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Inhibitory leukocyte immunoglobulin-like receptors in cancer development

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Inhibitory leukocyte immunoglobulin-like receptors (LILRB1-5) signal through immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their intracellular domains and recruit phosphatases protein tyrosine phosphatase, non-receptor type 6 (PTPN6, SHP-1), protein tyrosine phosphatase, non-receptor type 6 (PTPN6, SHP-2), or Src homology 2 domain containing inositol phosphatase (SHIP) to negatively regulate immune cell activation. These receptors are known to play important regulatory roles in immune and neuronal functions. Recent studies demonstrated that several of these receptors are expressed by cancer cells. Importantly, they may directly regulate development, drug resistance, and relapse of cancer, and the activity of cancer stem cells. Although counterintuitive, these findings are consistent with the generally immune-suppressive and thus tumor-promoting roles of the inhibitory receptors in the immune system. This review focuses on the ligands, expression pattern, signaling, and function of LILRB family in the context of cancer development. Because inhibition of the signaling of certain LILRBs directly blocks cancer growth and stimulates immunity that may suppress tumorigenesis, but does not disturb normal development, LILRB signaling pathways may represent ideal targets for treating hematological malignancies and perhaps other tumors.

immunoreceptor tyrosine-based inhibitory motifs, immunoreceptor tyrosine-based activation motif, leukocyte immunoglobulin-like receptor subfamily B, immunoglobulin-like transcript, leukocyte immunoglobulin-like receptor, phosphatase, ITIM, ITAM, LILRB, CD85, ILT, LIR, SHP-1, SHP-2, SHIP, MHC, HLA, signal transduction, leukemia, cancer

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1 Introduction to ITIM-containing receptors and LILRBs

First described in 1995, the immunoreceptor tyrosine-based inhibitory motifs (ITIMs) are conserved 6 amino acid stretches (S/I/V/LxYxxI/V/L) found in the cytoplasmic tails of certain surface transmembrane receptors [1]. Ligand binding or interplay with other signaling receptors leads to a conformational change that is accompanied by the phosphorylation of tyrosines in the ITIM by Src kinases. This leads to the recruitment of SH2 domain-containing phosphatases. With the exception of IgG Fc receptor II-B

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(FcγRIIB), which is the ITIM-containing receptor that only recruits the inositol-phosphatase SHIP, these receptors all bind tyrosine phosphatases SHP-1 or SHP-2 [2–4]. An isoleucine at the first position of the ITIM (IxYxxL/V) favors binding to SHP-1, whereas a leucine in the same position (LxYxxL/V) favors SHIP binding [5]. These phosphatases are known to negatively regulate immune cell activation. For example, SHP-1 dephosphorylates the activated immunoreceptor tyrosine-based activation motif (ITAM), Src proto-oncogene non-receptor tyrosine kinase (Src), spleen tyrosine kinase (Syk), zeta-chain associated protein kinase 70 kD (ZAP70), Lck/Yes-related novel protein tyrosine kinase (Lyn), phosphatidylinositol-4-phosphate 3-kinase (PI3K), phospholipase C gamma (PLC-γ), or Vav 1 guanine nucleotide exchange factor (Vav1) [6–9]. The consequences of signaling through ITIMs are not well defined. ITIMs may inhibit signaling from immune activating receptors, cytokine receptors, and tyrosine kinase receptors. A proteome-wide analysis performed a decade ago identified 109 human ITIM-containing receptors [10].

ITIMs have antagonistic functions relative to immunoreceptor tyrosine-based activation motifs (ITAMs). ITAM is a conserved amino acid sequence of YxxL/Ix(6-8)YxxL/I in the cytoplasmic tails of membrane proteins. ITIM transmits signals from various membrane receptors including T cell receptors, B cell receptors, activating leukocyte Ig-like receptors (LILRs), certain activating natural killer (NK) cell receptors and Fc receptors, and other activating receptors [11]. Like to ITIM-containing receptors, ligand engagement at a relevant receptor results in the activation of Src kinases, leading to the phosphorylation of tyrosines within the ITAM. Subsequent recruitment and activation of Syk or ZAP-70 tyrosine kinases in myeloid cells and lymphoid cells, respectively, initiate further downstream signaling events, usually resulting in immune activation [11].

The leukocyte Ig-like receptor subfamily B (LILRB) is an important group of ITIM-containing receptors that were cloned in 1997 [12–15] (see schematic in Figure 1). These receptors, also known as cluster of differentiation 85 (CD85), Ig-like transcripts (ILTs), or leukocyte Ig-like receptors (LIRs), are type I transmembrane glycoproteins containing extracellular ligand-binding Ig-like domains and intracellular ITIMs, and are thus classified as immune inhibitory receptors. The name LILRB was officially designated in 2001 to classify a group of inhibitory receptors, whereas LILRA refer to activating receptors [16]. It is known that LILRBs, including LILRB1, LILRB2, LILRB3, LILRB4, and LILRB5, are expressed on myeloid cells and certain other hematopoietic cells and can exert immunomodulatory effects on a wide range of immune cells [17]. It is noteworthy that LILRB1-5 are primate and human specific, with paired immunoglobulin-like receptor B (PirB) [18] and leukocyte immunoglobulin-like receptor, subfamily B, member 4 (Lilrb4, gp49B1) [19] as the only two mouse orthologs. The human genes encoding these receptors are found in a gene cluster known as the leukocyte receptor complex at chromosomal region 19q13.4 [12,20].

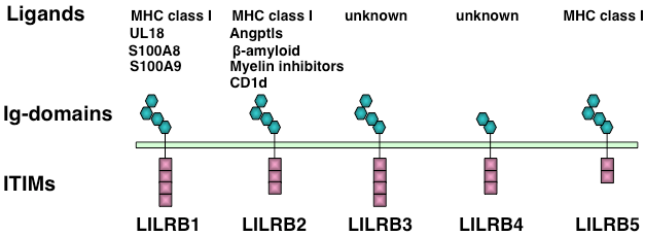

Figure 1 (color online) Schematic of the ligands, extracellular Ig-domains (hexagons), and intracellular ITIMs (boxes) of human LILRBs.

LILRBs are encoded by rapidly evolving genes, and it is thus difficult to study their function in animal models, so the biological function and clinical significance of these receptors have remained poorly understood. Recently, we demonstrated that LILRB2 and a mouse ortholog PirB are not only expressed by immune cells but are also expressed by hematopoietic stem cells [21]. Moreover, several LILRBs, PirB, and LAIR1 (a close related ITIM-containing receptor) [22–25] are expressed by primitive and differentiated acute leukemia cells that support leukemia development [21,26]. A number of reports also showed that LILRBs are expressed in hematopoietic and solid cancer cells and in most cases exert tumor-promoting functions [27–41]. Ligands and signaling pathways mediated through LILRBs have also been identified [21,26,38,39,41–44]. Although counterintuitive, the direct tumor-supportive roles of LILRBs are consistent with the generally immune-suppressive and thus tumor-promoting functions of the inhibitory receptors in the immune system [45]. The roles of LILRBs in immune and neuronal diseases have been recently reviewed [2,4,46,47]. Here we will focus on the ligands, expression pattern, signaling, and the immune-modulatory and direct tumor-supportive roles of LILRBs in cancer development.

2 Ligands for ITIM-containing receptors

Different types of molecules were identified as ligands for ITIM-containing receptors. These include integrated membrane proteins (e.g., major histocompatibility complex (MHC) class I for LILRB1-2 [48,49]), extracellular matrix proteins (e.g., collagens for LAIR1 [22]), and soluble proteins (e.g., antibodies for FcγRIIB).

Not all the ligands for LILRBs were identified. LILRB1-2 binds classical and non-classical MHC molecules [48–50]. LILRB5 was shown to bind to MHC- or human leukocyte antigen (HLA)-Class I heavy chains [44]. LILRB1 and 2 were also shown to bind to non-HLA ligands, including S100A8 and S100A9 for LILRB1 [51], and CD1d [52], several angiopoietin-like proteins (Angptls) [21,42], oligo-

meric β-amyloid [43], and myelin inhibitors reticulon 4 (RTN4, Nogo66), myelin associated glycoprotein (MAG), and oligodendrocyte myelin glycoprotein (OMgp) for LILRB2 [53]. We recently demonstrated that multimeric Angptls bind and activate LILRB2 more effectively than does HLA-G [42]. The ligands for LILRB3 and 4 have not been identified. Although integrin αvβ3 was shown to bind to mouse gp49B1, a possible ortholog of human LILRB4 [54], this dimeric integrin does not bind to human LILRB4.

3 Relevance to cancer

We performed an *in silico* analysis of the relationship between gene expression and the overall survival of acute myeloid leukemia (AML) patients using the TCGA database of AML patients (https://tcga-data.nci.nih.gov/tcga/). The expression of two out of 58 ITIM-containing receptors is positively correlated with the overall survival of AML patients; 20 of these receptors have negative correlation between expression and patient survival [26]. Importantly, we found that silencing of certain receptors individually lead to reduced cell growth [26]. These results suggest that some ITIM-containing receptors directly support human leukemia cell growth.

The *in silico* analysis further indicated that the levels of expression of several closely related LILRB family members, LILRB 1, 2, 3, and 4, and the related LAIR1 are higher in monocytic AML (M5 subtype) cells and inversely correlate with the overall survival of AML patients [26]. LAIR1 is a type I transmembrane glycoprotein that shares the same domain organization as LILRBs, containing one extracellular Ig-like domain that binds collagens or surfactant protein D and two intracellular ITIMs that recruit SHP-1 and SHP-2 [22–25]. LAIR1 is expressed on various lineages of hematopoietic cells and hematopoietic progenitor $CD34^+$ cells [55]. The silencing of expression of LILRB2, 3, 4 or LAIR1 individually in human AML cell lines inhibits cell growth *in vitro* [26]. LILRB4 and LAIR1 are more highly expressed on primary human AML cells than on normal counterparts [26,56]. Importantly, the $LAIR1^+$ cells are enriched for the activity of AML stem cells (AML SCs). Inhibition of expression of LAIR1 in human AML cell lines almost completely abolished leukemia development in xenografted NOD/SCID/IL2Rγ null (NSG) mice [26]. In contrast, the studies of individual knockout mouse lines of *PirB* and *gp49B1* (the only mouse orthologs) and of the *LAIR1*-knockout mouse revealed no overt defects in normal hematopoiesis [21,57,58].

With the exception of LILRB1, LILRBs are not expressed at significant levels by adaptive immune T and B cells, although several *LILR* mRNAs are detected in subset of B cells. This pattern of cell expression suggests that LILRBs may primarily be involved in the innate immune response. Also, the expression of LILRBs in tumor cells implies possible cancer-related functions. Below we summary the cancer related roles of individual LILRBs.

3.1 LILRB1 (CD85J, ILT2, LIR1, MIR7)

LILRB1 contains four extracellular immunoglobulin domains, a transmembrane domain, and four cytoplasmic ITIMs. Among the LILRBs, LILRB1 has the most extensive cellular distribution. It is expressed on primary NK cells, B cells, various populations of T cells, monocytes/macrophages, eosinophils and basophils, dendritic cells [4], decidual macrophages [59], and osteoclasts [17]. LILRB1, 2, 3, and 4 are expressed on progenitor mast cells but not on mature mast cells [60]. LILRB1 binds various HLA class I molecules, including HLA-A, HLA-B, HLA-C, HLA-E, and HLA-G, with μmol L^{-1} affinities [48,50]. It also binds the cytomegalovirus UL18 protein, which is a HLA class I homolog [13]. In addition, LILRB1 was reported to bind two calcium-binding proteins S100A8 and S100A9 [51]. Dimerized HLA-G induces more efficient LILRB1 signaling than the monomeric form [61]. LILRB1-mediated signaling is thought to control inflammatory responses and cytotoxicity to focus the immune response and limit autoreactivity, and LILRB1 functions as an inhibitor of immunity of T cells, B cells, NK cells, and macrophages. HLA-G is upregulated in human breast tumors, and LILRB1-expressing immune cells infiltrate breast cancer tissues, which may contribute to tumor development [62]. Soluble anti-LILRB1 stimulates, but crosslinked antibody inhibits, T cell proliferation [63]. Tumor-cell-expressed HLA-G interacts with LILRB1 on Vγ9Vδ2 T cells to inhibit cytotoxicity of these T cells [64]. Cross-linking of LILRB1 inhibits antigen-induced B cell activation and suppresses antibody production [65]. Interaction of HLA-G with LILRB1 on NK and macrophages inhibits cytotoxicity and inflammation toward trophoblasts, circumventing undesired anti-fetus immune responses during pregnancy [66,67]. In addition, LILRB1, like LILRB3 and 4, inhibits differentiation of osteoclasts [17].

LILRB1 is also expressed on certain AML cells [26], especially in M5 monocytic AML cells, neoplastic B cells (including B-cell leukemia, B-cell lymphoma, and multiple myeloma cells [27,28]), and T cell leukemia and lymphoma cells [29]. LILRB1 expression on primary cutaneous $CD8^+$ and $CD56^+$ T cell lymphomas may protect these tumor cells from apoptosis [29]. LILRB1 is also expressed in human gastric cancer cells and may enhance tumor growth [30]. In contrast, soluble or nanoparticle-aggregated HLA-G binds LILRB1 on neoplastic B cells and inhibits their proliferation [27]. Antibody blocking of LILRB1 on myeloma or lymphoblastic cells in culture did not change NK cell-mediated cell lysis [31]. Therefore the roles of LILRB1 in different contexts will need further investigations.

3.2 LILRB2 (CD85D, ILT4, LIR2, MIR10)

LILRB2 contains four extracellular immunoglobulin domains, a transmembrane domain, and three cytoplasmic ITIMs. The receptor is expressed on monocytes, macrophages, dendritic cells, hematopoietic stem cells, and the basophils of some individuals but not on lymphoid cells [21,49,68]. LILRB2 was also reported to be expressed by endothelial cells [69], decidual macrophages [59], and osteoclasts [17]. Progenitor mast cells express this receptor whereas mature mast cells do not [60]. LILRB2 binds to multiple types of ligands, including HLA class I molecules [49], CD1d [52], myelin inhibitors (including Nogo66, MAG, and OMgp [53]), Angptls [21,42], and β-amyloid [43]. *In vitro* cross-linking of LILRB2 with FcγRI inhibits Fc receptor (FcR)-mediated signaling in monocytes [68] and serotonin release in LILRB2-transfected basophilic leukemia cells [49]. Upregulation of LILRB2 induces dendritic cell tolerance [70]. LILRB2 suppresses axonal regeneration [53] and promotes Alzheimer's disease [43]. We showed that LILRB2 is required for *ex vivo* expansion of hematopoietic stem cells (HSCs) [21]. PirB, the mouse ortholog of human LILRB2 and LILRB3, suppresses myeloid-derived suppressor cell differentiation into M1 macrophages that inhibit regulatory T cell activities and tumor development [45]. PirB expression increases upon myeloid and B cell differentiation [71].

LILRB2 is expressed on various cancerous cells. AML cells, especially monocytic AML cells express LILRB2 [26]. The mouse ortholog PirB is also expressed on MLL-AF9 AML cells, including AML SCs [21]. LILRB2 expression is induced by some chronic lymphoblastic leukemia (CLL) cells [32]. LILRB2 expression is not limited to hematologic malignancies. LILRB2 is expressed in breast cancer cell lines and in 60.7% of primary ductal and lobular breast cancer tissues but is absent from normal breast tissues [33]. The receptor is also expressed on human non-small cell lung cancer cells [34,39–41] and supports the their survival and cancer development [39].

3.3 LILRB3 (CD85A, ILT5, LIR3, HL9)

LILRB3 contains four extracellular immunoglobulin domains, a transmembrane domain, and four cytoplasmic ITIMs. LILRB3 is constitutively expressed on monocytes, monocyte-derived osteoclasts, neutrophils, eosinophils, basophils, and osteoclasts [17]. LILRB3 is expressed on progenitor mast cells but not on mature mast cells [60]. Co-ligation of LILRB3 with FcεRI *in vitro* inhibits Fc receptor-mediated cell activities in human basophils [72]. LILRB3 inhibits differentiation of osteoclasts [17].

LILRB3 is expressed by certain myeloid leukemia, B lymphoid leukemia, and myeloma cells, and is co-expressed with leukemia stem cell marker CD34 or myeloma marker CD138 [35]. We showed that inhibition of LILRB3 expression in certain human leukemia cell lines inhibited growth of these cells [26]. An anti-LILRB3 antibody induces cytotoxicity of LILRB3-expressing cells via complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity [35].

3.4 LILRB4 (CD85K, ILT3, LIR5, HM18)

LILRB4 contains two extracellular immunoglobulin domains, a transmembrane domain, and three ITIMs. It is the only LILRB that contains two Ig domains. The receptor is expressed on dendritic cells, monocytes and macrophages [15,73], progenitor mast cells [60], endothelial cells [74], and osteoclasts [17]. LILRB4 is expressed on progenitor mast cells but not on mature mast cells [60]. Like other inhibitory LILRB proteins, the intracellular ITIMs of LILRB4 recruit SHP-1, which transduces a negative signal that inhibits stimulation of an immune response. LILRB4 was shown to inhibit cell activation of monocytes and macrophages [15]. Up-regulation of LILRB4 and LILRB2 expression in tolerogenic antigen presenting cells, cells that may regulate the functions of suppressor and regulatory T cells, leads to immune tolerance [70,75]. Both membrane-bound and soluble LILRB4 induce anergy of T helper cells and differentiation of $CD8^+$ T suppressor cells, contributing to rejection of allogeneic tumor transplants [36,76]. LILRB4 inhibits differentiation of osteoclasts [17]. LILRB4 is one of the most polymorphic receptors with at least 15 identified single nucleotide polymorphisms (SNPs) [77].

LILRB4 has been shown to play important roles in cancer development, transplantation, and autoimmune diseases [73]. LILRB4 expression marks monocytic AML cells, and it is co-expressed with stem cell marker c-Kit or CD34 in 50% and 39% of cases, respectively [56]. We showed that inhibition of LILRB4 expression in human leukemia cell lines inhibited growth of these cells [26]. LILRB4 is also expressed on CLL B cells but not normal B cells and may have prognostic value in CLL [32]. LILRB4 is expressed in human gastric cancer cells and may enhance tumor growth by inhibiting NK cell activity [30]. Soluble LILRB4 is present in more than 40% of serum samples from patients with colorectal carcinoma, pancreatic carcinoma, and melanoma, and inhibits T-cell responses *in vitro* [36,37]. The supportive role of LILRB4 in development and/or progression of these cancers was verified by treatment of anti-LILRB4 or by depletion of LILRB4 [36].

3.5 LILRB5 (CD85C, LIR8)

LILRB5 is expressed in subpopulations of monocytes, NK cells, and mast cell granules [12,60]. It was suggested to play a role in clearance of creatine kinase via the mononuclear phagocytic system in the liver [78]. LILRB5 is unique among LILRBs in that it is the only LILRB that is not highly expressed by M5 AML cells and in that its expres-

sion does not correlate with the overall survival of AML patients based on our analysis of TCGA database of AML patients (https://tcga-data.nci.nih.gov/tcga/).

3.6 Mechanisms that underlie the roles of LILRB signaling in cancer cells

The signaling mediated by LILRBs starts by the activation of ITIMs via the phosphorylation of tyrosines in these motifs by Src kinases, leading to the recruitment of SH2 domain-containing phosphatases or the inositol-phosphatase SHIP [2–4]. The interaction between phospho-tyrosine in ITIMs and phosphatases are exemplified by an analysis of the mouse PirB [79,80]. The known substrates of ITIM-recruited phosphatases include ITAMs, Src, Syk, ZAP70, Lyn, PI3K, PLC-γ, and Vav1 [6–9]. However, it is likely that LILRBs have additional substrates, and the downstream signaling molecules have not been well characterized. It is also likely that ITIM receptors have diverse signaling branches instead of a linear signaling cascade like that of the janus kinase and signal transducer and activator of transcription (JAK/STAT) pathway. In addition, the LILRBs and different ligands presumably induce distinct signaling networks in a manner that is cell context dependent.

We studied the downstream signaling of PirB, LILRB2, and LAIR1 as representative ITIM-containing receptors in retrovirus transplantation acute leukemia models and in the lung cancer implantation model.

3.7 Signaling downstream of LILRB2 and PirB in cancer cells

We have studied the role of the LILRB2 mouse homolog PirB in MLL-AF9 AML cells and found that PirB is associated with SHP-1 and SHP-2. Defective PirB signaling results in decreased levels of phosphorylation of SHP-1 and SHP-2 in AML cells [21]. We also found that phosphorylation of CAMKIV can be induced by Angptl binding to LILRB2 in human cord blood $CD34^+$ cells, and that p-CAMKIV levels are decreased in PirB-deficient AML cells [21]. These results suggest that LILRB2-mediated signaling is transmitted through SHP-1 and SHP-2 and is coupled with CAMKIV in AML cells.

Several papers showed that both Angptl2 and LILRB2 are highly expressed in non-small cell lung cancer (NSCLC) samples, and levels of these proteins are negatively correlated with the patient prognosis [39–41]. In one case, it was shown that ILT4 drives B7-H3 expression via PI3K/ AKT/ mTOR signalling and ILT4/B7-H3 co-expression correlates with poor prognosis in non-small cell lung cancer [41]. In another case, inhibition of LILRB2 resulted in a drastic decrease in proliferation, colony formation, and migration of NSCLC cells, and Angptl2 binding to LILRB2 supports lung cancer development via the SHP2/CaMKI/ CREB axis [39]. These studies suggest that LILRB2 signaling represents a novel target for lung cancer treatment.

3.8 Signaling downstream of LAIR1 in AML cells and B-ALL cells

LAIR1 does not affect normal hematopoiesis but is essential for leukemia development [26,38,57]. LAIR1 is a type I transmembrane glycoprotein that shares the same domain organization as LILRBs, containing one extracellular Ig-like domain that binds collagens or surfactant protein D and two intracellular ITIMs that recruit SHP-1 and SHP-2 [22–25]. LAIR1 is known to be expressed on various lineages of hematopoietic cells and hematopoietic progenitor $CD34^+$ cells [55]. Previous studies demonstrated that LAIR1 engagement with antibodies induced apoptosis and prevented NF-κB nuclear translocation of AML cell lines [81], and also blocked AKT and NF-κB activation and thus cell division of CLL cells [82]. We showed that LAIR1 is highly expressed on human AML and B cell ALL (B-ALL) cell lines, as well as on primary AML cells especially monocytic M5 AML cells [26]. Inhibition of LAIR1 expression decreases leukemia development *in vitro* and in xenograft experiments [26,38]. In retrovirus transplantation models, including MLL-AF9 (AML) [83,84], AML1-ETO9a (AML) [85], BCR-ABL1 (B-ALL) [38], and N-Myc (B-ALL) [86], LAIR1 deficiency blocks leukemia development in primary or serial transplantation [26,38], suggesting that LAIR1 is critical for maintenance of the activity of AML stem cells [26].

Importantly, we also found that SHP-1, but not SHP-2, mediates LAIR1 signaling in AML cells and prevents exhaustion of AML SCs *in vitro* and *in vivo*. Furthermore, we demonstrated that SHP-1 is a negative signaling molecule for normal myeloid differentiation but acts as a phosphatase-independent adaptor to recruit CAMKI for activation of the downstream transcription factor CREB in AML cells. The LAIR1/SHP-1/CAMKI axis may represent a target for treating AML [26].

Müschen's group [38] demonstrated that LAIR1, as well as other ITIM-containing receptors, supports development and relapse of Philadelphia chromosome-positive (Ph^+) B-ALL. LAIR1 acts through SHP-1 and SHIP, which dephosphorylate Syk to support this particular type of B-ALL. Hyperactive Syk tyrosine kinase activity is required and sufficient to induce death of these B-ALL cells. This suggests that the basic mechanism of negative selection of B cells with over activation is functional in transformed B-ALL cells. Therefore, activating Syk and a negative B-cell selection strategy may overcome drug resistance in Ph^+ B-ALL [38].

Together, these results suggest that ITIM-containing receptors support the development, drug resistance, relapse, or cancer stem cell activity of different types of cancer, even

though the downstream signaling differs. When SHP-1 acts as a mediator of the signaling, both the phosphatase-dependent and -independent mechanisms can be used by the cancer cells (Figure 2).

4 Perspectives and future work

Effective elimination of all cancer cells, including cancer stem cells, remains a major challenge in oncology. Our finding that LILRBs and related receptor LAIR1 directly support the survival and self-renewal of leukemia cells opened a new research front in cancer research, diagnosis, and treatment. Below we discuss several research questions of ITIM-containing receptors that should be addressed in the near future.

4.1 Identification of ligands

Cloning of unknown ligands for LILRBs is a key effort to elucidate how these receptors work. The ligands for LILRB3 and LILRB4 have not yet been identified. In addition, based on fact that LILRB2 and LAIR1 each bind to more than one ligand, it is possible that multiple ligands exist for certain ITIM-containing receptors. For example, the binding between LILRB1-2 and MHC-I occurs with low μmol L^{-1} affinity, suggesting the possibility of the existence of additional high-affinity ligands, co-ligands, or binding proteins. To identify LILRB ligands, various methods can be applied including expression cloning, crosslinking followed by co-immunoprecipitation and mass spectroscopy, protein array, and candidate screening. Recently developed

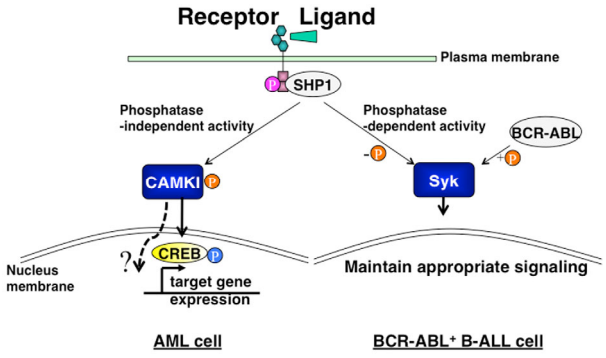

Figure 2 (color online) ITIM-containing receptor downstream signaling in different leukemia cells. In LILRB-expressing AML cells, the ligand binding or interplays among different types of receptors activates LILRB as reflected by the phosphorylation of the tyrosines in ITIMs. SHP-1 is recruited and acts as a phosphatase-independent scaffolding protein to form a complex with the kinase CAMKI. The activated CAMKI then induces phosphorylation of the transcription factor CREB that translocates into nucleus to regulate the self-renewal, survival, and differentiation of AML cells. By contrast, in BCR-ABL$^+$ B-ALL cells, BCR-ABL induced phosphorylation of Syk is balanced by the ITIM-containing receptor mediated SHP-1 (or SHIP, not shown here) phospatase-dependent dephosphorylation. This avoids the hyperphosphorylation of Syk, thus preventing the negative selection of over-activated malignant B cells.

cloning methods employing cell microarrays [87] and ligand-based receptor capture technologies [88] may accelerate the progress.

4.2 Cell type-dependent signaling

A key question is why some inhibitory receptors have supportive effects on cancer development. The tumor-supportive role of SHP-1 in certain acute leukemia provides an explanation. A role for SHP-1 in AML development is supported by evidence from previous studies: human LAIR1 is mainly associated with SHP-1 but not SHP-2 [89]; SHP-1 suppresses differentiation in some leukemia cells [90], concordant with the reported anti-differentiation activity of LAIR1 [91]; and SHP-1 inhibits apoptosis in freshly isolated leukemia cells [92]. While SHP-1 is capable of binding to Grb2 in a phosphatase-independent manner [93], the CAMKI recruitment of SHP-1 represents a different phosphatase-independent mechanism to sustain AML SC activity in AML. By contrast, SHP-1 utilizes a phosphatase-dependent mechanism to support Ph$^+$ B-ALL [38].

Furthermore, it is known that SHP-1 is a negative regulator of growth of normal hematopoietic progenitors and that overexpression of SHP-1 inhibits growth of cancer cell lines [94–98]. It was also suggested that LAIR1 signaling negatively regulates myeloid leukemia and CLL [81,82]. Therefore, it will be interesting to determine the cell specificity for ITIM-containing receptors and SHP-1 to exert the tumor-supportive and tumor-suppressive functions.

The potentially overlapping but distinct roles of SHP-1. SHP-2, and SHIP that mediate ITIM-containing receptors' signaling in different cells are largely unknown. It is generally agreed that SHP-2 plays a positive signaling role in the hematopoietic system, whereas SHP-1 is a negative regulator of cell signaling. Therefore, the finding that SHP-1, but not SHP-2, mediates LAIR1 signaling to support AML development is surprising and deserves attention. In addition, SHP-2 is also known to have tumor-specific functions, acting as an oncogene or a tumor-suppressor in different types of cancers [99]. A similarly interesting observation is that, while SHP-1 appears to be solely responsible for LAIR1's tumor-promoting function in AML cells [26], both SHP-1 and SHIP are capable of supporting the development of Ph$^+$ B-ALL [38]. Identification of the cell context for SHP-1, SHP-2, and SHIP's signaling roles and their respective substrates or interacting proteins in different cell types will be critical.

Better understanding of the likely divergent downstream signaling of LILRBs in cancer is another area that deserves further investigations. For example, SHP-1 and CAMKI may have downstream effectors other than CREB in LAIR1-expressing AML cells. The identification of the full spectrum of LILRB downstream signaling in various cells is an important task.

4.3 Roles of LILRBs and additional ITIM-containing receptors in solid cancer

The supportive role of LILRBs is unlikely to be limited to acute leukemia. While our *in silico* analyses indicate that expression of a number of ITIM inhibitory receptors negatively correlates with AML patient survival, it is possible that some of these receptors may also play positive roles in development of other types of cancer. Indeed, LILRB1, 2, and 4 are expressed in solid cancer cells such as gastric cancer, breast cancer, and lung cancer cells [30,33,34,39–41]. Because there are numerous types of ITIM-containing receptors, different receptors may have different expression patterns in different types or subtypes of cancers. It will be interesting to determine the individual and combined effects of these receptors in the same cancer cells, and the extent to which LILRB signaling in acute leukemia cells can be generalized to other immune inhibitory receptors and other types of cancer.

4.4 Therapeutic development

The identification of LILRBs and their downstream signaling as potential therapeutic targets may reshape our views regarding how cancer develops, how cancer cells differ from other cells, and how to treat this difficult disease. Our study suggests that some leukemia cells have unique signaling pathways downstream of ITIM-containing receptors. These inhibitory receptors may enable the leukemia cells to survive conventional therapies, resulting in tumor relapse.

Because inhibition of the signaling of certain LILRBs directly blocks cancer growth, and stimulates immunity that may suppress tumorigenesis but does not disturb normal development, these receptors may represent ideal targets for treating cancer.

The blockade of inhibitory receptor signaling in combination with conventional therapies may prove to be an effective strategy for elimination of leukemia cells as well as other types of cancer cells. Inhibition of factors involved in the intracellular signaling will represent a more powerful means of blocking the effects of ITIM-containing receptors. In particular, the identification of the specific inhibitors of the phosphatase-dependent and -independent SHP-1 activity for cancer treatment may have significant benefits. Therapeutic modalities for LILRBs may also include recombinant soluble extracellular domains of these receptors and blocking antibodies against the receptors' signaling. Moreover, it will be interesting to test whether chimeric antigen receptors [100] engineered to target LILRBs and other ITIM-containing receptors are effective in treating certain types of cancer especially hematopoietic malignancies.

The author(s) declare that they have no conflict of interest.

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