-renewal and chemoresistance. Cell Metab. 2018;27(1):136–50.e5.
67. Hu G, He N, Cai C, Cai F, Fan P, Zheng Z, et al. HDAC3 modulates cancer immunity via increasing PD-L1 expression in pancreatic cancer. Pancreatology. 2019;19(2):383–9.
68. Chen J, Jiang CC, Jin L, Zhang XD. Regulation of PD-L1: a novel role of pro-survival signalling in cancer. Ann Oncol. 2016;27(3):409–16.
69. Miska J, Lee-Chang C, Rashidi A, Muroski ME, Chang AL, Lopez-Rosas A, et al. HIF-1alpha is a metabolic switch between glycolytic-driven migration and oxidative phosphorylation-driven immunosuppression of Tregs in glioblastoma. Cell Rep. 2019;27(1):226–37.e4.
70. Wang Y, Wang H, Yao H, Li C, Fang JY, Xu J. Regulation of PD-L1: emerging routes for targeting tumor immune evasion. Front Pharmacol. 2018;9:536.
71. Tsytsykova AV, Tsitsikov EN, Geha RS. The CD40L promoter contains nuclear factor of activated T cells-binding motifs which require AP-1 binding for activation of transcription. J Biol Chem. 1996;271(7):3763–70.
72. Sumimoto H, Takano A, Teramoto K, Daigo Y. RAS-mitogen-activated protein kinase signal is required for enhanced PD-L1 expression in human lung cancers. PLoS One. 2016;11(11):e0166626.
73. Sayour EJ, Grippin A, De Leon G, Stover B, Rahman M, Karachi A, et al. Personalized tumor RNA loaded lipid-nanoparticles prime the systemic and intratumoral milieu for response to cancer immunotherapy. Nano Lett. 2018;18(10):6195–206.
74. Gonzalez MB, Lane M, Knight EJ, Robker RL. Inflammatory markers in human follicular fluid correlate with lipid levels and body mass index. J Reprod Immunol. 2018;130:25–9.
75. Devitt A, Griffiths HR, Milic I. Communicating with the dead: lipids, lipid mediators and extracellular vesicles. Biochem Soc Trans. 2018;46(3):631–9.
76. Zeng H, Yang K, Cloer C, Neale G, Vogel P, Chi H. mTORC1 couples immune signals and metabolic programming to establish T(reg)-cell function. Nature. 2013;499(7459):485–90.
77. Wu Q, Tian Y, Zhang J, Zhang H, Gu F, Lu Y, et al. Functions of pancreatic stellate cell-derived soluble factors in the microenvironment of pancreatic ductal carcinoma. Oncotarget. 2017;8(60):102721–38.
78. Sun Z, Mao A, Wang Y, Zhao Y, Chen J, Xu P, et al. Treatment with anti-programmed cell death 1 (PD-1) antibody restored postoperative CD8+ T cell dysfunction by surgical stress. Biomed Pharmacother. 2017;89:1235–41.
79. Prima V, Kaliberova LN, Kaliberov S, Curiel DT, Kusmartsev S. COX2/mPGES1/PGE2 pathway regulates PD-L1 expression in tumor-associated macrophages and myeloid-derived suppressor cells. Proc Natl Acad Sci U S A. 2017;114(5):1117–22.
80. Gorchs L, Fernandez Moro C, Bankhead P, Kern KP, Sadeak I, Meng Q, et al. Human pancreatic carcinoma-associated fibroblasts promote expression of co-inhibitory markers on CD4(+) and CD8(+) T-cells. Front Immunol. 2019;10:847.
81. Chou JP, Ramirez CM, Ryba DM, Koduri MP, Effros RB. Prostaglandin E2 promotes features of replicative senescence in chronically activated human CD8+ T cells. PLoS One. 2014;9(6):e99432.
82. Iqubal A, Iqubal MK, Sharma S, Ansari MA, Najmi AK, Ali SM, et al. Molecular mechanism involved in cyclophosphamide-induced cardiotoxicity: old drug with a new vision. Life Sci. 2019;218:112–31.
83. Cummins G, Yung DE, Cox BF, Koulaouzidis A, Desmulliez MPY, Cochran S. Luminally expressed gastrointestinal biomarkers. Expert Rev Gastroenterol Hepatol. 2017;11(12):1119–34.
84. Basta-Jovanovic G, Bogdanovic L, Radunovic M, Prostran M, Naumovic R, Simic-Ogrizovic S, et al. Acute renal failure—a serious complication in patients after kidney transplantation. Curr Med Chem. 2016;23(19):2012–7.
85. McKillop IH, Girardi CA, Thompson KJ. Role of fatty acid binding proteins (FABPs) in cancer development and progression. Cell Signal. 2019;62:109336.
86. Lin R, Zhang H, Yuan Y, He Q, Zhou J, Li S, et al. Fatty acid oxidation controls CD8(+) tissue-resident memory T-cell survival in gastric adenocarcinoma. Cancer Immunol Res. 2020;8(4):479–92.
87. Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. Nat Rev Drug Discov. 2008;7(6):489–503.
88. Field CS, Baixauli F, Kyle RL, Puleston DJ, Cameron AM, Sanin DE, et al. Mitochondrial integrity regulated by lipid metabolism is a cell-intrinsic checkpoint for Treg suppressive function. Cell Metab. 2020;31(2):422–37. e5
89. Kawashima M, Tokiwa M, Nishimura T, Kawata Y, Sugimoto M, Kataoka TR, et al. High-resolution imaging mass spectrometry combined with transcriptomic analysis identified a link between fatty acid composition of phosphatidylinositols and the immune checkpoint pathway at the primary tumour site of breast cancer. Br J Cancer. 2020;122(2):245–57.
90. Smathers RL, Petersen DR. The human fatty acid-binding protein family: evolutionary divergences and functions. Hum Genomics. 2011;5(3):170–91.
91. Dobson CM. Protein folding and misfolding. Nature. 2003;426(6968):884–90.
92. Song MJ, Malhi H. The unfolded protein response and hepatic lipid metabolism in non alcoholic fatty liver disease. Pharmacol Ther. 2019;203:107401.
93. Lee S, Min KT. The interface between ER and mitochondria: molecular compositions and functions. Mol Cells. 2018;41(12):1000–7.
94. Lebeaupin C, Vallee D, Hazari Y, Hetz C, Chevet E, Bailly-Maitre B. Endoplasmic reticulum stress sig-

nalling and the pathogenesis of non-alcoholic fatty liver disease. J Hepatol. 2018;69(4):927–47.
95. Calfon M, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, et al. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. Nature. 2002;415(6867):92–6.
96. Oakes SA. Endoplasmic reticulum proteostasis: a key checkpoint in cancer. Am J Physiol Cell Physiol. 2017;312(2):C93–C102.
97. Yao H, Xu J. Regulation of cancer immune checkpoint: mono- and poly-ubiquitination: tags for fate. Adv Exp Med Biol. 2020;1248:295–324.
98. Dias-Teixeira KL, Calegari-Silva TC, Medina JM, Vivarini AC, Cavalcanti A, Teteo N, et al. Emerging role for the PERK/eIF2alpha/ATF4 in human cutaneous leishmaniasis. Sci Rep. 2017;7(1):17074.
99. Sidibeh CO, Pereira MJ, Abalo XM, Gretha JB, Skrtic S, Lundkvist P, et al. FKBP5 expression in human adipose tissue: potential role in glucose and lipid metabolism, adipogenesis and type 2 diabetes. Endocrine. 2018;62(1):116–28.
100. D'Arrigo P, Russo M, Rea A, Tufano M, Guadagno E, Del Basso De Caro ML, et al. A regulatory role for the co-chaperone FKBP51s in PD-L1 expression in glioma. Oncotarget. 2017;8(40):68291–304.
101. Strasser R. Protein quality control in the endoplasmic reticulum of plants. Annu Rev Plant Biol. 2018;69:147–72.
102. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature. 2016;529(7586):326–35.
103. Jansen G, Maattanen P, Denisov AY, Scarffe L, Schade B, Balghi H, et al. An interaction map of endoplasmic reticulum chaperones and foldases. Mol Cell Proteomics. 2012;11(9):710–23.
104. Ibrahim IM, Abdelmalek DH, Elfiky AA. GRP78: a cell's response to stress. Life Sci. 2019;226:156–63.
105. Gupta AP, Singh P, Garg R, Valicherla GR, Riyazuddin M, Syed AA, et al. Pancreastatin inhibitor activates AMPK pathway via GRP78 and ameliorates dexamethasone induced fatty liver disease in C57BL/6 mice. Biomed Pharmacother. 2019;116:108959.
106. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest. 2002;109(9):1125–31.
107. Cook KL, Soto-Pantoja DR, Clarke PA, Cruz MI, Zwart A, Warri A, et al. Endoplasmic reticulum stress protein GRP78 modulates lipid metabolism to control drug sensitivity and antitumor immunity in breast cancer. Cancer Res. 2016;76(19):5657–70.
108. Larbi A, Fortin C, Dupuis G, Berrougui H, Khalil A, Fulop T. Immunomodulatory role of high-density lipoproteins: impact on immunosenescence. Age (Dordr). 2014;36(5):9712.
109. Ma X, Li SF, Qin ZS, Ye J, Zhao ZL, Fang HH, et al. Propofol up-regulates expression of ABCA1, ABCG1, and SR-B1 through the PPARgamma/LXRalpha signaling pathway in THP-1 macrophage-derived foam cells. Cardiovasc Pathol. 2015;24(4):230–5.
110. Han F, Wang G, Li Y, Tian W, Dong Z, Cheng S, et al. Investigation of T-cell immunoglobulin- and mucin-domain-containing molecule-3 (TIM-3) polymorphisms in essential thrombocythaemia (ET). Hematology. 2017;22(6):361–7.
111. O'Sullivan D, van der Windt GJ, Huang SC, Curtis JD, Chang CH, Buck MD, et al. Memory CD8(+) T cells use cell-intrinsic lipolysis to support the metabolic programming necessary for development. Immunity. 2014;41(1):75–88.
112. Patsoukis N, Bardhan K, Chatterjee P, Sari D, Liu B, Bell LN, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat Commun. 2015;6:6692.
113. Haselbarth L, Ouwens DM, Teichweyde N, Hochrath K, Merches K, Esser C. The small chain fatty acid butyrate antagonizes the TCR-stimulation-induced metabolic shift in murine epidermal gamma delta T cells. EXCLI J. 2020;19:334–50.
114. Xie Z, Ago Y, Okada N, Tachibana M. Valproic acid attenuates immunosuppressive function of myeloid-derived suppressor cells. J Pharmacol Sci. 2018;137(4):359–65.
115. Folkl A, Bienzle D. Structure and function of programmed death (PD) molecules. Vet Immunol Immunopathol. 2010;134(1–2):33–8.
116. Toprak U, Hegedus D, Dogan C, Guney G. A journey into the world of insect lipid metabolism. Arch Insect Biochem Physiol. 2020;104(2):e21682.
117. Yessoufou A, Ple A, Moutairou K, Hichami A, Khan NA. Docosahexaenoic acid reduces suppressive and migratory functions of CD4+CD25+ regulatory T-cells. J Lipid Res. 2009;50(12):2377–88.
118. Chikuma S, Imboden JB, Bluestone JA. Negative regulation of T cell receptor-lipid raft interaction by cytotoxic T lymphocyte-associated antigen 4. J Exp Med. 2003;197(1):129–35.
119. Li X, Wu Y, Zhao J, Wang H, Tan J, Yang M, et al. Distinct cardiac energy metabolism and oxidative stress adaptations between obese and non-obese type 2 diabetes mellitus. Theranostics. 2020;10(6):2675–95.
120. Lu Y, Zheng Y, Coyaud E, Zhang C, Selvabaskaran A, Yu Y, et al. Palmitoylation of NOD1 and NOD2 is required for bacterial sensing. Science. 2019;366(6464):460–7.
121. Yang Y, Hsu JM, Sun L, Chan LC, Li CW, Hsu JL, et al. Palmitoylation stabilizes PD-L1 to promote breast tumor growth. Cell Res. 2019;29(1):83–6.
122. Yao H, Lan J, Li C, Shi H, Brosseau JP, Wang H, et al. Author correction: inhibiting PD-L1 palmitoylation enhances T-cell immune responses against tumours. Nat Biomed Eng. 2019;3(5):414.

123. Han L, Lam EW, Sun Y. Extracellular vesicles in the tumor microenvironment: old stories, but new tales. Mol Cancer. 2019;18(1):59.
124. Flaherty SE 3rd, Grijalva A, Xu X, Ables E, Nomani A, Ferrante AW Jr. A lipase-independent pathway of lipid release and immune modulation by adipocytes. Science. 2019;363(6430):989–93.
125. Lawler SE, Nowicki MO, Ricklefs FL, Chiocca EA. Immune escape mediated by exosomal PD-L1 in cancer. Adv Biosyst. 2020;4:e2000017.
126. Yu J, Lin Y, Xiong X, Li K, Yao Z, Dong H, et al. Detection of exosomal PD-L1 RNA in saliva of patients with periodontitis. Front Genet. 2019;10:202.
127. Whiteside TL. Exosomes carrying immunoinhibitory proteins and their role in cancer. Clin Exp Immunol. 2017;189(3):259–67.
128. Gao J, Qiu X, Li X, Fan H, Zhang F, Lv T, et al. Expression profiles and clinical value of plasma exosomal Tim-3 and Galectin-9 in non-small cell lung cancer. Biochem Biophys Res Commun. 2018;498(3):409–15.
129. Ferris SP, Kodali VK, Kaufman RJ. Glycoprotein folding and quality-control mechanisms in protein-folding diseases. Dis Model Mech. 2014;7(3):331–41.
130. Breitling J, Aebi M. N-linked protein glycosylation in the endoplasmic reticulum. Cold Spring Harb Perspect Biol. 2013;5(8):a013359.
131. Alter G, Ottenhoff THM, Joosten SA. Antibody glycosylation in inflammation, disease and vaccination. Semin Immunol. 2018;39:102–10.
132. Li CW, Lim SO, Xia W, Lee HH, Chan LC, Kuo CW, et al. Glycosylation and stabilization of programmed death ligand-1 suppresses T-cell activity. Nat Commun. 2016;7:12632.
133. Zhang J, Bu X, Wang H, Zhu Y, Geng Y, Nihira NT, et al. Cyclin D-CDK4 kinase destabilizes PD-L1 via cullin 3-SPOP to control cancer immune surveillance. Nature. 2018;553(7686):91–5.
134. Serman TM, Gack MU. FBXO38 drives PD-1 to destruction. Trends Immunol. 2019;40(2):81–3.
135. Lim SO, Li CW, Xia W, Cha JH, Chan LC, Wu Y, et al. Deubiquitination and stabilization of PD-L1 by CSN5. Cancer Cell. 2016;30(6):925–39.
136. Mezzadra R, Sun C, Jae LT, Gomez-Eerland R, de Vries E, Wu W, et al. Identification of CMTM6 and CMTM4 as PD-L1 protein regulators. Nature. 2017;549(7670):106–10.
137. Han D, Liu J, Chen C, Dong L, Liu Y, Chang R, et al. Anti-tumour immunity controlled through mRNA m(6)A methylation and YTHDF1 in dendritic cells. Nature. 2019;566(7743):270–4.
138. Burr ML, Sparbier CE, Chan YC, Williamson JC, Woods K, Beavis PA, et al. CMTM6 maintains the expression of PD-L1 and regulates anti-tumour immunity. Nature. 2017;549(7670):101–5.
139. Yao H, Lan J, Li C, Shi H, Brosseau JP, Wang H, et al. Inhibiting PD-L1 palmitoylation enhances T-cell immune responses against tumours. Nat Biomed Eng. 2019;3(4):306–17.
140. Fujita Y, Tinoco R, Li Y, Senft D, Ronai ZA. Ubiquitin ligases in cancer immunotherapy—balancing antitumor and autoimmunity. Trends Mol Med. 2019;25(5):428–43.
141. Imbert C, Montfort A, Fraisse M, Marcheteau E, Gilhodes J, Martin E, et al. Resistance of melanoma to immune checkpoint inhibitors is overcome by targeting the sphingosine kinase-1. Nat Commun. 2020;11(1):437.
142. Strauss L, Mahmoud MAA, Weaver JD, Tijaro-Ovalle NM, Christofides A, Wang Q, et al. Targeted deletion of PD-1 in myeloid cells induces antitumor immunity. Sci Immunol. 2020;5(43):eaay1863.
143. Wang H, Tang Y, Fang Y, Zhang M, Wang H, He Z, et al. Reprogramming tumor immune microenvironment (TIME) and metabolism via biomimetic targeting codelivery of Shikonin/JQ1. Nano Lett. 2019;19(5):2935–44.
144. Wang B, Tontonoz P. Liver X receptors in lipid signalling and membrane homeostasis. Nat Rev Endocrinol. 2018;14(8):452–63.
145. Lee S, Dong HH. FoxO integration of insulin signaling with glucose and lipid metabolism. J Endocrinol. 2017;233(2):R67–79.