Sugar-free transforming growth factor β1 increases the fitness of myelodysplastic neoplasm/acute myeloid leukemia cells

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Myelodysplastic neoplasms (MDS) are clonal disorders driven by somatic genetic aberrations accumulating in hematopoietic stem and progenitor cells (HSPC). Increasing evidence indicates a contribution of the bone marrow microenvironment (BMME) to the emergence of MDS and their evolution toward acute myeloid leukemia (AML). The molecular mechanisms driving interactions between genetically altered hematopoietic cells and their BMME are being increasingly deciphered.[1,2] One of them is a deregulation of cellular interactions through post-translational modification of proteins such as glycosylation, especially when it affects membrane-bound and secreted proteins.[3]

In this issue of *Haematologica*, Feng *et al*. involve an increased secretion of transforming growth factor β1 (TGFβ1) by mesenchymal stromal cells of the bone marrow niche (BMMSC) in myeloid leukemia cell expansion through generating an immunosuppressive niche. The depicted mechanism involves a decrease in bisecting N-acetylglucosamine (GlcNAc) structures in some BMMSC. The decreased glycosylation of TGFβ1 prevents its lysosomal degradation. The cytokine, secreted in excess, reshapes macrophages that, in turn, alter the adaptive immune response.[4]

The starting point of this study is the immunosuppressive phenotype of bone marrow macrophages in patients with MDS or AML, as identified by single cell RNA sequencing and immuno-phenotyping. Feng *et al*. correlate the presence of macrophages expressing CD163, MRC1 (mannose receptor C-type 1), arginase-1 and PD-L1 (programmed death-ligand 1) with a decreased number of $CD8^+$ T cells.[3]

The authors explore the role of BMMSC in this phenotype. They set up co-culture experiments using primary BMMSC collected from healthy donors (HD) and MDS/AML patients, and monocyte-derived macrophages or phorbol 12-myristate 13-acetate (PMA)-treated THP1 leukemic cells as substitutes of monocyte-derived macrophages. In the presence of patient-derived BMMSC, these cells acquire some markers of immunosuppressive macrophages. When added to these co-cultures, activated $CD8^+$ T cells demonstrate reduced secretion of tumor necrosis factor (TNF) and gamma interferon (IFN-γ).

A proteomic analysis of BMMSC identifies the latency-associated protein-TGFβ1 (L-TGFβ1) as one of the 29 proteins up-regulated in MDS/AML patient BMMSC, correlating with an increased secretion of mature TGFβ1. In co-culture experiments described above, recombinant TGFβ1 (rTGFβ1) mimics the effects of patient BMMSC, i.e., rTGFβ1 promotes an increase in CD163 and PD-L1 expression by PMA-treated THP1 cells and decreases the secretion of TNF and IFN-γ by activated $CD8^+$ T cells.

These effects are validated by *in vivo* experiments in which a GFP-labeled murine leukemic cell line is co-transplanted with rTGFβ1-treated macrophages in bone marrow macrophage-depleted mice, which promotes leukemic cell expansion. Importantly, these *in vivo* effects, that include a decrease in CD8 T-cell number and TNF / IFN-γ plasma levels, are prevented by an anti-PD-L1 therapy.

At this step, the authors connect with their recently reported study in which they identified a decrease in bisecting GlcNAc structures in BMMSC of MDS/AML patients.[5] They show that modulation of bisecting GlcNAc expression in BMMSC interferes with TGFβ1 secretion, macrophage polarization and activated CD8 T-cell functions in co-culture experiments. One thing leading to another, the authors combine mixed anion exchange (MAX) and liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) to identify a Glc-NAc-modified peptide on L-TGFβ1.

Asparagine 176 is shown to be the primary site of Glc-NAc modification of L-TGFβ1. This modification is dramatically

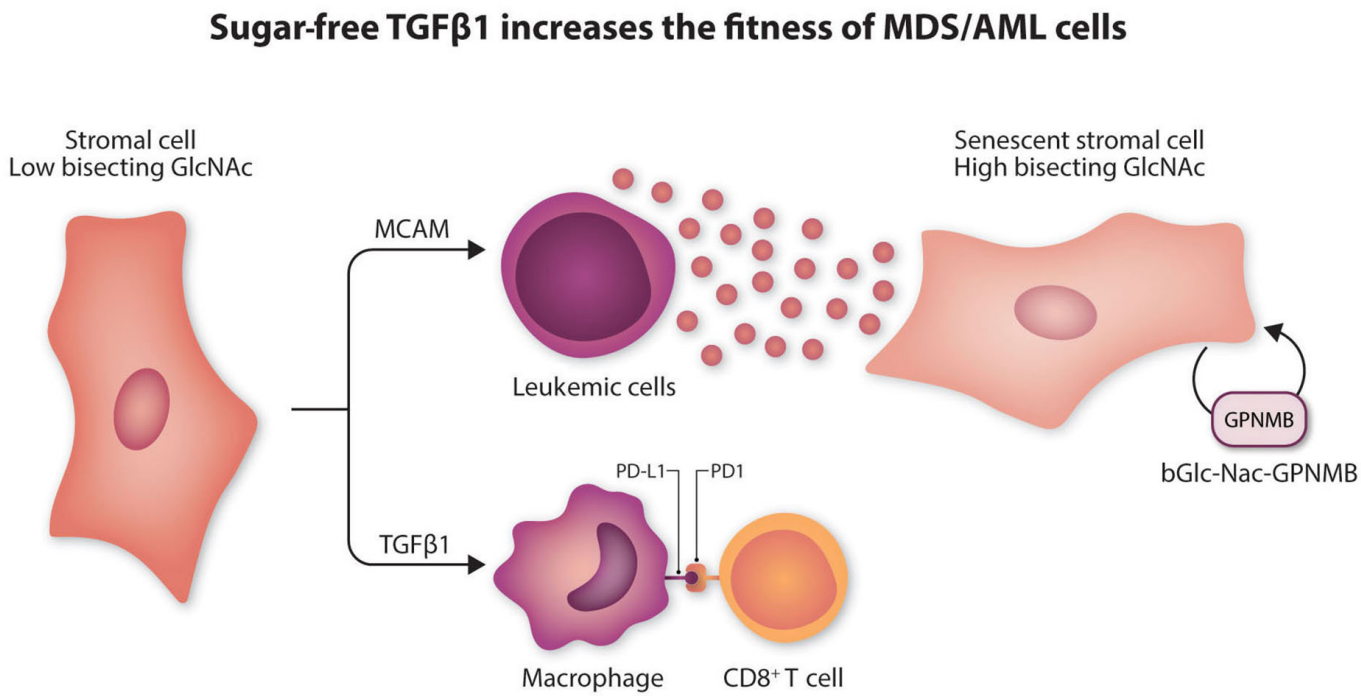

Figure 1. Heterogeneous features of bone marrow mesenchymal stromal cells in myelodysplastic neoplasms / acute myeloid leukemia patients. Bisecting GlcNAc is decreased in a fraction of bone marrow mesenchymal stromal cells (BMMSC), modifying the glycosylation of various proteins including MCAM at the cell surface, interacting with CD13 at the surface of myeloid leukemia cells, and TGFβ1 whose secretion is increased, generating PD-L1 expressing macrophages and reducing activated $CD8^+$ T-cell functions. Senescent BMMC release in extracellular vesicules GPNMB with increased bisecting GlcNAc modification that, in turn, amplifies BMMSC senescence and the associated release of inflammatory proteins. MCAM: melanoma cell adhesion molecule; GPNMB: glycoprotein non-metastatic melanoma protein B; bGlcNAc: bisecting GlcNAc; TGFβ1: transforming growth factor β1.

reduced when the N176D mutant of L-TGFβ1 is expressed in a murine stromal cell line. In turn, the secretion of the mature protein increases and reproduces the immunomodulating effects of patient BMMSC in co-culture and in the above-described mouse model. The authors also identify an interaction of asparagine 176 with the small GTPase protein Rab7, a late endosomal and phagosomal protein that modulates the transport of proteins to lysosomes. Deletion of Rab7 promotes TGFβ1 secretion by BMMSC.

Together, interaction of bisecting GlcNAc and Rab7 with asparagine 176 of L-TGFβ1 may promote the lysosomal degradation of the protein in healthy BMMSC, a mechanism altered by the decrease in bisecting GlcNAc in BMMSC of myeloid leukemia patients, leading to an increased secretion of mature TGFβ1. In their previous report, these authors incriminated a toxic interaction between leukemia cells and BMMSC in the decrease in bisecting GlcNAc structures detected in the later cells. Leukemia cell-derived exosomes dialog with BMMSC by delivering miR-188-5p that down-regulates *MGAT3* (β-1,4-mannosyl-glycoprotein 4-β-N-acetylglucosaminyl transferase) gene expression.[5]

In addition to phosphorylation, acetylation and ubiquitination, glycosylation is a common post-translational modification of proteins that regulates their folding, localization, trafficking, stability, and activity. Glycan structures are diverse and dynamically regulated. In contrast with single O-glycosylation, that targets serine and threonine residues of intracellular proteins,[6] N-glycosylation mostly targets an asparagine residue in a membrane-bound or a secreted protein. N-glycosylation generates a variety of complex structures. Bisecting GlcNAc, biosynthesized by a glycosyltransferase encoded by the *MGAT3* gene, is a subtype involved in the fine regulation of N-glycan structures.[7] Another Glc-NAc-bearing protein identified by Feng *et al*. as being altered in MDS/AML BMMSC is CD146 (also known as melanoma cell adhesion molecule [MCAM]) expressed at the surface of stromal cells. Modification of MCAM glycosylation increases stromal cell interaction with CD13 at the surface of myeloid leukemia cells.[5]

The age-associated accumulation of senescent stromal cell secreting pro-inflammatory proteins may contribute to the transition of clonal hematopoiesis to overt MDS or AML.[2] Interestingly, senescent BMMSC also release extracellular vesicles containing the transmembrane protein GPNMB (glycoprotein non-metastatic melanoma protein B) in a bisecting GlcNAcylated form that further amplifies their senescent phenotype in an autocrine or paracrine loop,[8] indicating that deregulated glycosylation can also promote disease evolution through increasing bisecting GlcNAc structures in some BMMSC.

Together, these results illustrate the heterogeneity of stromal cells. BMMSC subsets with increased or decreased bisecting GlcNAc structures may diversely contribute to myeloid leukemia cell expansion through modifying various proteins (Figure 1), illustrating the complexity of these regulatory mechanisms.[3,9] Further study will bet-

ter decipher BMMSC heterogeneity in MDS/AML patients and evaluate the contribution of abnormally glycosylated proteins released by these cells to myeloid leukemia cell expansion. The objective will be to determine if these changes deserve to be targeted therapeutically in an attempt to control MDS/AML progression.

Disclosures

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