LILRB4, an immune checkpoint on myeloid cells

Ting Yang¹, Yixin Qian², Xiaoting Liang³, Jianbo Wu², Ming Zou², Mi Deng²,⁴,⁵

¹Peking University International Cancer Institute, Health Science Center, Peking University, Beijing, China; ²Peking University Cancer Hospital and Institute, Peking University, Beijing, China

Abstract
Leukocyte immunoglobulin-like receptor B4 (LILRB4) is an inhibitory receptor in the LILR family mainly expressed on normal and malignant human cells of myeloid origin. By binding to ligands, LILRB4 is activated and subsequently recruits adaptors to cytoplasmic immunoreceptor tyrosine inhibitory motifs to initiate different signaling cascades, thus playing an important role in physiological and pathological conditions, including autoimmune diseases, microbial infections, and cancers. In normal myeloid cells, LILRB4 regulates intrinsic cell activation and differentiation. In disease-associated or malignant myeloid cells, LILRB4 is significantly correlated with disease severity or patient survival and suppresses T cells, thereby participating in the pathogenesis of various diseases. In summary, LILRB4 functions as an immune checkpoint on myeloid cells and may be a promising therapeutic target for various human immune diseases, especially for cancer immunotherapy.

Keywords: Autoimmune disease, Cancer, Immune checkpoint, Immunotherapy, Inhibitory receptor, LILR, LILRB4, MDSC, Myeloid cell, TAM

1. INTRODUCTION

The leukocyte immunoglobulin-like receptor (LILR) family comprises inhibitory and activating receptors expressed among human hematopoietic cells. The LILR genes are located on human chromosome 19q13.4 within the leukocyte receptor complex, which is a genomic region that contains several other innate immune system multigenic families belonging to the Ig superfamily.¹ The LILR family has 13 members, including six activating receptors (LILRA1–LILRA6), five inhibitory receptors (LILRB1–LILRB5), and two pseudogenes (Table 1). The extracellular structures of LILRAs and LILRBs are highly similar to C2-type Ig-like domains, but the cytoplasmic tails are different, which extends into opposing signaling pathways in their cytoplasmic domains. LILRAs possess a shorter cytoplasmic tail lacking intrinsic signaling capacity but possessing a positively charged arginine residue (R) in the transmembrane domain, which enables association with the FcγR chain that contains immunoreceptor tyrosine-based activation motifs (ITAMs), while LILRBs have a long cytoplasmic tail with immunoreceptor tyrosine-based inhibition motifs (ITIMs).² LILRs are mainly expressed on the myeloid lineage, such as monocytes, macrophages, neutrophils and dendritic cells, and some LILRs are also found on B cells, NK cells and T cells.²,³ LILRB4 is one of the inhibitory receptors in this family. It was an orphan receptor until recent discoveries and has become a potential target for cancer immunotherapy.

LILRB4, also known as ILT3, LIR5, CD85K, and HM18, was identified in 1997 as an inhibitory receptor on monocytes and considered to be a homolog of mouse glycoprotein (gp49B).⁴,⁵ The LILRB4 gene is highly polymorphic, which may be related to susceptibility to autoimmune diseases.⁶,⁷ LILRB4 bears two extracellular C2-type Ig domains and three cytoplasmic ITIMs that recruit phosphatases for downstream signal transduction. Interestingly, LILRB4 is unique because family phylogenetic analysis shows that LILRB4 appears as an “outlier” and structural feature analysis shows that LILRB4 contains only two extracellular Ig domains (designated D1, D2), while most family members contain four Ig domains (designated D1, D2, D3, and D4) in their extracellular domains.⁸ Unlike other LILRBs, LILRB4 is not conformationally and electrostatically suitable for the interaction with major histocompatibility complex (MHC), as well as hormone and virus products (Table 1). Thus, LILRB4 may not be directly involved in regulation of MHC-mediated antigen-specific T cell activation,⁹ synergetic inhibition of phagocytosis to CD47,¹⁰ activation of NK cells,¹¹ immune response to special pathogens¹² and stemness of hematopoietic stem cells.¹³ In this review, we will mainly discuss LILRB4 functions in homeostasis, inflammation disorders and tumors, and particularly focus on its extracellular interaction patterns, intracellular signaling and functions in different cell types. (Fig. 1).

1.1. LILRB4 functions in homeostasis

1.1.1. Monocytes. Monocytes originate from hematopoietic progenitor cells in the bone marrow and are able to further differentiate into macrophages and dendritic cells (DCs).¹⁴ FcγRI, expressed on the surface of monocytes and macrophages,
is a high-affinity human IgG receptor composed of a ligand-binding α-chain and an ITAM-bearing γ-chain. Three ITAMs in the γ-chain of FcγRI are necessary for eliciting immune functions such as phagocytosis, cytotoxicity, degranulation, antigen presentation and cytokine production.44,52 FcγRI signal initiation occurs after cross-linking of the receptor complex, leading to phosphorylation of the ITAM on the FcγRI chain by Src-family tyrosine kinases, which enables them to recruit Src homology 2(SH2) domain phosphatases (SHPs), such as SHP-1 or SHP-2, and SH2-containing inositol phosphatase tyrosine-based inhibitory motif(PTIM), LILR/LIR=leukocyte immunoglobulin-like receptor, MIR=myeloid inhibitory receptor, Mo=monocyte, Neu=neutrophil, NK=natural killer cell, Omp=oligomeric myelin protein ligase Cbl. Furthermore, the extracellular matrix protein ligand binds to LILRB4 and may regulate FcγRI-dependent intrinsic activation of monocytes in an ITIM-dependent mechanism.

| Gene   | Species | Alias           | Ig domain | Tail | Ligand                                      | Physiological expression | Reference |
|--------|---------|-----------------|-----------|------|---------------------------------------------|--------------------------|-----------|
| Activation receptor | LILRA1 | Human | LIR-6, CD85 | 4   | R HLA-B27, HLA-C free heavy chain, BCG, M.Bovis | B cells, Mac, Mo         | 2,14-16  |
|        | LILRA2 | Human | ILT1, LIR-7, CD85h | 4   | R Degraded IgM, IgG3, IgG4, IgG1, IgG2 | Mo, Mac, DC, NK, Gr (Neu, Eos, Baso), T cells, Mo, NK, T cells, B cells pDC | 17-22    |
|        | LILRA3 | Human | ILT6, LIR-4, CD85e | 4   | R HLA-C free heavy chain, Nogo66 | BST2, Mo, Neu | 2,15,22,23 |
|        | LILRA4 | Human | ILT7, CD85g | 4   | R | | pDC | 24,25 |
|        | LILRA5 | Human | ILT1, LIR-9, CD85f | 2   | R Glandular epithelial cells cytookeratin 8 | Mo | 2,26    |
|        | LILRA6 | Human | ILT8, CD85b | 4   | R | | Mo | 27    |
| Inhibitory receptor | gp49A | Mouse | | 2 | R Integrin, Vβ3 | Mast cells, NK | 28 |
|        | LILRB1 | Human | ILT2, LIR-1, CD85j | 4   | 4 ITIM HLA-I, UL18, dengue virus product, S100A8/9, S.aureus, RRFN, E.coli | Mo, Mac, DC, Gr (Eos, Baso), B cees, T cells, NK, Mast cells progenitor, Osteoclasts, Placental stromal cells | 2,23,30 |
|        | LILRB2 | Human | ILT4, LIR-2, CD85d, MR10 | 4   | 3 ITIM HLA-1, UL18, CD1d, ANGPTLs, oligomeric β-amyloid, RTN4, MAG, OMPg | Mo, Mac, DC, Gr, Mast cells progenitor, Osteoclasts, Endothelial cells, Placental vascular smooth muscle, HSC, Neuron | 2,22,31 |
|        | LILRB3 | Human | ILT5, LIR-3, CD85a | 4   | 4 ITIM ANGPTLs, glandular epithelial cells cytookeratin 8 | Mo, DC, Gr, Mast cells progenitor, Osteoclasts, B cells, Gr (Neu, Eos, Baso) | 2,32,33 |
|        | LILRB4 | Human | ILT3, LIR-5, CD85k, HM18 | 2   | 3 ITIM ALCAM, APOE, fibronectin | DC, Mo, Mac, Plasmablast/ plasma cells, Memory B, Progenitor mast cell, Osteoclasts, Microglia, Endothelial cells, Neutrophils, Mast cells granules, Mast cells progenitor | 5,34-41 |
|        | LILRB5 | Human | LIR-8, CD85c | 4   | 2 ITIM HLA class-I heavy chains, HLA-B7, HLA-B27 dimers, BCG | DC, Mac, Gr (Neu, Eos), B cells, Osteoclasts, Microglia | 14,16    |
|        | PIRB   | Mouse | | 6   | 4 ITIM MHC class-I, ANGPTLs, oligomeric β-amyloid, MAG, OMPg, APOE | DC, Mac, Gr (Neu, Eos), B cells, Osteoclasts, Microglia | 42-45    |
|        | gp49B  | Mouse | Mouse LILRB4 | 2   | 2 ITIM Fibronectin, Integrin, Vβ3 | Mast cells, DC, Mo, Mac, NK, Marginal zone and memory B cells | 46-52    |

**Table 1**
Characterizations of LILR family members.
dependent manner. In addition, osteoclasts are multinucleated giant cells derived from bone marrow monocytes. Osteoclast precursor cells enter the blood circulation under the chemotaxis of chemokines, reach the bone tissue in the resorption state, and differentiate into osteoclasts under the activation of M-CSF and NFκB ligand (RANKL). LILRB4 is expressed on osteoclast precursor cells derived from peripheral blood monocytes, and in the presence of RANKL and M-CSF, the ITIM of LILRB4 constitutively recruits SHP-1 to inhibit the development of osteoclasts in vitro. Together, LILRB4, as an inhibitor of monocyte activation, plays an important role in immune regulation by abrogating FcyRI-dependent monocyte activation.

1.2. LILRB4 functions in inflammation disorders

1.2.1. Macrophages. Macrophages, derived from blood monocytes, are a key component of the innate immune system. As tissue-resident cells, macrophages act as immune sentinels and can be equipped to sense and respond to tissue invasion by infectious microorganisms and tissue injury. LILRB4 is expressed in macrophages, and as an endogenous negative regulator of macrophage activation, coligation of LILRB4 and FcyRI inhibits the FcyRI-mediated production of TNFs in vitro. In Toxoplasma gondii infection during pregnancy, LILRB4 expression is downregulated on macrophages, which enhances M1 activation function but attenuates M2 tolerance function. The decrease in LILRB4 results in downregulation of the arginine catabolism enzyme arginase-1 (ARG-1) and upregulation of inducible nitric oxide synthase (iNOS) to suppress placental vascular development, which contributes to abnormal pregnancy outcomes. In atherosclerosis, LILRB4 is expressed on macrophages located in atherosclerotic plaque atherosclerotic lesions of human coronary arteries. In mice, gp49B deficiency reduces SHP-1 phosphorylation and subsequently promotes the activation of the NFκB signaling pathway to aggravate atherosclerosis and increase the inflammatory response of macrophages. In an acute lung injury (ALI) model induced by lipopolysaccharide, the expression of gp49B is upregulated, and its deficiency increases the macrophage-dependent inflammatory response of ALI through the activation of the NFκB signaling pathway. In human chronic obstructive pulmonary disease (COPD), the percentage of LILRB4-positive lung interstitial macrophages is increased, which correlates with the severity of emphysematous lesions. In the mouse model of COPD induced by elastase, the expression of gp49B on interstitial macrophages was also increased. In gp49B-deficient mice, elastase-induced emphysema and the production of matrix metalloprotease-12 (MMP-12) are increased, which suggests that upregulation of gp49B on pulmonary interstitial macrophages of COPD may have a protective effect on emphysema formation. In short, LILRB4 plays an important role in the activation, differentiation and polarization of macrophages during pathogenesis.
1.2.2. Dendritic cells. Classical dendritic cells (DCs) are professional antigen presenting cells (APCs) that drive T cell priming and differentiation. Under disease conditions, DCs have tolerogenic functions, which are characterized by high expression of inhibitory receptors, such as LILRB2 and LILRB4. Tolerogenic DC (tDC) overexpression of LILRB4 recruits SHP-1 or SHIP-1 phosphatases to decrease the phosphorylation and degradation of IκB, thus preventing NFκB nuclear translocation and the signaling cascade. tDCs suppress the activation and maturation of CD4+ T cells and induce the generation of regulatory T (Treg) cells and CD8+ T suppression (Ts) cells from naïve T cells. On the other hand, Treg and Ts cells induce the differentiation of tDCs from immature DCs. The tolerogenic crosstalk between Treg/Ts cells and tDCs is partly carried out by the inhibitory receptor LILRB4. In addition, IL-6, IL-10, IFNα, IFNβ, vitamin D3, low tryptophan and aspirin also induce DC tolerance through upregulation of LILRB4. The fine balance of DC regulation between tolerogenic and inflammatory states may contribute to the development of autoimmune diseases, tolerance of transplanting, and tumor immune evasion. Treatment with recombinant human LILRB4-ECD-Fc proteins or upregulation of mouse endogenous gp49B induces DC tolerance to inhibit a variety of autoimmune diseases, such as systemic lupus erythematosus, collagen-induced arthritis, autoimmune encephalomyelitis and inflammatory bowel disease; in contrast, blocking LILRB4 or gp49B deficiency exacerbates autoimmune diseases. In human patients without transplant rejection have more circulating T cells to upregulate the expressions of LILRB4 and LILRB2 in donor DCs, rendering tolerance. In malignancies, tumor cells secrete IL-6 and IL-10 to induce tDCs with high expression of LILRB4, which attenuates the response of T cells to tumor-associated antigens. Together, LILRB4 may induce tolerance of DCs to promote immunological tolerance in autoimmune diseases and transplant rejection; blocking LILRB4 may reverse DC tolerance to treat these diseases.

1.2.3. Myeloid-derived suppressor cells. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature myeloid cells derived from the bone marrow. In general, MDSCs include early-stage MDSCs (eMDSCs), monocytic MDSCs (M-MDSCs), and granulocytic or polymorphonuclear MDSCs (G-MDSCs or PMN-MDSCs). In chronic infections and inflammation, MDSCs are strongly expanded and are able to inhibit the activity of T cells. LILRB4 is associated with the disease severity of coronavirus disease-19 (COVID-19) infection. In severe COVID-19 patients, PMN-MDSCs and M-MDSCs are strongly expanded and related to poor T cell responses, which suggests that MDSCs may exert immunosuppressive functions through LILRB4 in COVID-19 patients.

1.3. LILRB4 functions in tumors

1.3.1. Myeloid-derived suppressor cells. In tumor immune microenvironment, MDSCs are strongly expanded and are able to inhibit the activity of T cells. In non-small-cell lung cancer (NSCLC), LILRB4 is expressed on both PMN-MDSCs and M-MDSCs. Particularly in PMN-MDSCs, the expression of LILRB4 is correlated with poor outcomes in NSCLC patients. M-MDSCs may exhibit a stronger immunosuppressive potential than PMN-MDSCs. In GM-CSF/IL-6-induced M-MDSCs from human normal monocytes in vitro, treatment with prostaglandin E2 expands M-MDSCs and enhances the ability to induce IL-10-producing Treg cells through the effect of LILRB4 on M-MDSCs. Furthermore, in vitro coculture with SK-MEL-5 cancer cells induces human normal monocytes from peripheral blood to M-MDSCs with high expression levels of LILRB4 on the cell surface. Treatment of these M-MDSCs with anti-LILRB4 antibodies impairs the ability to induce T cell suppression. In addition, the fibronectin expressed by stromal cells in tumor microenvironment binds and activates LILRB4 on MDSCs. The fibronectin-LILRB4 interaction recruits SHP-1 to inhibit Syk-mediated FcγR signalings and immunosuppressive activities of MDSCs. Together, MDSCs may inhibit the activity of T cells via LILRB4.

1.3.2. Tumor-associated macrophages. In addition to MDSCs, monocytes in the circulation can be recruited to the tumor microenvironment and further differentiate into tumor-associated macrophages (TAMs). TAMs, similar to alternatively activated macrophages (M2), lack phagocytic activities and promote tumor cell evasion from immune surveillance and metastasis to other tissues and organs. Intensive studies have shown that LILRB4 is expressed on TAMs in human lung cancer, melanoma, colon carcinoma, pancreatic carcinoma, mouse melanoma and colon cancer models. Either blocking LILRB4 with antibodies or gp49b deficiency increases the infiltration of anti-tumor immune cells into the tumor microenvironment and decreases the inhibitory effect of Treg cells via regulation of the production of IL-1β and iNOS from TAMs. Together, these studies imply that LILRB4 expressed on TAMs may be an intriguing target for cancer immunotherapy.

1.3.3. Malignant cells. Expression of LILRB4 was found in a variety of tumor cells, including solid tumors and hematological tumors. The expression of LILRB4 in acute myeloid leukemia (AML) has been extensively studied. Independent studies have shown that LILRB4 is specifically expressed on monocytic AML cells but not on other subtypes of AML cells. In AML, apolipoprotein E (APOE) binds to LILRB4, in turn recruiting SHP-2 to the intracellular ITIMs of LILRB4, which further activates the NFκB signaling pathway to promote leukemia cell infiltration via the urokinase receptor (uPAR) and inhibit T cell activation via ARG-1. Mutation analyses have shown that phosphorylation of tyrosine 412 and 441 in LILRB4 ITIMs by Src-family tyrosine kinases is required for T cell suppression and leukemia cell infiltration. Treatment with anti-LILRB4 antibodies, which interrupts the APOE-LILRB4 interaction in a competitive manner, unleashes T cell suppression and inhibits leukemia cell infiltration and AML development. The expression of LILRB4 is regulated at multiple levels, such as the transcriptional, posttranscriptional and epigenetic levels, which may provide more therapeutic options. Vitamin D3 and its nuclear receptor bind to the promoter region of LILRB4 and drive LILRB4 expression. Fat mass and obesity-related protein (FTO), an RNA N6-methyladenosine (m6A) demethylase, positively regulates the expression of LILRB4 in mononuclear AML cells by inhibiting YTH N6-methyladenosine RNA binding protein 2 (YTHDF2)-mediated decay of LILRB4 mRNA m6A modification. Furthermore, FTO-specific inhibitors reduce the expression of LILRB4 and PD-L1/2 on AML cells, substantially increasing the sensitivity of AML cells to be killed by activated T cells. In addition, protein arginine methyltransferase 5 (PRMT5),
an enzyme that catalyzes symmetric demethylation of protein arginine residues, regulates epigenetic activity by targeting histone proteins.108,109 LILRB4 expression is upregulated by PRMT5 in AML cells, which results in activation of the mTOR pathway to enhance the invasion ability of AML cells.109 LILRB4 is specifically expressed on monocytic AML cells but not on normal hematopoietic progenitor cells and anti-tumor T cells, which makes LILRB4 a promising therapeutic target to overcome the disadvantages of previous AML antigens, such as CD123 and CD33, causing severe bone marrow toxicity.35,102 Therefore, several therapeutic strategies have been developed, including anti-LILRB4 humanized monoclonal antibodies105 anti-LILRB4 chimeric antigen receptor (CAR)-T cells110 and anti-LILRB4 antibody-drug conjugates (ADCs).111 and biomimetic inhibitors.112 Although these therapies have shown anti-tumor efficacy in pre-clinical experiments, the most promising therapeutic strategies that could be utilized to target LILRB4 on myeloid cells to improve disease outcome will be validated in human patients.

Similar to AML, LILRB4 is expressed and positively correlated with immune checkpoint, cytokotic T-lymphocyte associated protein 4 (CTLA-4), in chronic myelomonocytic leukemia (CML).113 Although LILRB4 is not expressed on naive B or memory B cells, it is ectopically expressed on plasmablasts and plasma cells, that might be related to the pathogenesis of systemic lupus erythematosus.37,114 as well as B cell malignancies. In B-cell chronic lymphocytic leukemia (B-CLL) cells, LILRB4 recruits SHP-1 to form a dynamic aggregation inhibitory cluster to the B cell receptor (BCR), thereby inhibiting BCR-dependent Akt activation.115 Beyond hematopoietic malignancies, LILRB4 may be expressed on solid cancer cells, such as non-small cell lung cancer (NSCLC) and gastric cancer cells.100 In NSCLC, LILRB4 is activated by APOE binding and further recruits SHP-2 and SHP-1 to activate ERK1/2 signaling and increase the expression of vascular endothelial growth factor (VEGF) to promote cancer metastasis.116 Furthermore, LILRB4 may interact with membrane-bound molecules and modulate their function. CD166/activated leukocyte cell adhesion molecule (ALCAM) is a transmembrane protein on either tumor cells or plasma cells, that might be related to the pathogenesis of various diseases, especially cancers.

2. CONCLUSION

T cell immune checkpoint blockade as an immunotherapy, like PD-1 and CTLA-4, has elicited impressive therapeutic responses in the treatment of many cancers.119,120 However, existing immunotherapies are not effective in most cancer patients and may develop de novo or adaptive resistance.121 Therefore, exploring novel immune checkpoint molecules is emergent. Here, we summarized the expression, ligands, intracellular signaling and biological functions of LILRB4 in myeloid cells. In normal and disease-associated myeloid cells, such as monocytes, macrophages, and DCs, LILRB4 may prefer to recruit SHP-1 as a signaling adaptor to inhibit cell activation and cytokine production; however, in malignant cells, LILRB4 is likely to recruit SHP-2 or SHP-1 to conduct its downstream signaling.

More importantly, expression of LILRB4 is 40- to 50-fold higher than that of PD-L1 and PD-L2 in AML cells.107 LILRB4 blockade can unleash anti-tumor T cells and suppress the disease progression of AML.35 In solid tumor, especially in “cold” tumors, such as pancreatic ductal adenocarcinoma, LILRB4 is expressed in MDSCs and TAMs and alleviated the suppression of LILRB4 to tumor immunity in TME may remodel TME and provide antitumor efficacy. Together, LILRB4 is a promising therapeutic target that modulates immune activities in the pathogenesis of various diseases, especially cancers.

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