Function and characteristics of TIM-4 in immune regulation and disease (Review)

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Abstract. T-cell/transmembrane immunoglobulin and mucin domain containing 4 (TIM-4) is a phosphatidylserine receptor that is mainly expressed on antigen-presenting cells and is involved in the recognition and efferocytosis of apoptotic cells. TIM-4 has been found to be expressed in immune cells such as natural killer T, B and mast cells and to participate in multiple aspects of immune regulation, suggesting that TIM-4 may be involved in a variety of immune-related diseases. Recent studies have confirmed that TIM-4 is also abnormally expressed in a variety of malignant tumor cells and is closely associated with the occurrence and development of tumors and the tumor immune microenvironment. The present study aimed to describe the expression and functional characteristics of TIM-4 in detail and to comprehensively discuss its role in pathophysiological processes such as infection, allergy, metabolism, autoimmunity and tumor immunity. The current review provided a comprehensive understanding of the functions and characteristics of TIM-4, as well as novel ideas for the diagnosis and treatment of diseases.

Contents

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1. Introduction

The T-cell immunoglobulin and mucin domain (TIM) family, also known as the transmembrane immunoglobulin and mucin domain (TIM) family, was discovered in 2001 (1). The names reflect their function, properties and expression patterns (2). All TIM members are composed of a cell surface type I membrane protein, with an N-terminal cysteine rich IgV-like domain, a mucin domain, a transmembrane domain and an intracellular domain (3). The TIM family consists of eight members on mouse chromosome 11B1.1 (TIM-1-8) and three members on human chromosome 5q33.2 (TIM-1, TIM-3 and TIM-4) (4). The mouse chromosome 11B1.1 region is associated with various diseases, including asthma, allergies and autoimmunity (5). In mice, TIM genes include hepatitis A virus cellular receptor (HAVCR)1 (also known as TIMD1, which encodes TIM-1), TIMD2 (encoding TIM-2), HAVCR2 (also known as TIMD3, which encodes TIM-3) and TIMD4 (which encodes TIM-4) and four predicted TIM genes, namely TIM-5, TIM-6, TIM-7 and TIM-8 (6). The human 5q33.2 region is also a chromosomal region associated with autoimmunity (4). There are three TIM genes expressed in humans: HAVCR1 (which encodes TIM-1), HAVCR2 (which encodes TIM-3) and TIMD4 (which encodes TIM-4) (6) (Fig. 1).

TIM-4 was first identified in 2004 in stromal cells of the marginal zone and lymph nodes of the spleen (7). TIM-4, a type I membrane protein of 60 kDa, contains a conserved arginine-glycine-aspartic acid (RGD) sequence, which is a possible integrin protein binding sequence (8). The mucin domain of TIM-4 is the longest among the TIM family members and is rich in threonine, serine and proline residues (9). The intracellular domain of TIM-4 contains 42-77 amino acids, while the intracellular domain of TIM-4 lacks tyrosine-activating residues and cannot transmit signals to the cytoplasm, which is the difference between TIM-4, TIM-1 and TIM-3 (10,11). Therefore, the unique structure of TIM-4 suggests that it may serve unique roles.

TIM-4 has multiple expression profiles. TIM-4 is highly expressed in common peripheral lymphoid tissues (including

tonsils, thymus, spleen and lymph nodes), while it is expressed at low levels in lung, liver and kidney tissues (12-15). Previously, it was considered that TIM-4 was mainly expressed in antigen-presenting cells (APCs) such as macrophages and mature dendritic cells (DCs), but it has also been found that peritoneal B1 and natural killer T (NKT) cells also express TIM-4 (13,15). TIM-4 is also expressed in mast cells after stimulation with flagellin (16). These results suggest that TIM-4 is involved in the regulation of immune function at multiple levels through a variety of immune cells. Thus, it is worth investigating the potential therapeutic role of TIM-4 in the treatment of diseases such as autoimmune diseases, organ transplantation and inflammatory diseases. Notably, in addition to immune cells expressing TIM-4, a variety of tumor cells also express TIM-4, such as lung, kidney and colorectal cancer and malignant glioma (17-20). Moreover, TIM-4 has different effects on tumor cell function and the tumor microenvironment in different tumors, which reflects the variety of functions of TIM-4.

The diverse expression of TIM-4 leads to its functional diversity. TIM-4 is considered to be a costimulatory molecule regulating T cell function and a phosphatidylserine receptor (PS) involved in the recognition of apoptotic cells (6,21). Its functional characteristics change with expressing cells and sites. TIM-4 exerts a bimodal regulatory effect according to the activation state of T cells and it may regulate T cell function by crosslinking different receptors (such as TIM-1 and PS) (9). Lipopolysaccharide (LPS), cholera toxin, cytokines, concanavalin A and danger-associated molecule pattern also stimulate the expression of TIM-4; by contrast, certain probiotics inhibit the expression of TIM-4 by inhibiting the transcription factor STAT6 in DCs (22-24). APCs expressing TIM-4 change their function following different stimuli. As a PS receptor, TIM-4 transmits the signal of 'eat me' to phagocyte cells and then initiates efferocytosis (21). The recognition of apoptotic cells is a complex process that involves various surface molecules, bridging molecules and phagocytic receptors. Furthermore, unlike TIM-1 and TIM-3, TIM-4 is markedly sensitive to the density of PS, which is a mechanism for phagocytes to recognize apoptotic and non-apoptotic cells (25). However, as aforementioned, the intracellular structure of TIM-4 lacks tyrosine sequences. Therefore, TIM-4 cannot transmit secondary signals to the cell; thus, it is unclear how it transmits the 'eat me' signal. Although the concept of a 'tethered molecule' has been proposed (26), it is insufficient to fully explain the role of TIM-4 in efferocytosis. Thus, the present review discussed this topic in detail.

In summary, due to its unique expression and functional characteristics, TIM-4 participates in the regulation of immune function and the process of malignant tumors at multiple levels. The present study aimed to clarify the role of TIM-4 in various immune diseases and tumors and provided useful ideal for the development of new treatment strategies.

2. Regulatory role of TIM-4 in efferocytosis

Phagocytes possess a unique mechanism for distinguishing apoptotic from living cells (27,28). PS, which is exposed on the outer side of apoptotic cell membranes, is a key molecule in this mechanism. TIM-4 binds to the PS molecules that are expressed in apoptotic cells to promote efferocytosis (29).

There are two pathways to initiate phagocytic signal transduction. First, receptors of apoptosis signals on the surface of phagocytes directly bind to apoptotic signal molecules on the surface of apoptotic cells and directly transmit signals to the interior of phagocytes to initiate the process of apoptosis. Second, receptors called tethering molecules on the surface of phagocytes bind directly or indirectly to apoptotic signaling molecules on the surface of apoptotic cells to fix apoptotic cells around phagocytes, so that other apoptotic signal recognition receptors can interact with them to initiate efferocytosis (29-33). TIM-4 cannot directly transmit phagocytic signals due to the lack of a tyrosine sequence in its intracellular domain (34). Previous experimental evidence suggested that the ability of TIM-4 mutants (lacking transmembrane and intracellular domains) to mediate efferocytosis was the same as that of wild type TIM-4 (10,26,35). Therefore, TIM-4 is considered to be a tethered receptor that promotes the efferocytosis of apoptotic cells by effectively anchoring them to phagocytic cells, which have the same function as phagocytic receptors (such as CD14 and CD36) (26). Using a parental Ba/F3 mouse pre-B-cell line model that does not phagocytose apoptotic cells, it was demonstrated that Ba/F3 transformants expressing TIM-4 could efficiently bind apoptotic cells in a PS-dependent manner but did not phagocytose them, while Ba/F3 transformants expressing the TIM-4 and integrin $\alpha v\beta 3$ complex could bind and phagocytose apoptotic cells in the presence of milk fat globule EGF factor 8 (MFG-E8), which showed that TIM-4 played a role in the binding step of the two-step efferocytosis process of apoptotic cells (32) (Fig. 2A).

Although TIM-4 has been widely recognized as a tethered receptor, it can still interact with certain molecules to enhance the active participation of TIM-4 in the process of efferocytosis not only as a physical tether but also as a bridge in signal recognition and transduction. It was previously observed that a surrogate, even more effective transmembrane tether, failed to compensate for the absence of TIM-4 in the efferocytosis of the PS-dependent pathway, which showed that TIM-4 not only played a physical role in tethering but also played a synergistic effect on efferocytosis (36). Fibronectin 1 (Fn1) interacts with TIM-4. Fn1 contains three domains: Types I, II and III and the last is responsible for cell-to-cell binding by interacting with various integrins through the RGD motif (37,38). A single type III domain can interact with the RGD motif of the TIM-4 IgV domain and Fn1 contains multiple type III domains (37). Therefore, multiple TIM-4 molecules can gather to recruit different types of phagocytic receptors. Fn1 participates in the formation of the TIM-4/Fn1/integrin ($\alpha v\beta 5$) trimer complex by acting as a bridge. Integrin can directly transmit phagocytic signals and this trimer complex effectively participates in the tethering and efferocytosis of apoptotic cells (39) (Fig. 2A).

In addition to serving as a receptor for PS, TIM-4 is also involved in the formation of phagosomes. In a zebra fish model, it was found that TIM-4 and brain-specific angiogenesis inhibitor 1 (BAI1) on microglia mediate the clearance of dead neurons, a process that can be divided into three steps: Recognition, phagosome formation and engulfment. In fact, TIM-4 is not required for the recognition of apoptotic cells, a result that does not support the notion that TIM-4 act

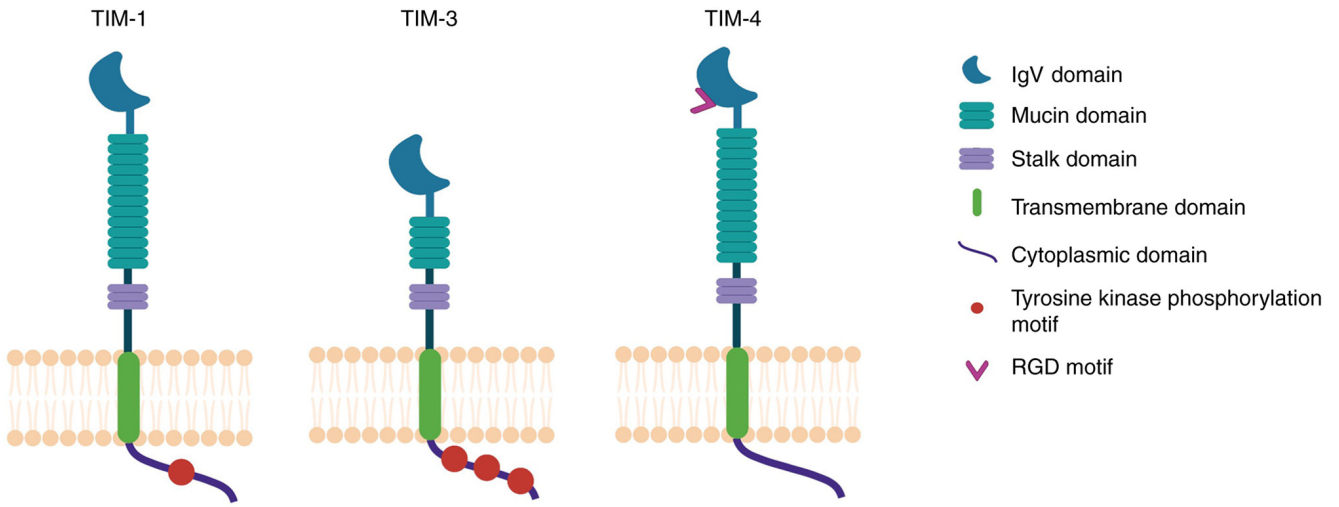

Figure 1. The structure and main domains of human TIM gene family members. The structure of the TIM family is conserved from in mouse and human. TIM molecules are type-I cell-surface glycoproteins that comprise an extracellular IgV domain, a mucin-like domain, a transmembrane domain and an intracellular cytoplasmic tail. TIM-4 is unique because it has an RGD (integrin-binding) motif, but no tyrosine phosphorylation motif. TIM, transmembrane immunoglobulin and mucin domain; RGD, arginine-glycine-aspartic acid.

as a tethered molecule in efferocytosis (32,40). Indeed, in microglia, TIM-4 stabilizes phagosomes by allowing actin to polymerize around the phagosome (41) (Fig. 2B).

Some pathogens simulate apoptotic cells by exposing PS to viral envelopes or presenting membrane-derived vesicles containing bacteria. TIM-4 on the surface of host cells can combine with these apoptotic mimetic cells to promote efferocytosis, which promotes the cell-to-cell spread of bacteria or viruses (42-44). For example, *Listeria* is released from cells in the form of PS^+ vesicles, while macrophages mediate the internalization of PS^+ vesicles through TIM-4, which promotes the transmission of bacteria between cells (44) (Fig. 2A). Similar to the above mechanism, targeted drug delivery can be achieved by PS containing liposome and TIM-4-PS interaction. The affinity between liposomal PS and TIM-4 can be regulated by the membrane fluidity of PS-containing liposomes and a zwitterionic helper lipid affects this regulation mechanism by modulating membrane fluidity. An increase in membrane fluidity was found to increase the affinity between PS and TIM-4. This result shown that TIM-4 can be used to rationally optimize nanotechnology for targeted drug delivery (45).

3. Functional characteristics of TIM-4 in different immune cells

Role of TIM-4 in macrophages. One of the most important functions of TIM-4 is to express in macrophages and participate in immune regulation. The functions of TIM-4 in macrophages can be summarized into five parts: Efferocytosis, tethering, receptor-ligand binding, defining macrophages with unique functions and regulating the release of cytokines (Table I).

In adaptive immune regulation phase, the highly expressed TIM-4 on the phagocytes rapidly recognizes and phagocytizes antigen-specific T cells through PS. Through this mechanism, TIM-4 regulates immunity by balancing the number and proportion of antigen-specific T and memory T cells (46). It has been observed that $F4/80^+TIM\text{-}4^+$ macrophages engulfed antigen-specific T cells expressing PS, resulting in a decrease in antigen-specific T cells entering the periphery, which finally induced immune tolerance (47-49) (Fig. 2C).These results demonstrate that TIM-4 on the surface of macrophages participates in immune regulation through efferocytosis.

In addition to directly regulating immunity through efferocytosis, TIM-4 on the surface of macrophages indirectly promotes efferocytosis and participates in immune regulation through tethering. The most striking example is the efficient efferocytosis by resident macrophages in the abdominal cavity through TIM-4 and protein S/MerTK (31). In the pulmonary lymph nodes, $F4/80^+$ macrophages not only engulf apoptotic T cells but also retain apoptotic T cells that have not yet entered the apoptotic pathway completely in the medulla through the TIM-4-PS pathway and ultimately promote the clearance of antigen-specific T cells (47) (Fig. 2C). In fact, the expression of PS on the cell membrane is not only a sign of apoptosis but also represents in the activation of various immune cells, including T cells (50,51). The immune system is able to distinguish the PS in apoptotic and non-apoptotic cells. TIM-4 initiates highly selective and complete clearance of apoptotic T cells by binding to high levels of PS on the cytomembrane. In other words, TIM-4 recognizes apoptotic cells by recognizing different densities of PS (14,29,47). In addition, this tethering effect of TIM-4 on the surface of macrophages leads to functional isolation of $TIM\text{-}4^+$ macrophages from cytotoxic CD8+T cells and inhibition of their proliferation, which in turn affects the effect of anti-PD-1 therapy (52).

TIM-4 on the surface of macrophages can also serve an immunomodulatory role by binding to its ligands on the surface of T cells (Fig. 3). It has been previously shown that TIM-4 binds to TIM-3 on the surface of Th1 cells and induces the apoptosis of Th1 cells in a dose-dependent manner by enhancing the expression of Fas ligand (53). The role of TIM-4 in the immune response is complex and depends on the expression level of TIM-4 and the type of tissues and cells and is also closely associated with the type of target cells. A previous study has shown that a low concentration of TIM-4 fusion

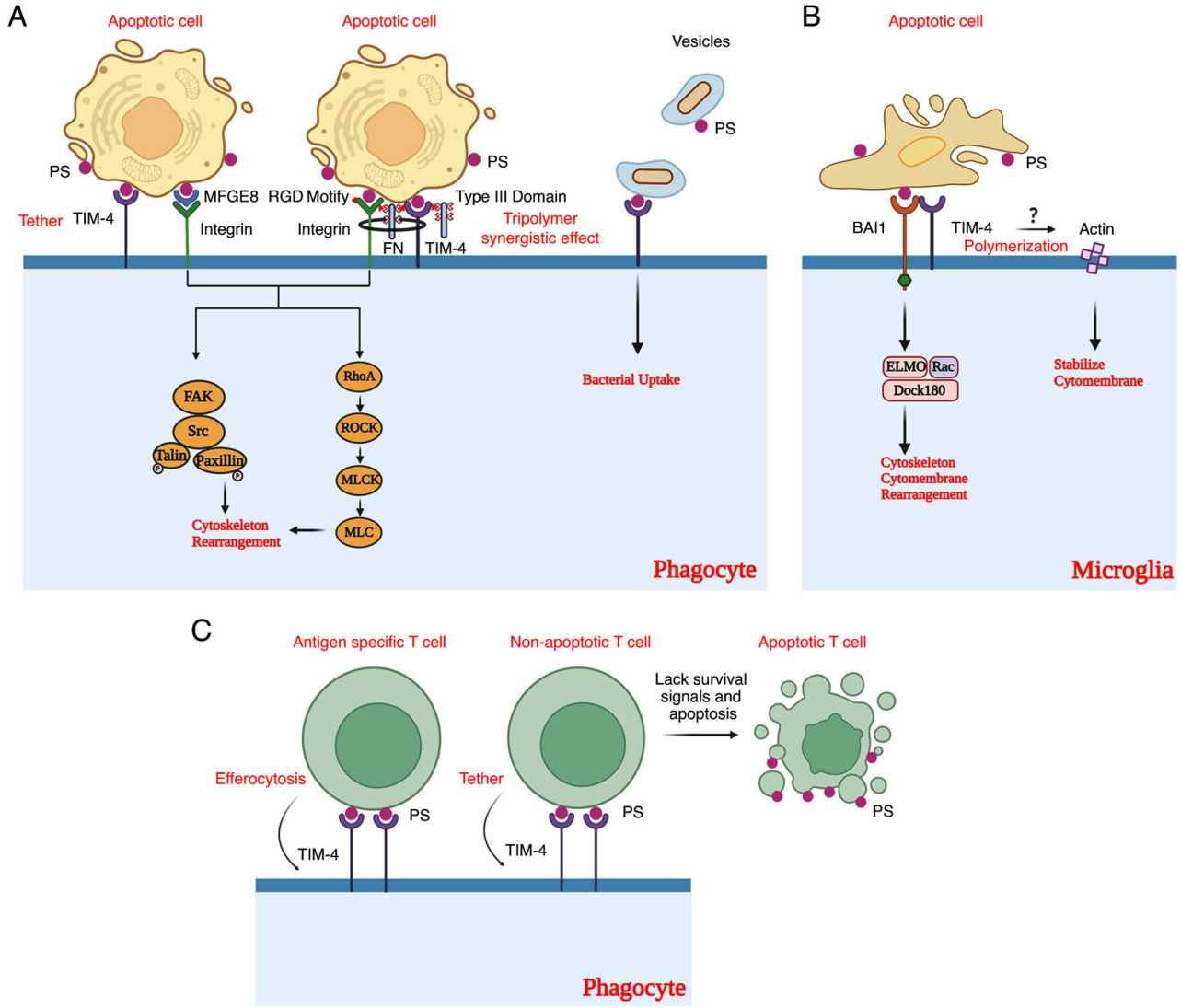

Figure 2. Several patterns for the TIM-4 in efferocytosis. (A) Pattern 1: TIM-4 acts as a tethering molecule to fix apoptotic cells around phagocytes and directly transmit phagocytic signals to phagocytes to cause cytoskeleton or cell membrane remodeling to initiate efferocytosis. Pattern 2: As a cooperative molecule, TIM-4 recruits integrins to form tripolymer through connecting molecules and integrins directly transmit phagocytic signals to phagocytes to initiate cytoskeleton or cell membrane remodeling. Pattern 3: TIM-4 directly promotes the engulfment of bacterial vesicles by recognizing PS on the surface of bacterial vesicles and accelerates the spread of bacteria *in vivo* through apoptotic mimicry. (B) When the apoptosis recognition receptor BAI1 recognizes PS and initiates the efferocytosis of apoptotic neurons by microglia, TIM-4, as a direct or indirect signaling molecule, promotes the polymerization of actin through a certain mechanism to stabilize the phagosome. (C) In the immune contraction stage, TIM-4 promotes the efferocytosis of antigen-specific T cells by interacting with PS. For non-apoptotic T cells, TIM-4 serves a tethering role and remains non-apoptotic T cells in the medulla of the lymph node. Therefore, antigen-specific T cells unable to enter the periphery to exert their effects because of lacking survival signals and undergoing apoptosis. TIM-4, T-cell/transmembrane immunoglobulin and mucin domain containing 4; PS, phosphatidylserine; MFGE8, Milk Fat Globule EGF Factor 8; RGD, arginine-glycine-aspartic acid; FN, Fibronectin; FAK, focal adhesion kinase; Src, Src-family kinases; P, phosphorylation; RhoA, Ras homolog gene family member A; ROCK, Rho-associated kinase; MLCK, Myosin Light Chain Kinase; BAI1, brain-specific angiogenesis inhibitor 1; ELMO, Engulfment and Cell Motility Protein; RAC, Ras-related C3 botulinum toxin substrate; DOCK, dedicator of cytokinesis proteins.

protein inhibits the T cell response, while the proliferation of T cells increases continuously with increasing concentrations of TIM-4 fusion protein (12). This dose-dependent effect can be explained by different mechanisms: TIM-4 can bind to another high-affinity ligand on the surface of T cells and transmit negative regulatory signals to T cells. Alternatively, depending on the density of TIM-1, TIM-1 can participate in the transmission of inhibitory signals at lower levels of TIM-4 and promote signals at higher TIM-4 concentrations, which is similar to the association between different effects and different dose thresholds induced by T-cell receptor (TCR) ligands with bilateral agonist/antagonist characteristics in T cell populations (54). That is, the change in ligand density can be transformed into the difference in T cell response. Furthermore, TIM-4 is a highly glycosylated protein and TIM-4 has different glycosylation states in different cells, resulting in different binding affinities to receptors (5). The activation state of T cells is the key feedback of TIM-4 regulation. It has been found that the expression of TIM-4 on activated macrophages or mature DCs was increased. TIM-4 on these cells bound to TIM-1 on the surface of T cells to induce TIM-1 phosphorylation, which activated the PI3K/Akt signaling pathway, selectively increased the expression of Bcl-2 protein and finally regulated the proliferation of T cells (55).

Table I. Functional characteristics of TIM-4 in different immune cells.

Author(s), year	Cell	Classification	Characteristic	Function	(Refs.)
Albacker *et al*, 2013 del Rio *et al*, 2007 Tsitoura *et al*, 1999	Macrophage	Pulmonary lymph nodes-resident Macrophage	F4/80+TIM-4+ macrophages engulfed antigen-specific T cells expressing PS	Efferocytosis	(47-49)
Nishi *et al*, 2014 Albacker *et al*, 2013 Hilligan *et al*, 2016		Abdominal cavity-resident Macrophage	Not only engulf apoptotic T cells but also retain apoptotic T cells	Efferocytosis/ Tethering	(31,47,52)
Ge *et al*, 2016 Gilliet *et al*, 2002 Rodriguez-Manzanet *et al*, 2008 Mizui *et al*, 2008		Activated Macrophage	Negative regulation of immature T cells and positive regulation of activated T cells	Receptor-ligand binding	(53-56)
Hashimoto *et al*, 2013		Intestinal-resident Macrophage	CD4+TIM-4+ resident macrophages are not replenished by blood monocytes which breaking the stereotype	Redefine a population of resident macrophage	(46)
Osorio *et al*, 2019		Abdominal cavity-resident Macrophage	CD169+ macrophages with high TIM-4 reduce the survival rate of total T cells, disrupt the balance between regulatory T cells and effector T cells	Redefine a population of resident macrophage	(59)
Ouimet *et al*, 2019 Magalhaes *et al*, 2021		Adipose tissue-resident macrophages	TIM-4+/Lyvel+ adipose tissue resident macrophages promote the outflow of cholesterol and phospholipids to form new HDLs, and finally promotes cholesterol excretion	Redefine a population of resident macrophage	(60,61)
Yeung *et al*, 2013 Bouwens *et al*, 1986 Tosello-Trampont *et al*, 2012		Kupffer cell	Through the NF-κB/p38/MAPK signaling pathways and IL-4/ STAT6/GATA3 signaling pathway to promote Treg differentiation	Regulating the release of cytokines	(62-64)
Qin *et al*, 2022		Inflammatory associated macrophages	Via the ROS/p38 MAPK/Egr-1 pathway to contributes to the EMT process and aggravates the development of CRSwNP	Regulating the release of cytokines	(65)
Xu *et al*, 2016 Liu *et al*, 2020 Rossaint *et al*, 2015 Boomer *et al*, 2011		Peripheral blood macrophage	TIM-4 alleviated LPS-induced endotoxic shock by inhibiting the production of cytokines [tumor necrosis factor (TNF-α) and IL-6] from macrophages	Regulating the release of cytokines	(66-69)
Liu *et al*, 2007 Yang *et al*, 2007	DCs	Peripheral blood DCs	Staphylococcal enterotoxin B increase the expression of TIM-4	Interacted with TIM-1 to promote the differentiation of CD4+ T cells into Th2 cells	(70,71)
Yang *et al*, 2007		Intestinal-resident DCs	Staphylococcal enterotoxin B, cholera toxin and ovalbumin increase the expression of TIM-4	Interacted with TIM-1 to promote the differentiation of CD4+ T cells into Th2 cells	(71)

Table I. Continued.

Author(s), year	Cell	Classification	Characteristic	Function	(Refs.)
Yang *et al*, 2018			Bifidobacterium reduce the expression of TIM-4	Improve allergic inflammation in the intestine	(24)
Feng *et al*, 2008		Bone marrow-derived DCs	Cholera toxin and peanut extract increase the expression of TIM-4	Inducing Th2 cell polarization and initiating intestinal allergy	(22)
Caronni *et al*, 2021		Lung type I DCs	TIM-4 is a necessary molecule in early lung cancer	Capture and present cell-associated antigens	(73)
Caronni *et al*, 2021			TIM-4 is not detected in advanced lung cancer	Decreased the efficiency of antigen acquisition and antigen presentation and weakened the response of effector T cells	(73)
Meyers *et al*, 2005 Li *et al*, 2014 Feng *et al*, 2008 Siracusa *et al*, 2013	Mastocyte	Bone marrow-derived mastocyte	Flagellin induce the expression of TIM-4	TIM-4 interacted with TIM-1 to promote the differentiation of CD4+ T cells into Th2 cells	(12,16,74,75)

TIM-4, transmembrane immunoglobulin and mucin domain containing 4; PS, phosphatidylserine; Lyvel, lymphatic vessel endothelial hyaluronan receptor 1; HDL, high density lipoprotein; GATA3, GATA-binding protein 3; CRSwNP, chronic rhinosinusitis with nasal polyps; LPS, lipopolysaccharide; DCs, dendritic cells.

However, it was found that immature T cells did not express TIM-1, but TIM-1 was induced during T cell activation (56). In the immature T cell stage, TIM-4 decreased the proliferation and IL-2 production of immature T cells by downregulating the phosphorylation of p70 S6 kinase in a dose-dependent manner through the non-TIM-1 pathway. However, along with the activation of immature T cells and the initiation of the immune effect phase, activated and effector T cells begin to express TIM-1 (56). Therefore, TIM-4 may have two different functions according to the activated state of T cells: i) Negative regulation of immature T cells and ii) positive regulation of activated T cells (56). These results suggest that TIM-4 can regulate T cell function according to the activated state of T cells and different receptors.

In addition, the TIM-4 on the surface of tissue resident macrophages can be used to define some macrophages with unique functions. It was previously considered that, unlike intraperitoneal resident macrophages, all intestinal macrophages were supplemented by blood monocytes (57). However, by using the intestinal macrophage markers TIM-4 and CD4, the definition of intestinal macrophages has been expanded. A previous study found that the maintenance of large numbers of $TIM\text{-}4^{+}/CD4^{+}$ intestinal-resident macrophages was independent of monocytes, whereas the $TIM\text{-}4^{-}/CD4^{+}$ subset was replenished at a slower rate from monocytes, with only $TIM\text{-}4^{-}/CD4^{-}$ macrophages supplemented from blood monocytes (46). One of the important roles of tissue resident macrophages is efferocytosis and TIM-4, as an important apoptotic signal receptor, is logically a marker of intestinal macrophage subsets (58). In the resident macrophages of the abdominal cavity, CD169+ macrophages with high TIM-4 expression express low levels of antigen presentation molecules (MHC-II) and costimulatory molecules (CD80 and CD86) and high levels of anti-inflammatory factors (CD39, CD73, lactose lectin-9 and TGF-β) (58). This makes for a profound effect on the proliferation, survival and differentiation of CD4+ T cells and induces the high expression of the transcription factor FOXP3 in T cells (58). CD169+ macrophages with high TIM-4 reduced the survival rate of total T cells, disrupted the balance between regulatory and effector T cells and were more conducive to the differentiation of regulatory T cells (58). This also explains why the peritoneum is a common site of cancer metastasis and the reason is that macrophages in the abdominal cavity weaken the anti-tumor activity (59). In addition, the TIM-4+/lymphatic vessel endothelial hyaluronan receptor 1 (Lyvel)+ adipose tissue resident

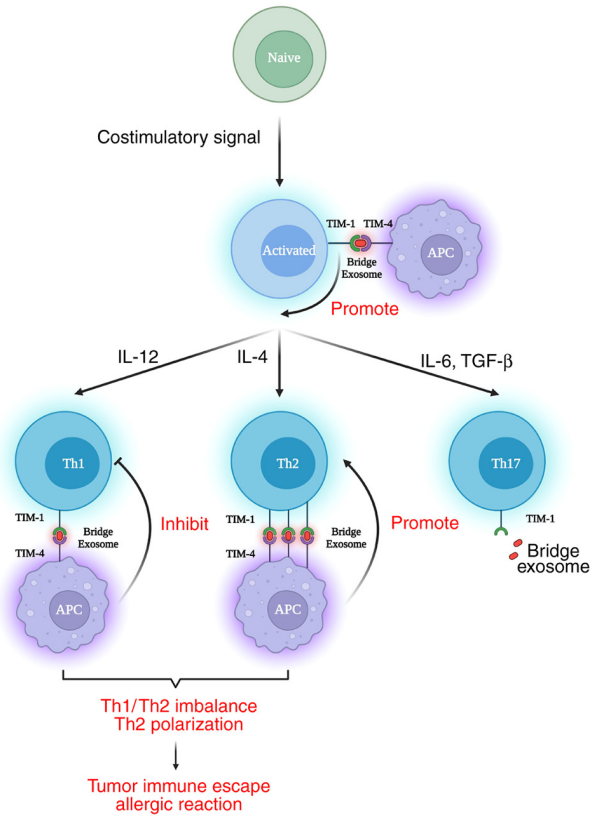

Figure 3. The regulatory role of TIM-4 in T cell-mediated adaptive immunity. TIM-4 on the surface of APC cells binds to TIM-1 on the surface of activated CD4+ T cells through a bridging molecule to promote the proliferation and differentiation of CD4+ T cells. TIM-1 is lowly expressed on the surface of Th1 and Th17 cells, but highly expressed on the surface of Th2 cells. TIM-4 on the surface of APC cells can serve an opposite regulatory role with TIM-1 on the surface of Th1 and Th2 cells through the binding of bridging molecules and eventually lead to Th1/Th2 imbalance. →, promote; ┤, inhibit. TIM, T-cell/transmembrane immunoglobulin and mucin domain; APC, antigen-presenting cells.

macrophages were characterized by high transcriptional activity, lysosomal activity, neutral lipid content and ATP binding cassette subfamily A member 1 (ABCA1) expression. ABCA1 in these cells was affected by TIM-4, which promotes the outflow of cholesterol and phospholipids to form new high density lipoprotein (HDLs) and finally promotes cholesterol excretion (60,61). These data suggest that TIM-4 is a key regulator of postprandial cholesterol transport and adipose tissue macrophage and may be a new way to treat dyslipidemia.

Finally, the expression of TIM-4 on the surface of macrophages increases in some pathophysiological conditions, leading to changes of inflammatory factors secreted by macrophages, which then participate in the regulation of inflammation and immunity (58). Acute inflammatory reactions following orthotopic liver transplantation can increase the expression of TIM-4 on Kupffer cells (KCs) (62). Blocking TIM-4 can reduce the expression of inflammatory cytokines through the NF-κB and p38/MAPK signaling pathways and inhibit the IL-4/STAT6/GATA Binding Protein 3 (GATA3) signaling pathway to promote regulatory T cell differentiation (62-64). Therefore, TIM-4 in macrophages may be a therapeutic target to promote the survival of allografts. For the p38/MAPK pathway, in the immune microenvironment of chronic inflammation of chronic rhinosinusitis with nasal polyps (CRSwNP), TIM-4 was increased in the patients with CRSwNP and, especially, in macrophages. Mechanistically, TIM-4 upregulates TGF-β1 expression in macrophages via the ROS/p38 MAPK/Egr-1 pathway to contribute to the epithelial-to-mesenchymal transition (EMT) process and aggravate the development of CRSwNP (65). In the early stage of sepsis, LPS induces the increased expression of TIM-4. TIM-4 alleviates LPS-induced endotoxic shock by inhibiting the production of cytokines (TNF-α and IL-6) from macrophages (66). In the advanced stage of sepsis, the expression of TIM-4 in peripheral blood monocytes/macrophages decreases significantly (67), which leads to macrophage paralysis, efferocytosis disability and an immunosuppressive state; thus, secondary infection became the main cause of mortality in patients with advanced sepsis (68,69).

To sum up, TIM-4 expressed in macrophages participates in immune regulation and affects the outcome of various diseases through a variety of ways. Further, affecting the expression of TIM-4 at different stages can affect the process of immune response and ultimately improve the pathophysiological state of the disease.

Role of TIM-4 in DCs. DCs serve key roles in inflammatory regulation and immune responses (Table I). Numerous studies have highlighted the important role of TIM-4 in DCs. DCs derived from peripheral blood are stimulated by staphylococcal enterotoxin B to increase the expression of TIM-4, which interacts with TIM-1 to promote the differentiation of CD4+ T cells into Th2 cells (70). In addition, staphylococcal enterotoxin B, cholera toxin and ovalbumin can increase the expression of TIM-4 in intestinal mucosa resident DCs. Activated mucosal DCs promote the differentiation of Th2 cells by increasing the expression of TIM-4, which interacts with TIM-1 (71). However, the DCs in the intestinal mucosa are heterogeneous. Cholera toxin could induce the expression of TIM-4 on the surface of $CD103^{+}CD11b^{+}$ DCs in the intestinal lamina propria (52). It is important that the expression and activation of TIM-4 are limited to this subset, while DCs with high expression of TIM-4 selectively express high levels of costimulatory molecules, thus promoting the initiation of mucosal immunity (52). In addition, bone marrow-derived DCs exposed to both cholera toxin and peanut extract can increase the expression of TIM-4 and promote the maturation of DCs, thus inducing Th2 cell polarization and initiating intestinal allergy (22). Cigarette smoke extract can stimulate the expression of TIM-4 in DCs through the ERK kinase signaling pathway, thus increasing the ratio of CD4+ T cells (72). This evidence suggests that activated DCs with high expression of TIM-4 serve an important role in T cell proliferation and Th2 cell polarization. Notably, it is not only the factor that induces the increase in TIM-4 in DCs. *Bifidobacterium* can reduce the expression of TIM-4 and improve allergic inflammation in the intestine by inhibiting the binding of the transcription factor STAT6 and the TIM-4 promoter region (24).

In normal and early cancer lung tissues, TIM-4 in lung type I DCs (cDC1) is a necessary molecule to capture and present cell-associated antigens (73). The expression of TIM-4 on tumor cells has not been detected in advanced tumor lung tissues, however, in early lung cancer, cDC1s are the key

factor to uptake tumor antigens and initiate tumor-specific cytotoxic T lymphocyte (CTL) responses (73). By contrast, in the advanced lung cancers, a decrease in TIM-4 results in the inactivation of cDC1s in lung tumors, which decreases the efficiency of cDC1 antigen acquisition and antigen presentation and weakens the response of effector T cells (73). This difference may be due to the different mechanisms of DCs in dealing with antigens in different tissues and immune response stages. In summary, the above studies emphasize the role of TIM-4 in capturing tumor-associated antigens in cDC1s, which is helpful in studying tumorigenesis and tumor-associated immune responses.

Role of TIM-4 in mastocytes. Mastocytes are the main effector cells of allergic diseases and are often accompanied by an abnormal increase in the number of Th2 cells and/or Th2 cell-related cytokines in the pathogenesis of allergic diseases, while the initiation of Th2 cell polarization requires the participation of mastocytes (74,75). The receptor of TIM-4 is TIM-1, which is expressed on the surface of Th2 cells. The interaction between TIM-1 and TIM-4 can initiate the polarization of Th2 cells (12). Flagellin can induce the expression of TIM-4 in mastocytes and then initiate the polarization of Th2 cells through TIM-1. Notably, when mastocytes are not exposed to proper stimulation, the expression level of TIM-4 is markedly low (16). This finding reflects the participation of TIM-4 in inflammatory reactions and allergic reactions (Table I). In the past, APCs were considered to be the main source of TIM-4 expression and these results further enriched the expression pattern of TIM-4.

4. Regulatory role of TIM-4 in the occurrence and development of diseases

Role of TIM-4 in malignant tumors. It was previously considered that the expression of TIM-4 was limited to DCs and macrophages. Further studies showed that TIM-4 was also expressed in mast cells and B lymphocytes and participated in immune regulation (8,9). Numerous studies have shown that tumor cells also express TIM-4 and have unique distribution and functional characteristics (Table II) (16,70,76). Multiorgan tumor tissue microarray immunohistochemistry staining has been used to detect the expression of TIM-4 in multiple types of tumor tissue. The results showed that the expression level of TIM-4 in esophageal, colon, rectal, pancreatic, breast and lung cancer was higher than that in adjacent tissues, indicating a potential association between TIM-4 and tumors (77). Langerhans cell sarcoma is a rare malignant tumor derived from epidermal DCs (78). It has been found that TIM-4 exists in the cell membrane and cytoplasm of tumor cells (78). This expression pattern suggests that TIM-4 serves an important role in the tumor immune microenvironment and tumor cell function regulation. By comparing the expression characteristics of TIM-4 in the renal cancer cell line 786-O and renal clear cell carcinoma tissue, it was demonstrated that TIM-4 was expressed in the cytoplasm in the 786-O cell line but not on the cell membrane; however, in tumor tissue samples from patients, it was found that TIM-4 was expressed on the cell membrane of cancer cells (17). The reason for this apparent discrepancy may be that certain cytokines in the tumor microenvironment led to the translocation of TIM-4. This shows the diversity of TIM-4 expression, which may be related to tumorigenesis and immune regulation. Although the expression of TIM-4 in colorectal cancer and malignant glioma is upregulated compared with that in adjacent tissues, previous studies have not fully clarified the cellular location of TIM-4 (19,20). In malignant glioma, macrophages are the main source of TIM-4 (79). Therefore, when studying the expression characteristics of TIM-4 in tumor tissues, the cellular localization should be clearly defined. Through colocalization analysis of cytokeratin 18 and TIM-4, previous studies confirmed that TIM-4 was expressed in non-small cell lung cancer cells (77,80). Notably, only a low level of TIM-4 was detected in non-small cell lung cancer cell lines, while stimulation with LPS, IL-6, TGF-β and TNF-α significantly increased TIM-4 expression in the A549 and NCI-H1975 cell lines. This result suggested that inflammatory factors may upregulate the expression of TIM-4 (81). Further experiments found that IL-6 promotes the expression of TIM-4 in non-small cell lung cancer cells through the NF-κB signaling pathway and the increased expression of TIM-4 can increase the production of IL-6, thus forming a positive cycle (18). This suggests that the tumor microenvironment is one of the important reasons for changing the expression pattern of TIM-4 in tumor cells.

The expression level of TIM-4 in tumor tissue is closely associated with the clinical characteristics of the tumor. High expression of TIM-4 in renal clear cell carcinoma is correlated to reduced tumor progression-free survival, suggesting that TIM-4 is involved in the metastasis of renal clear cell carcinoma (17). In diffuse large B-cell lymphoma, high TIM-4 expression in tumor tissues is closely associated with poor patient prognosis (82). The increased expression of TIM-4 in colorectal cancer is closely associated with distant metastasis, TNM staging and reduced overall survival (19). High TIM-4 expression in lung cancer tissue is negatively correlated with the 5-year overall survival rate of patients (77). High expression of TIM-4 is involved in tumor progression as an unfavorable factor. Therefore, it is particularly important to further clarify how TIM-4 participates in the occurrence and development of tumors. TIM-4 also offers a possibility for the diagnosis and differential diagnosis of malignant tumors. Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a malignant primary T-cell lymphoma. Delay in diagnosis and a high misdiagnosis rate affect the prognosis and survival of patients (83). Through single-cell RNA sequencing, TIM-4 was identified as a potential novel marker for SPTCL, which was specifically differentially expressed in the SPTCL cells. This offers insight into the heterogeneity of SPTCL (83). TIM-4 is involved in a variety of cellular biological functions of tumor cells. *In vitro*, it was found that high expression of TIM-4 promoted the proliferation of diffuse large B-cell lymphoma cells and inhibited cell apoptosis through the Wnt/β-catenin signaling pathway (82). In the human glioma cell line LN-18, high expression of TIM-4 promoted the proliferation of LN18 cells, inhibited their apoptosis and enhanced their clone formation ability (20). Downregulating TIM-4 expression in LN-18 cells led to a significant increase in caspase-3 activity, but caspase-8, a marker of the death receptor pathway, was not detected, suggesting that the signaling pathway of

Table II. Expression and function of TIM-4 in malignant tumors.

Author(s), year	Tumor type	Expression position	Expression characteristic	Clinical characteristic	Molecular mechanism	(Refs.)
Li *et al*, 2013	Langerhans cell sarcoma	Tumor cell	/	/	/	(78)
Yano *et al*, 2017	Renal clear cell carcinoma	Tumor cell	Upregulated	Negatively associated with survival; promote tumor metastasis	/	(17)
Tan *et al*, 2018	Colorectal cancer	/	Upregulated	Negatively associated with survival; promote tumor metastasis	Promotes angiogenesis by VEGF; increases cell migration and metastasis of by activating PI3K/AKT/mTOR signaling; promotes proliferation and tumor interstitial remodeling	(19)
Li *et al*, 2016 Xu *et al*, 2011 Akl *et al*, 2014	Malignant gliomas	Macrophage	Upregulated	/	Promotes the proliferation and inhibits apoptosis	(20,79,84)
Liu *et al*, 2020 Siracusa *et al*, 2013 Zhang *et al*, 2015 Danhier *et al*, 2012	Non-small cell lung cancer	Tumor cell	/	Negatively associated with survival; Promote tumor metastasis	Downregulated the expression of E-cadherin; upregulated the expression of N-cadherin, vimentin and Slug	(18,75,77,89)
Li *et al*, 2020	Diffuse large B-cell lymphoma	/	Upregulated	Negatively associated with survival	Promotes the proliferation and inhibits apoptosis through the Wnt/β-catenin pathway	(82)
Zhang *et al*, 2015	Esophagus cancer	/	Upregulated	/	/	(77)
Zhang *et al*, 2015	Breast cancer	/	Upregulated	/	/	(77)
Zhang *et al*, 2015	Pancreatic cancer	/	Upregulated	/	/	(77)

TIM-4, transmembrane immunoglobulin and mucin domain containing 4; /, no data.

TIM-4-induced apoptosis in LN-18 cells may be mediated by mitochondria (20). This result is inconsistent with the results of a previous study showing that tumor cell apoptosis is regulated by caspase-8 (84). This difference may be related to different sensitivities of different tumor cells to external stimuli (85). In addition, it was hypothesized that TIM-4 may directly or indirectly activate the mitochondrial-mediated signaling pathway to initiate the process of apoptosis (86). In malignant gliomas, TIM-4 serves diverse roles. Hypoxia can induce high expression of TIM-4 in tumor-associated macrophages in malignant gliomas and hypoxia inducible factor-1 (HIF1) and HIF1α are the key factors for macrophages to upregulate TIM-4 under hypoxic conditions. T cells in the malignant glioma microenvironment express PS. The interaction between TIM-4 and PS induces macrophages to phagocytose PS^+T cells to obtain immune tolerance and induces the enrichment of tumor-specific regulatory T cells, which inhibits the activation of tumor-specific CD8+ T cells (79). In colorectal cancer,

TIM-4 promotes angiogenesis by upregulating VEGF, significantly hastening the development of colorectal cancer *in vivo,* and TIM-4 increases the migration and metastasis of CT26 cells by activating PI3K/AKT/mTOR signaling, while TIM-4 promotes the migration of macrophages to tumor tissues by increasing the levels of colony stimulating factor-1, C-C motif chemokine ligand 2/5 and VEGF and participates in colorectal cancer cell proliferation and tumor interstitial remodeling (19). Through the study of clinical tissue samples, the expression level of TIM-4 was determined to be an independent predictor of lymph node metastasis in lung cancer (18). In cell experiments, TIM-4 downregulated the expression of E-cadherin, a marker of the EMT and upregulated the expression of N-cadherin, vimentin and Slug, which confirmed that TIM-4 promoted the migration, invasion and EMT of lung cancer cells *in vitro* (18). In non-small cell lung cancer (NSCLC) cells membrane, TIM-4 was extensively N-glycosylated at Asn291 and after the removal of N-glycosylation, the stability of TIM-4 protein was decreased and TIM-4 was more susceptible to degradation by ER-localized ubiquitin ligase-mediated ERAD (87). Thus, the expression of TIM-4 on the cell surface was decreased, which suppressed TIM-4 mediated metastasis in NSCLC, which could provide a valuable biomarker for developing drugs targeting N-glycosylation at Asn291 on TIM-4 (87). In addition, overexpression of TIM-4 promoted the proliferation of lung cancer cells and upregulated the expression of proliferating cell nuclear antigen (PCNA) and cyclins A, B1 and D1, resulting in the accumulation of lung cancer cells in the S phase of the cell cycle (77).

The molecular mechanism by which TIM-4 mediates cancer progression remains unclear. Due to the lack of tyrosine phosphorylation sites in the cytoplasmic tail, TIM-4 cannot mediate signal transduction directly (10). Unlike other TIM proteins, TIM-4 contains an RGD motif in its IgV domain, which is a marker of adhesion proteins (4). The RGD sequence can be recognized by the integrin family on the cell surface and the RGD sequence preferentially binds to avb3 integrin (88,89). The interaction between TIM-4 and avb3 in non-small cell lung cancer cells was confirmed by a coimmunoprecipitation technique (77). The RGD motif and integrin avb3 signaling pathway are important reasons why TIM-4 promotes the progression of lung cancer (77). In renal clear cell carcinoma, it was found that TIM-4 was not associated with the proliferation, invasion or migration of 786-O cells. By knocking down TIM-4 in the 786-O cell line, it was found that the cell line became sensitive to the targeted therapy of sorafenib, but the specific mechanisms involved remain to be further studied (17). This result suggests that TIM-4 may be involved in the process of immunotherapy.

A study provided a possible way to perform tumor target therapy of TIM-4 in malignant melanoma and a novel anti-TIM-4 monoclonal antibody achieved antitumor effects by recruiting $CD8^+$ T cells (90). In addition, compared with the use of a single monoclonal antibody, the combined use of anti-TIM-3 and anti-TIM-4 monoclonal antibodies increased the number and killing effect of NK and $CD8^+$ T cells in tumors, which further improved the efficacy of tumor vaccines (90). These findings suggest that monoclonal antibodies targeting TIM-3 and TIM-4 provide a new strategy for improving the antitumor efficacy of tumor vaccines.

In conclusion, the expression of TIM-4 in tumor tissues exhibits polymorphism. Although investigating the role of TIM-4 in tumors has achieved certain results, due to the diversity of TIM-4 expression, its expression and location is not limited to the tumor cells of the tumor tissue. The characteristics are also different among various tumors and, as a result, TIM-4 also serves different roles in the regulation of cell functions of different tumor cells. Moreover, the changes in the subcellular localization of TIM-4 also suggest that the tumor microenvironment induces changes in the expression characteristics of TIM-4. Therefore, when studying the role of TIM-4 in different tumor tissues, it is particularly important to detect in which cells TIM-4 is expressed. TIM-4 has been preliminarily determined to exhibit upregulated expression in various malignant tumor tissues through tissue chip technology, but further research is necessary considering the important role of TIM-4 in malignant tumors.

Role of TIM-4 in viral infection. TIM-4 can promote the entry of envelope viruses into the host, but the mechanism of the interaction between viruses and TIM-4 requires further investigation (Table III) (43). Ebola virus (EBOV) is a member of the filamentous virus family. EBOV can infect a variety of cells, including macrophages and DCs (91). TIM-4, a member of the TIM family, is expressed in macrophages and DCs. EBOV interacts with the TIM-4 IgV domain on the surface of macrophages or DCs through the PS on its surface and enters the cell by apoptotic mimicry (92). By using single-molecule force spectroscopy, a previous study found that the interaction between TIM-4 and PS on the surface of macrophages was mechanically stable and even more resistant to mechanical stress than other adhesion molecules, which helped to maintain the adhesion of EBOV to host cells in blood vessels (93). Another important envelope virus, human immunodeficiency virus type 1 (HIV-1), mainly infects cells through the classical receptor (CD4, C-C chemokine receptor type 5 and C-X-C motif chemokine receptor 4) pathway, but HIV-1 viral particles are also found in cells without classical receptors, indicating that there are non-traditional infective pathways (94-97). One of the main phospholipid components of the HIV-1 envelope is PS. TIM-4 is the receptor of PS and exosomes express TIM-4, which can interact with HIV-1 (98). Exosomes participate in the transport of virus as the carriers of HIV-1 (99). A previous study indicated that the uptake of exosomes by phagocytes was dependent on TIM-4 (100). Therefore, blocking TIM-4 can reduce the ability of HIV-1 to enter the host (99). In conclusion, the interaction between TIM-4 and PS serves an important role in the infection of enveloped viruses and future studies need to determine whether TIM-4 itself initiates viral efferocytosis or whether other PS receptors or cytokines are potential interacting molecules for TIM-4-mediated viral entry into the host.

Role of TIM-4 in autoimmune diseases. TIM-4 is a potential regulator of the immune system and serves a key role in multiple autoimmune diseases (Table III). Ulcerative colitis (UC) is a subtype of inflammatory bowel disease and is a chronic, idiopathic and immune-mediated disease that mainly affects the rectum and colonic epithelium, resulting in severe intestinal damage (101). A recent study has shown that the proportion

Table III. Expression and function of TIM-4 in disease.

Author(s), year	Disease type	Expression position	Expression characteristic	Clinical characteristic	Mechanism	(Refs.)
Amara *et al*, 2015 Dragovich *et al*, 2019	EBOV infection	Macrophage and DCs	/	Promote the entry of viruses into the host	Apoptotic mimicryand enhanced adhesion	(92,93)
Aloia *et al*, 1993 Sims *et al*, 2017 Kassu *et al*, 2011	HIV-1 infection	Exosomes	/	Promote the entry of viruses into the host	Exosome-dependent non-classical route of infection	(98-100)
Xue *et al*, 2021	Ulcerative colitis	Monocytes	Upregulated	Positively associated with disease severity	Regulate the proliferation and differentiation of regulatory T cells	(102)
Zhao *et al*, 2010 Xue *et al*, 2021	Systemic lupus erythematosus	Monocytes	Upregulated	Associated with the active phase of disease	/	(81,102)
Ye *et al*, 2020	Allergic rhinitis	DCs	Upregulated	Associated with the active phase of disease	/	(116)
Dambach *et al*, 2002 Ji *et al*, 2014	Liver ischemia-reperfusion injury	Macrophage	Upregulated	Positively correlated with the degree of inflammatory injury	Macrophages and DCs activate and release a large number of inflammatory factors	(107,110)
Li *et al*, 2015 Ji *et al*, 2014		DCs(108)	Upregulated	Positively correlated with the degree of inflammatory injury		(109,110)
Lambertsen *et al*, 2012 Fang *et al*, 2019	Ischemic stroke	Monocytes	Upregulated	Positively correlated with the degree of inflammatory injury; negatively correlated with prognosis	Regulates the early activation of T cells by IL-6	(114,115)
Hansson *et al*, 2006	Atherosclerosis	Macrophage	/	Negatively correlated with lesion area	Enhance the recognition and phagocytosis of apoptotic cells in atherosclerotic Plaques	(118)

TIM-4, transmembrane immunoglobulin and mucin domain containing 4; EBOV, Ebola virus; HIV-1, human immunodeficiency virus type 1; DCs, dendritic cells; /, no data.

of $CD14^+TIM\text{-}4^+$ monocytes in patients with UC is increased and that the proportion of monocytes expressing TIM-4 is related to the activity and severity of UC (102). In addition, Pearson's correlation analysis showed that the proportion of $CD14^+TIM\text{-}4^+$ monocytes was positively correlated with the proportion of bone marrow-derived inhibitory cells, while

the proportion of $CD14^+TIM\text{-}4^+$ monocytes was negatively correlated with the number of regulatory T cells (102). This suggests that the high expression of TIM-4 in monocytes may reduce the proliferation and differentiation of regulatory T cells in UC (102). As a regulator of immune homeostasis, TIM-4 serves an important role in numerous immune diseases. For example, the expression of TIM-4 in monocytes and the level of plasma TIM-4 in patients with ankylosing spondylitis were significantly higher than those in the control group and related to the severity of the disease (103). The level of TIM-4 mRNA in peripheral blood mononuclear cells of patients with systemic lupus erythematosus (SLE), particularly those with active SLE, was significantly higher than that of healthy individuals (81). Furthermore, in patients with allergic rhinitis, the expression of TIM-4 in DCs decreased significantly after treatment (104).

T cells are considered to be the core of the pathophysiological process of autoimmune diseases. It is generally considered that experimental autoimmune encephalomyelitis (EAE) is a disease mediated by T cells, in which Th1 and Th17 subsets serve a key role in its pathogenesis and severity (104). The EAE model was established in a previous study by immunizing mice with brain-derived MOG35-55 peptide and, after anti-TIM-4 treatment, it was observed that the clinical symptoms of EAE were markedly improved (8). Rheumatoid arthritis (RA) is a common chronic autoimmune disease (105). Using a model of collagen-induced arthritis (CIA), it was found that injection of an anti-TIM-4 monoclonal antibody into CIA could aggravate the development of RA. The main mechanism is the significant increase in IFN-γ and IL-17 production. By contrast, an anti-TIM-4 monoclonal antibody could inhibit the development and progression of RA after the onset of CIA, at least partly by inhibiting the production of proinflammatory cytokines by macrophages (106). These results show that TIM-4 has dual functions depending on the different stages of CIA. Therefore, in autoimmune diseases, TIM-4 may be a promising target for disease prediction and treatment; however, at present, limited data are derived from animal models, which suggests that animal models may not be able to simulate complex human situations in all cases. Therefore, further research is needed not only on animals but also on humans.

Role of TIM-4 in liver ischemia-reperfusion injury (IRI). Liver IRI is an immune-driven inflammatory response. The liver has the largest number of tissue resident macrophages, namely KCs, which can recognize hepatocyte injury, initiate inflammation and maintain and amplify local inflammation (107,108). After IRI of the liver, KCs, endothelial cells and hepatic parenchymal cells release inflammatory factors such as TNF-α and IL-1, resulting in circulating macrophages, DCs and neutrophils infiltrating the liver, which results in further inflammatory response (109). In a previous study, inflammation led to an increase in the PS exposed to the surface of apoptotic/necrotic cells, which induced a gradual increase in TIM-4 on the surface of macrophages and the expression of TIM-4 was related to the degree of hepatocyte injury (110). In the case of IRI, infiltrating macrophages in the liver lost their ability to maintain homeostasis and became highly activated, which could effectively and quickly phagocytize cell fragments and release a large number of inflammatory factors to promote hepatocyte injury (Table III). Blocking TIM-4 may reduce the inherent efferocytosis of macrophages to further reduce IRI-induced liver inflammation (110). Anti-TIM-4 antibodies can improve liver IRI, which suggests that anti-TIM-4 antibodies affects KCs *in vivo*. Anti-TIM-4 antibodies depletes the phagocytic function of $TIM\text{-}4^+$ macrophages. Compared with $TIM\text{-}4^+$ KCs, the phagocytic ability of $TIM\text{-}4^-$ KCs was significantly lower and the levels of the corresponding inflammatory factors were also significantly decreased. After 2 h of anti-TIM-4 antibody treatment, the phagocytic function of $TIM\text{-}4^+$ KCs was eliminated and the inflammatory reaction of the liver was improved. When the $CD11b^+$ macrophages were renewed after 48 h, the liver inflammatory response gradually increased. These new macrophages released inflammatory factors more easily than the original KCs. In this process, the infiltrating macrophages renewed themselves into new KCs and gradually acquired high expression of TIM-4 (111). In addition to macrophages, DCs in the liver also serve an important role in IRI (Table III) and the expression of TIM-4 is also increased (108). Therefore, blocking TIM-4 with an anti-TIM-4 antibody can also alleviate liver injury and inflammation through DCs and these effects can be attributed to blocking TIM-4 to inhibit Th2 differentiation and hinder the IL-4/STAT6 signaling pathway. Therefore, targeting TIM-4 should be considered a therapeutic method to improve liver ischemia-reperfusion injury (109,110).

Role of TIM-4 in cardiovascular and cerebrovascular diseases. Inflammation and immunity are involved in the occurrence and development of acute ischemic stroke (112). When using thrombolysis for reperfusion therapy, IRI is an important problem for convalescent patients and anti-inflammatory therapy is one of the auxiliary methods (113). Following ischemic stroke, the percentage of TIM-4 expression increases significantly in circulating total, classical and non-classical monocytes because non-classical monocytes have a higher ability to infiltrate from blood to injured tissue and serve an important role in the inflammatory response of ischemic stroke (Table III) (114). Therefore, a positive correlation has been reported between the expression of TIM-4 in non-classical monocytes and plasma IL-6 levels in patients with acute ischemic stroke. The mechanism may be attributed to TIM-4 regulating the activation amplitude of naïve T cells by regulating the threshold of TCR signal activation in the initial stage of the immune response (114). Furthermore, the expression of TIM-4 is closely associated with the prognosis of patients, which suggests that TIM-4 expressed by monocytes in the circulation can be regarded as an adaptive molecule in the regulation of inflammation (115). By establishing a model of cerebral ischemia-reperfusion in C57/BL6 mice, it was found that the expression of TIM-4 in monocytes increased gradually with prolonged reperfusion time and that blocking TIM-4 could reduce the release of inflammatory cytokines, such as IL-6, C-X-C motif chemokine ligand 1/2, IL-1β and TNF-α, decrease the inflammatory response mediated by microglia and reduce the infarct area. This suggested that TIM-4 is a potential target for the treatment of ischemic stroke (116).

TIM-4 serves a different role in atherosclerosis (Table III). At present, the main treatment of atherosclerosis is to reduce

blood lipids, but in the face of the concurrence of lipid accumulation and inflammatory immunity, this method is not satisfactory in controlling disease progression or pathological state (117). Innate and adaptive immunity as well as apoptotic cell clearance serve a central role in the development of atherosclerosis and TIM-4 may affect this process. TIM-4 in macrophages can enhance the recognition and efferocytosis of apoptotic cells in atherosclerotic plaques and increase the proportion of regulatory T cells by controlling the polarization of Th2 cells to improve local inflammation. The area of atherosclerotic lesions significantly increases after blocking TIM-4 (118). In summary, whether as a costimulatory molecule or a PS receptor, TIM-4 serves different or even opposite regulatory roles in different immune cells and stages, which reflects the diversity exhibited byTIM-4 in immune regulation (62).

5. Conclusion

In recent years, the expression and function of TIM family on different cells and the identification of new ligands have suggested that the TIM family serves a crucial role in immune regulation (119). Simultaneously, TIM-4 has attracted more and more attention because of its unique expression characteristics and its role in immune regulation. Liu *et al* (9) collectively evaluated the role of TIM-4 in health and diseases; but this review focuses on clinical diseases and lacks the classification and summary of TIM-4 in the regulation of immune cell, especially for macrophages. In the review of Kim *et al* (120), efferocytosis of TIM-4 is described in detail, but the review lacks the mechanism of immune regulation and its role in clinical diseases. By contrast, McGrath (121) emphasized the role of TIM-4 in immune regulation. Finally, Evans and Liu (122) summarized the characteristics of TIM-4 from the perspective of virus-host interaction. In conclusion, recent reviews have failed to comprehensively and systematically summarize the expression characteristics, functional characteristics and the relationship between TIM-4 and disease. In the present review, the above characteristics of TIM-4 were generalized for the first time, in particular the functional characteristics of TIM-4 expressed on the surface of macrophages, which has important guiding significance for the study of immune microenvironment regulation of various diseases.

By summarizing the results of research on TIM-4, the present review found that TIM-4 was previously thought to be mainly expressed in APCs, but it was later demonstrated that TIM-4 could be expressed in a variety of other immune cells, such as mast and NKT cells and even in tumor cells, which supports the direction of further research on TIM-4. At the functional level, TIM-4 serves a multifaceted role in immune regulation. Whether as a PS receptor or a bridge in efferocytosis regulation, the core function of TIM-4 is immune regulation. On the other hand, the dual effect of TIM-4 on the regulation of T lymphocytes shows the diversity of TIM-4 in immunoregulation. This characteristic makes TIM-4 capable of playing a regulatory role in the occurrence and development of a variety of autoimmune diseases. The biological behavior of solid tumors is determined by the interaction between tumor cells and the surrounding microenvironment and the development of malignant tumors must escape autoimmune-mediated surveillance. Therefore, in view of the role of TIM-4 in immune regulation, researchers have found that TIM-4 participates in several types of malignant tumor immune microenvironment. The aforementioned forms the multifaceted functional basis of TIM-4, whose core function is to act as a PS receptor and to be involved in inflammation and tumor immune regulation. Thus, future studies on TIM-4, whether in the context of viral infection or lipid metabolism, would be a valuable new strategy to aim to treat diseases that currently do not have effective cures.

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Authors' contributions

NK and ZW contributed to study concept and design, ZW contributed to investigation and writing the original draft, CC contributed to investigation and the figures and tables and YS contributed to investigation and writing the original manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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