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Clinical roles of TIM-3 in myeloid malignancies and its importance in cellular therapy

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Abstract

Acute myeloid leukemia (AML), one of the most common hematological malignancies worldwide, is derived from a fraction of stem cells known as leukemic stem cells (LSCs), which possess self-renewal and high propagation capacities. Remaining quiescent and being resistant to conventional chemotherapy, residual LSCs after chemotherapy drive leukemia regrowth, leading to AML relapse. Therefore, the eradication of LSCs is critical for the treatment of AML. We previously identified hepatitis A virus cellular receptor 2 (HAVCR2/TIM-3) as an LSC-specific surface molecule by comparing gene expression in LSCs and hematopoietic stem cells (HSCs). TIM-3 expression clearly discriminated LSCs from HSCs within the $CD34^+CD38^-$ stem cell fraction. Furthermore, AML cells secrete galectin-9, a TIM-3 ligand, in an autocrine manner, leading to constitutive TIM-3 signaling that maintains the self-renewal capacity of LSCs via the induction of β-catenin accumulation. Thus, TIM-3 is an indispensable functional molecule for human LSCs. Herein, we review the functional aspects of TIM-3 in AML and evaluate minimal/measurable residual disease with a focus on $CD34^+CD38^-TIM\text{-}3^+$ LSCs. Using sequential genomic analysis of identical patients, we determined that $CD34^+CD38^-TIM\text{-}3^+$ cells in the complete remission (CR) phase after allogeneic stem cell transplantation (allo-SCT) are the LSCs responsible for AML relapse. We retrospectively evaluated the incidence of $TIM\text{-}3^+$ residual LSCs. All analyzed patients achieved CR and complete donor chimerism at the engraftment phase; however, the high frequency of residual $TIM\text{-}3^+$ LSCs within the $CD34^+CD38^-$ fraction at engraftment was a significant and independent risk factor for relapse. Residual $TIM\text{-}3^+$ LSC levels in the engraftment phase had a stronger impact on relapse than did pre-SCT disease status. Therefore, the evaluation of residual $TIM\text{-}3^+$ LSCs is a promising approach for predicting leukemia relapse after allo-SCT.

Key words leukemic stem cells, hematopoietic stem cells, TIM-3, allo-SCT

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1. Introduction

Acute myeloid leukemia (AML) originates from a small fraction of self-renewing leukemic stem cells (LSCs), which can repopulate human AML in immunodeficient mice after xenogeneic transplantation[1,2]. Leukemic stem cells possess self-renewal capacity and differentiation potential toward leukemic blasts, thereby maintaining the AML population. Acute myeloid leukemia LSCs were originally identified in the $CD34^+CD38^-$ fraction, and their phenotype is identical to that of normal hematopoietic stem cells (HSCs). Recent research advances in immunodeficient mice have enabled the isolation of LSCs from the $CD34^+CD38^-$ fraction[3]; however, quiescent LSCs with high self-renewal potential are concentrated within this fraction[4]. Given that LSCs originate from multipotent self-renewing pre-leukemic HSCs, in which somatic mutations or genetic events accumulate throughout their life cycle[5-7], LSCs and HSCs share multiple molecular mechanisms for the mainte-

nance of their stemness. Such similarities between LSCs and HSCs have hampered the development of LSC-specific therapeutic strategies while sparing the HSCs. We intensively investigated the gene expression of FACS-purified $CD34^+CD38^-$ HSCs and LSCs and identified T-cell immunoglobulin mucin-3 (TIM-3) as an LSC-specific surface molecule whose expression was not observed in HSCs. TIM-3 expression clearly discriminates LSCs from HSCs within the $CD34^+CD38^-$ stem cell fraction[8].

In this review, we discuss the function of TIM-3 in AML LSCs. Furthermore, we discuss the significance of the evaluation of minimal/measurable residual disease (MRD), focusing on $CD34^+CD38^-TIM\text{-}3^+$ LSCs in allogeneic stem cell transplantation (allo-SCT).

2. Role of TIM-3 in Hematopoiesis

TIM-3 belongs to the TIM family, and *HAVCR1*, *HAVCR2*, and *TIMD4* encode TIM-1, TIM-3, and TIM-4, respectively. TIM-3 consists of an N-terminal immunoglobulin (Ig) variable domain, mucin domain, transmembrane domain, and cytoplasmic tail. The cytoplasmic tail of TIM-3 contains conserved tyrosine residues and an Src homology 2 (SH2)-binding motif.

TIM-3 was originally identified as a 60 kDa surface molecule expressed on interferon-γ-producing $CD4^+$ and $CD8^+$ T cells in mice. Previous studies have demonstrated that TIM-3 is expressed in other types of blood cells such as monocytes, dendritic cells (DCs), mast cells, regulatory T (T_{reg}) cells, and natural killer cells[9, 10].

Four independent ligands of TIM-3 have been identified: galectin-9 (gal-9), phosphatidylserine (PtdSer), high-mobility group box 1 (HMGB1), and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1)[9]. Gal-9, an S-type lectin, is expressed and secreted by hematopoietic cells. Gal-9 ligation to TIM-3 induces apoptosis of Th1 cells and inhibits interferon-γ production[11]. PtdSer has been identified as a common ligand for all TIM family members based on the crystal structure analysis of TIM-1, TIM-3, and TIM-4[12, 13]. TIM-3 plays crucial roles in the phagocytosis of apoptotic cells and cross-presentation by DCs via its interaction with PtdSer[14]. CEACAM1 is considered to bind to the cleft framed by the FG and CC loops of the IgV domain of TIM-3, similar to PtdSer[15]. HMGB1 was identified as a TIM-3 ligand in DCs; $TIM\text{-}3^+$ tumor-infiltrating DCs attenuated the nucleic acid-mediated innate immune response by interacting with TIM-3 and HMGB1[16].

The interaction between ligands and TIM-3 results in the recruitment of Src family kinases to the SH2 binding motif of the cytoplasmic tail, leading to downstream signal transduction. In general, ligation of TIM-3 ligands suppresses the activation of $TIM\text{-}3^+$ T cells; therefore, TIM-3 has been extensively investigated as a "co-inhibitor" or "immune checkpoint" molecule of T cells like programmed cell death 1 and cytotoxic T-lymphocyte-associated protein 4.

Consistent with the suppressive function of TIM-3 in T cells, the most dysfunctional T-cell subset of tumor-infiltrating $CD8^+PD\text{-}1^+$ T cells expresses TIM-3[17-19]. The double blockade of programmed cell death 1 and TIM-3 by monoclonal antibodies showed a synergistic effect against tumors in murine models[17] and improved the tumor antigen-specific human $CD8^+$ T-cell response in vitro[18, 19]. Furthermore, recent studies have emphasized the significance of TIM-3 in tumor immunity by modifying T_{reg} function, with the majority of tumor-infiltrating human T_{reg} cells expressing TIM-3[20, 21]. Accordingly, antibody blockade of TIM-3 suppresses T_{reg} function[22] and attenuates tumor growth[23] in mice. Based on the results of these studies, anti-human TIM-3 monoclonal antibodies have been developed as "immune checkpoint" inhibitors, and first-in-human phase I/II studies are being conducted to test their safety and efficacy in cancer treatment[24].

3. TIM-3 Expression Marks Functional LSCs in Human Myeloid Malignancies

TIM-3 has been extensively investigated as a negative regulator of T-cell function. However, unlike other co-inhibitory molecules, TIM-3 lacks known inhibitory signaling motifs in the cytoplasmic domain. Therefore, we attempted to identify the LSC-specific functions of TIM-3 in AML cells. We first evaluated the serum concentration of gal-9, the most extensively investigated ligand of TIM-3, in humans and unexpectedly found that patients with AML exhibited a significant increase in serum gal-9 levels compared to healthy donors and patients with other hematological malignancies, such as acute lymphoblastic leukemia and lymphoma. Based on these observations, we hypothesized that $TIM\text{-}3^+$ AML cells secrete gal-9 in an autocrine manner in humans. Consistently, xenotransplantation of primary $TIM\text{-}3^+$ LSCs reconstituted human AML in recipient mice with high serum concentrations of human gal-9. Blockade of the TIM-3/gal-9 interaction using an anti-human gal-9 monoclonal antibody strongly suppressed the leukemia reconstitution potential of AML LSCs in xenotransplantation experiments, suggesting a critical role of the TIM-3/gal-9 autocrine loop in the maintenance of LSC properties[25]. Furthermore, we found that TIM-3/gal-9 interaction promoted the co-activation of the nuclear factor (NF)-κB and β-catenin pathways in human AML cells. Nuclear accumulation of β-catenin and subse-

quent activation of its target genes are involved in the maintenance of LSCs in murine AML models[26] and human chronic myelogenous leukemia[27]. Furthermore, co-activation of NF-κB and β-catenin coordinately promotes the development of cancer stem cells in a murine colorectal cancer model[28]. Therefore, we hypothesized that this TIM-3/gal-9 autocrine loop may be involved in the development of human myeloid LSCs. In humans, the frequencies of TIM-3$^+$ aberrant HSCs within the CD 34$^+$CD38- fraction increased along with disease progression toward AML in myeloid malignancies, including myelodysplastic syndrome and myeloproliferative neoplasm[25]. Importantly, purified-CD34$^+$CD38$^-$TIM-3$^+$ cells exclusively reconstituted AML, whereas CD34$^+$CD38$^-$ TIM-3$^-$ cells from identical patients reconstituted multilineage human normal hematopoiesis in xenotransplantation experiments[29], suggesting that TIM-3 clearly discriminates functional LSCs from HSCs. Consistent with the distinct reconstitution potential of CD34$^+$CD38$^-$ TIM-3$^+$ LSCs and CD34$^+$CD38$^-$TIM-3$^-$ HSCs, genetic analysis revealed a clonal relationship between CD34$^+$ CD38$^-$TIM-3$^+$ and CD34$^+$CD38$^-$TIM-3$^-$ cells; CD34$^+$CD 38$^-$TIM-3$^+$ LSCs represented clones harboring driver mutations derived from CD34$^+$CD38$^-$TIM-3$^-$ cells containing pre-leukemic HSCs[6]. Collectively, these results suggest that TIM-3 is a functional molecule required to maintain the properties of human myeloid LSCs.

We identified various unique molecules and downstream signaling cascades involved in TIM-3 signaling in AML LSCs (Sakoda, Kikushige et al., under revision). Interestingly, the downstream molecules in TIM-3$^+$ AML and TIM-3$^+$-exhausted T cells in humans are quite different. Thus, signaling molecules downstream of TIM-3 are critical for determining the effects of TIM-3 signaling. In this review, we discuss AML-specific TIM-3 signaling cascades.

4. Therapeutic Strategies Targeting TIM-3 in Human Myeloid Malignancies

We previously reported that a cytotoxic anti-human TIM-3 monoclonal antibody effectively killed LSCs without affecting normal human hematopoiesis *in vivo*[8].

A recent study demonstrated the efficacy of bispecific chimeric antigen receptor T cells targeting both CD13 and TIM-3 in the treatment of human AML *in vivo*[30].

In addition to cytotoxic therapeutic approaches against TIM-3$^+$ AML cells, Recent clinical studies have demonstrated the efficacy of blocking TIM-3$^+$ myeloid malignancies. As anti-human TIM-3 monoclonal antibodies have been developed as immune checkpoint inhibitors, first-in-human phase I/II studies have been initiated to test the safety and efficacy of these antibodies in cancer treatment[24]. The efficacy of anti-human TIM-3 monoclonal antibodies has been evaluated in combination with other immune checkpoint inhibitors such as anti-PD-1/PD-L1 monoclonal antibodies[31]. Sabatolimab (MBG453), an anti-human TIM-3 humanized IgG4 monoclonal antibody with the S228P mutation, has been tested for the treatment of myeloid malignancies, including AML, high-risk myelodysplastic syndromes, and chronic myelomonocytic leukemia. Combination therapy with sabatolimab and hypomethylating agents (HMAs) has shown preliminary efficacy and safety in recent clinical trials[32].

5. TIM-3 Marks Residual Leukemic Stem Cells Responsible for Relapse in allo-SCT

Acute myeloid leukemia relapse remains a major clinical challenge. Particularly, the prognosis of patients who experience relapse after allo-SCT is poor. Leukemic stem cells remain quiescent and are resistant to conventional chemotherapy. Residual LSCs after chemotherapy eventually drive leukemia regrowth, leading to AML relapse. Therefore, the evaluation of MRD, focusing on LSCs, can be a powerful strategy to improve the clinical outcome of AML. Consistently, the European Leukemia Network MRD Working Party recommends the evaluation of LSC markers in multiparameter flow cytometry-based MRD in addition to the conventional leukemia-associated immunophenotype and different-from-normal-based staining strategies[33]. TIM-3 clearly discriminated LSCs from HSCs within the CD 34$^+$CD38$^-$ stem cell fraction. In addition, TIM-3 expression in AML cells is stably preserved at the time of relapse[34, 35]. Based on these data, we consider TIM-3 an ideal surface marker for LSCs in MRD evaluation.

We conducted a retrospective cohort study to investigate whether the evaluation of residual LSCs using TIM-3 expression could predict AML relapse after allo-SCT. The frequency of residual TIM-3$^+$ LSCs within the CD34$^+$CD38$^-$ fraction at the time of engraftment was strongly correlated with relapse after allo-SCT, although all patients achieved hematological complete remission (hCR) and complete donor chimerism. The levels of residual TIM-3$^+$ LSCs in the engraftment phase had a stronger impact on relapse than did pre-SCT disease status, indicating the significance of response-oriented MRD evaluation strategies. Furthermore, CD34$^+$CD38$^-$TIM-3$^+$ cells in the hCR phase represent the LSCs responsible for relapse, as assessed by sequential genomic and transcriptome analyses of identical patients. Thus, evaluation of residual TIM-3$^+$ LSCs is a promising approach for predicting the clinical outcome of patients with AML after allo-SCT (Sakoda, Kikushige et al., manuscript in preparation).

6. Conclusion

TIM-3 expression clearly discriminated donor-derived normal HSCs and recipient-derived LSCs within the $CD34^+CD38^-$ cellular fraction. Therefore, evaluation of $CD34^+CD38^-TIM\text{-}3^+$ residual LSCs after allo-SCT would be a useful method for the risk stratification of AML relapse, as discussed in this review. Furthermore, the blockade of TIM-3 signaling represents a unique therapeutic strategy to eradicate LSCs via two independent mechanisms: blockade of the TIM-3/gal-9 autocrine loop in $TIM\text{-}3^+$ AML and restoration of T cell immunity against AML. Thus, the combination of TIM-3-targeted therapeutic approaches and TIM-3-based MRD assessment may be used to improve the clinical outcomes of patients with AML after allo-SCT. Further studies are necessary to determine the clinical role of TIM-3 in myeloid malignancies.

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Conflicts of Interest

The author declares no conflict of interest. a disclosure form provided by the author is available on the website.

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