The correlation between Flt3-ITD mutation in dendritic cells with TIM-3 expression in acute myeloid leukemia

Hooriyeh Shapoorian, Hamidrea Zalpoor, Mazdak Ganjalikhani-Hakemi

1Department of Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran; 2Paramedical School, Shiraz University of Medical Sciences, Shiraz, Iran; 3Acquired Immunodeficiency Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

Abstract

In general, acute myeloid leukemia (AML) is an aggressive and heterogeneous disease that is characterized by rapid cellular proliferation and high mortality. One of the mutations related to AML is the Flt3-ITD mutation, which is found in approximately 25% of patients. In this mini-review, we investigate the function of dendritic cells and T cells based on Flt3-ITD mutation and immune evasion as a result of this abnormality. Finally, we discuss some AML therapeutic strategies, including targeting Flt3 on DCs and TIM-3 on T cells as immune receptors to treat this hematopoietic malignancy.

Keywords: Acute myeloid leukemia, Dendritic cell, Flt3-ITD, TIM-3

1. INTRODUCTION

Acute myeloid leukemia (AML) is a type of cancer that manifests clonal cell proliferation, abnormal differentiation, and poor differentiation of hematopoietic cells inside the bone marrow, blood, and other tissues.1 Patients who are difficult to treat are mainly older adults. As a result, overall survival over 5 years is only about 27%.2 There are molecular markers and genetic mutations that have been identified in the genomes of AML patients and cytogenetic data indicates that they are: Runx1, Flt3-ITD, MLL-PTD, NPM1, CEBPA, and ASXL1.3 There are about 25% of cases of AML that have mutations in the Flt3 (FMS-like tyrosine kinase-3 receptor) gene. Mutations in this gene are associated with poor outcomes.4 Flt3 mutations can be classified into two types: internal tandem duplications of 3 to over 100 amino acids in the juxtamembrane domain (Flt3/ITD) and point mutations in the tyrosine kinase domain (Flt3/ITD).2,4,5 In this mini-review, we focused on these markers for the purpose of treating AML patients by inhibiting the Flt3 tyrosine kinase domain and blocking TIM-3 signaling.

2. FLT3 AND FLT3L

FMS-like tyrosine kinase-3 (Flt3; CD135) is a 993 amino acid single transmembrane type III receptor tyrosine kinase in the same family as macrophage colony-stimulating factor (M-CSF) receptor (M-CSFR), mast/stem cell growth factor receptor (SCFR/c-KIT/CD117), and platelet-derived growth factor receptors A and B (PDGFR-A and -B) that has one extracellular domain, five immunoglobulin folds, a juxtamembrane domain, and the cytoplasmic tyrosine kinase domain.4,6 Flt3 is expressed by hematopoietic stem cells,4,6 dendritic cells (DCs)7 and its signaling is crucial for hematopoiesis of CD34+ hematopoietic stem cells.8 Flt3L is a transmembrane type I protein that is a ligand for Flt3 and belongs to the same family as SCFR and M-CSFR ligands. Five extracellular domains, a transmembrane domain and a cytosolic tail construct this protein. Contrary to Flt3 receptor, most hematopoietic and non-hematopoietic tissues express Flt3L mRNA.7 The CD4+ T cells responsible for the hematopoietic source make Flt3L. Specifically, when CD4+ T cells are stimulated with anti-CD3ε and anti-CD28 antibodies, they secrete more Flt3L than CD8+ T cells.10 Flt3L has three main isoforms; one of them is cleavable membrane-bound Flt3L that can be converted to soluble Flt3L. The second form lacks a transmembrane domain and is a soluble protein. Lastly, the third is not cleavable and is membrane-tethered. Each form is found in different abundances among different species; however, all are biologically active. A membrane-bound version of Flt3L is the most common type observed in humans.8

3. DCs AND ANTIGEN PRESENTING

The DC is an important antigen-presenting cell.11 Through acquisition, processing, and presentation of the peptide of antigen, DCs trigger immune responses and activate T lymphocytes. It is possible to develop immunotherapies based upon the biology of DCs to combat infection, cancer, and autoimmune disease. Numerous studies have shown that Flt3 plays a significant role in DC maintenance and development. Thus, Flt3 is applied to manipulate DCs to produce high-efficiency immunotherapy in vitro and in vivo.6 DCs can take up dead tumor cells,12 pathogens, apoptotic cells, and infected cells and
process antigens derived from these particles into peptides, and load these peptides into major histocompatibility complex (MHC) class I and MHC class II molecules.\textsuperscript{11}

As part of MHC class II (HLA-II) antigen presentation, exogenously derived antigens must be processed and loaded into the endosomal/lysosomal pathway. Newly synthesized HLA class II α and β heavy chains dimerize and associate with the invariant chain (Ii or CD74).\textsuperscript{20} The cytoplasmic tail of Ii is then specifically targeted by endosomal proteases (cathepsins) and is degraded to a small component that is called class II-associated invariant chain peptide (CLIP). CLIP is converted with exogenous peptides by HLA-DM (HLA-like chaperone molecules). After peptide exchange, the HLA class II molecules are recruited onto the plasma membrane, where it is introduced to CD4\textsuperscript{+} T cells.\textsuperscript{12}

In myeloid DCs, it has been demonstrated that TAAs (tumor-associated anti-gens) can be taken up as exogenous material (for instance, tumor cell fragments) and presented by HLA class I molecules via a process known as cross-presenting\textsuperscript{12} and also, recently, studies have shown that antigen-presenting cells such as DCs can process endogenous antigens on HLA class II molecules, which is known as “reverse cross-presentation.”\textsuperscript{13}

4. FLT3 MUTATION AND DCS

\textsuperscript{21} In bone marrow and peripheral blood of AML patients, exhausted T cells have different phenotypes than those from normal controls. Not only T cells but also DCs and monocytes can express TIM-3.\textsuperscript{22} The autocrine or paracrine signaling pathways of TIM-3 promote leukemia cell proliferation and resistance to apoptosis.\textsuperscript{24} It is also secreted together with galectin-9, which blocks the activity of T-cells, contributing to immune evasion.\textsuperscript{22,23,25} The secreted form of TIM-3 may exert a distant inhibitory effect on immune cells away from the leukemia cells. Because the TIM-3 gene is located on chromosome 5, the aberrant TIM-3 mRNA level might be caused by chromosome 5 duplication seen in the karyotype of an AML patient. The TIM-3 transcript appears to be correlated with CLIP expression, this suggests that immune escape mechanisms are frequently activated simultaneously.\textsuperscript{20} Additionally, Flt3-ITD is linked to a higher positivity of CD4\textsuperscript{+} T cells for TIM-3, an immunosuppressive molecule.\textsuperscript{22,23} Kuželová et al showed that high levels of TIM-3 and CLIP are correlated with worse prognosis. To accomplish this, in immune resistant cells, inhibitory receptors are expressed, antigen presentation is reduced, as well as inhibitory molecules such as TIM3 are secreted (Fig. 1).\textsuperscript{20}

In recent days, there have been new therapies for treating AML patients, including improved chemotherapy, antibodies that
target immune checkpoints,2,3 cell cycle checkpoint inhibitors, epigenetic regulators, and microenvironment therapies.2 TIM-3 has been identified as an immune checkpoint target for both solid tumors and hematologic malignancies.26 A blockade of TIM-3 has been shown to improve the proliferation and activity of tumor antigen-specific T cells.27 The use of anti-TIM-3 antibodies, either alone or in combination with other drugs in clinical trials, has been found to be effective in treating a variety of cancers. Early clinical trials have been reported for three molecules as TIM-3 blockers so far: TSR-022 (Tesaro), LY3321367 (Eli Lilly and Company), and Sabatolimab (Novartis Pharmaceuticals; developed as MBG453).26 Flt3 TKIs (Flt3 tyrosine kinase inhibitors) have been demonstrated to inhibit the constitutive kinase activity of Flt3 mutations both in vitro and in vivo in preclinical and clinical studies. Zhu et al studied the combination of Flt3 TKIs (Gilteritinib or Sorafenib) with BCL-2 selective inhibitor (BCL-2i) that synergistically enhanced apoptosis and reduced cell proliferation in Flt3-ITD cell lines and primary AML samples.5 One of the potential therapeutic agents for AML is Flt3L. While it has been reported that Flt3L may cause lymphoproliferative malignancies with the described mutations of Flt3 in AML, no clinical trial has confirmed this issue when Flt3L has been administered to patients.28 The use of retroviral vectors expressing Flt3L has shown the stimulation of the immune system in some AML murine models29 despite the fact that it is a controversial therapy. Therefore, further clinical trials and research are needed to determine if Flt3L could be considered as a potential treatment for AML in humans.

7. CONCLUSIONS

Many studies have shown that AML patients have molecular mutations such as Flt3-ITD that are evident in their karyotypes. DCs as immune cells play significant roles in priming T cells and struggling with cancers, but in AML, most DCs and myeloid precursors are malignant. As a result of Flt3-ITD mutation, DC antigen presentation capacity is reduced and T cells present exhausted phenotypes. Leukemia cells express TIM-3, which may be used as a biomarker and therapeutic target for AML. However, some clinical trials have found that blocking TIM-3 alone does not provide clinical benefit for the majority of patients with AML. As Flt3-ITD is associated with a higher expression of TIM-3 on T cells, so combining immune therapies that target
both the Flt3 receptor and TIM-3 may prove more effective. Despite this, future clinical trials and research will be needed to prove the efficacy and safety of these immunotherapies. Flt3L, as the ligand of this receptor, is expressed on most hematopoietic and non-hematopoietic cells. Flt3L administration for AML remains controversial and hence, there is a need for more studies to confirm whether it can be used as a potential treatment for AML patients.

REFERENCES

[1] Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med 2015;373 (12):1136–1152.
[2] Wiener ES, Stone RM. Novel therapy in acute myeloid leukemia (AML): moving toward targeted approaches. Ther Adv Hematol 2019;10:2040620719860645.
[3] Grossmann V, Schnittger S, Kohlmann A, et al. A novel hierarchical prognostic model of AML solely based on molecular mutations. Blood 2012;120 (15):2963–2972.
[4] Sexauer AN, Tasian SK. Targeting Flt3 signaling in childhood acute myeloid leukemia. Front Pediatr 2017;5:248.
[5] Zhu R, Li L, Nguyen B, et al. FLT3 tyrosine kinase inhibitors synergize with BCL-2 inhibition to eliminate FLT3/ITD acute leukemia cells through BM activation. Signal Transduct Target Ther 2021;6 (1):1–11.
[6] Wilson KR, Villadangos JA, Mintern JD. Dendritic cell Flt3-regulation, roles and repercussions for immunotherapy. Immunol Cell Biol 2021.
[7] Miller JC, Brown BD, Shay T, et al. Deciphering the transcriptional network of the dendritic cell lineage. Nat Immunol 2012;13 (9):888–889.
[8] Small D, Levenstein M, Kim E, et al. STK-1, the human homolog of Flk-2/Flt-3, is selectively expressed in CD34+ human bone marrow cells and is involved in the proliferation of early progenitor/stem cells. Proc Natl Acad Sci 1994;91 (2):459–463.
[9] Lyman SD, James L, Bos TV, et al. Molecular cloning of a ligand for the Flt3/Flk-2 tyrosine kinase receptor: a proliferative factor for primitive hematopoietic cells. Cell 1993;75 (6):1157–1167.
[10] Chklovskaia E, Jansen W, Nissen C, et al. A novel hierarchical prognostic model of AML solely based on molecular mutations. Blood 2012;120 (15):2963–2972.
[11] Kuželová K, Brodská B, Marková J, et al. Associations between recurrent mutations and blast immunophenotype in acute myeloid leukemia. bioRxiv 2021.
[12] Van Luijn MM, van de Loosdrecht AA, Lampen MH, et al. Promiscuous binding of invariant chain-derived CLIP peptide to distinct HLA-A molecules revealed in leukemic cells. PLoS One 2012;7 (4):e34649.
[13] Li C, Chen X, Yu X, et al. TIM-3 is highly expressed in T cells in acute myeloid leukemia and associates with clinico-pathological prognostic stratification. Int J Clin Exp Pathol 2014;7 (10):6880.
[14] Van Luijn MM, Van de Loosdrecht AA, Lampen MH, et al. Promiscuous binding of invariant chain-derived CLIP peptide to distinct HLA-A molecules revealed in leukemic cells. PLoS One 2012;7 (4):e34649.
[15] Li C, Chen X, Yu X, et al. TIM-3 is highly expressed in T cells in acute myeloid leukemia and associates with clinico-pathological prognostic stratification. Int J Clin Exp Pathol 2014;7 (10):6880.
[16] Tan J, Yu Z, Huang J, et al. Increased PD-1+ Tim-3+ exhausted T cells in bone marrow may influence the clinical outcome of patients with AML. Biomark Res 2020;8 (1):1–9.
[17] Kikushige Y, Miyamoto T, Yada J, et al. A TIM-3/Gal-9 autocrine stimulatory loop drives self-renewal of human myeloid leukemia stem cells and leukemic progression. Cell Stem Cell 2015;17 (3):341–352.
[18] Zeidan AM, Komrokji RS, Brunner AM. TIM-3 pathway dysregulation in acute myeloid leukemia. Cell Stem Cell 2015;17 (3):341–352.
[19] Goonjalves Silva I, Rüegg L, Gibbs BF, et al. The immune receptor Tim-3 acts as a trafficker in a Tim-3/Gal-9 autocrine loop in human myeloid leukemia cells. Oncoimmunology 2016;5 (7):e1195353.
[20] Zeidan AM, Komrokji RS, Brunner AM. TIM-3 pathway dysregulation and targeting in cancer. Expert Rev Anticancer Ther 2021;21 (5):523–534.
[21] Gao X, Zhu Y, Li G, et al. TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression. PloS One 2012;7 (2):e30676.
[22] Karsunky H, Merad M, Cozzoio A, Weissman IL, Manz MG. Flt3 ligand regulates dendritic cell development from Flt3+ lymphoid and myeloid-committed progenitors to Flt3+ dendritic cells in vivo. J Exp Med 2003;198 (2):305–313.
[23] Audiger G, Lesage S. FLT3 ligand is dispensable for the final stage of type 1 conventional dendritic cell differentiation. J Immunol 2020;205 (8):2117–2127.
[24] Hosp M, Jarrossay D, Lafage-Pochitaloff M, et al. Circulating blood dendritic cells from myeloid leukemia patients display quantitative and cytogenetic abnormalities as well as functional impairment. Blood 2001;98 (13):3750–3756.
[25] Harrison BD, Adams JA, Briggs M, Berretton ML, Yin JAL. Stimulation of autologous proliferative and cytotoxic T-cell responses by “leukemic dendritic cells” derived from blast cells in acute myeloid leukemia. Blood 2001;97 (9):2764–2771.
[26] Panoskaltsis N. Dendritic cells in MDS and AML – cause, effect or solution to the immune pathogenesis of disease? Leukemia 2005;19 (3):354–357.
[27] Hetsh EM, Whitecar JP, McCreedie KB, Bodey GSP, Freireich EJ. Chemotherapy, immunocompetence, immunosuppression and prognosis in acute leukemia. N Engl J Med 1971;285 (22):1211–1216.
[28] Kuželová K, Brodská B, Marková J, et al. Associations between recurrent mutations and blast immunophenotype in acute myeloid leukemia. bioRxiv 2021.
[29] Van Luijn MM, Van de Loosdrecht AA, Lampen MH, et al. Promiscuous binding of invariant chain-derived CLIP peptide to distinct HLA-A molecules revealed in leukemic cells. PLoS One 2012;7 (4):e34649.
[30] Li C, Chen X, Yu X, et al. TIM-3 is highly expressed in T cells in acute myeloid leukemia and associates with clinico-pathological prognostic stratification. Int J Clin Exp Pathol 2014;7 (10):6880.
[31] Tan J, Yu Z, Huang J, et al. Increased PD-1+ Tim-3+ exhausted T cells in bone marrow may influence the clinical outcome of patients with AML. Biomark Res 2020;8 (1):1–9.
[32] Kikushige Y, Miyamoto T, Yada J, et al. A TIM-3/Gal-9 autocrine stimulatory loop drives self-renewal of human myeloid leukemia stem cells and leukemic progression. Cell Stem Cell 2015;17 (3):341–352.
[33] Zeidan AM, Komrokji RS, Brunner AM. TIM-3 pathway dysregulation in acute myeloid leukemia. Cell Stem Cell 2015;17 (3):341–352.
[34] Goonjalves Silva I, Rüegg L, Gibbs BF, et al. The immune receptor Tim-3 acts as a trafficker in a Tim-3/Gal-9 autocrine loop in human myeloid leukemia cells. Oncoimmunology 2016;5 (7):e1195353.
[35] Zeidan AM, Komrokji RS, Brunner AM. TIM-3 pathway dysregulation and targeting in cancer. Expert Rev Anticancer Ther 2021;21 (5):523–534.
[36] Gao X, Zhu Y, Li G, et al. TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression. PloS One 2012;7 (2):e30676.
[37] Karsunky H, Merad M, Cozzoio A, Weissman IL, Manz MG. Flt3 ligand regulates dendritic cell development from Flt3+ lymphoid and myeloid-committed progenitors to Flt3+ dendritic cells in vivo. J Exp Med 2003;198 (2):305–313.
[38] Audiger G, Lesage S. FLT3 ligand is dispensable for the final stage of type 1 conventional dendritic cell differentiation. J Immunol 2020;205 (8):2117–2127.
[39] Hosp M, Jarrossay D, Lafage-Pochitaloff M, et al. Circulating blood dendritic cells from myeloid leukemia patients display quantitative and cytogenetic abnormalities as well as functional impairment. Blood 2001;98 (13):3750–3756.
[40] Harrison BD, Adams JA, Briggs M, Berretton ML, Yin JAL. Stimulation of autologous proliferative and cytotoxic T-cell responses by “leukemic dendritic cells” derived from blast cells in acute myeloid leukemia. Blood 2001;97 (9):2764–2771.