Revealing the inner workings of human HSC adhesion

Cristina Lo Celso

In this issue of Blood, Rak et al identify cytohesin 1 (CYTH1) as an important intracellular mediator of human hematopoietic stem cell (HSC) adhesion that plays a role in HSC homing and lodging in the bone marrow and subsequent engraftment. This finding contributes to our understanding of the mechanisms involved in regulating HSC-niche interaction, which we know is critical in regulating HSC function.

Much effort over the years has revealed many cell surface players responsible for the ability of HSCs to interact with the bone marrow microenvironment. Integrins in particular have emerged as critical players in the adhesion of HSCs to the extracellular matrix, with integrin β1 (ITGβ1) knockout leading to severe homing defects. However, very little is known about the intracellular players that link integrin activation to HSC migration and localization.

CYTH1 is a guanine-nucleotide exchange factor for multiple guanosine triphosphate (GTP)-binding proteins. Known to form a complex with ITGβ1 and integrin αL to mediate adhesion to ICAM1, it was recently shown to cooperate with ITGβ2 in neutrophils, leading to Rho activation in dendritic cells, and regulate migration of natural killer cells. Still highly understudied in the hematopoietic system, CYTH1 was selected by the authors as the top hit of an original shRNA library in vitro screening. Rak and colleagues reasoned that the traditional approach of separating hematopoietic stem and progenitor cells (HSPCs) adhering to stroma by means of washing the medium is far from physiological because it introduces high shear stress, and decided to take advantage of gravity, which naturally acts on all cells in any organism, to identify genes that precisely regulate the adhesion properties of human HSPCs. Using the analogy of a car, which moves thanks to the interaction of the wheels with the road, while the steering wheel determines the direction of movement, this screening allowed the identification of wheel components, and the ensuing study focused on a factor akin to the wheels’ axis. All top hits identified, including CD90, ITGα5, MMRN1, NEDD9, PPFIA1, and ROBO1 had previously been described as components of cellular molecular motors. Importantly, known genes involved in HSPC localization but acting through different mechanisms (eg, chemotaxis in the case of CXCR4), were not highlighted by this screening.

In this study, CYTH1 is knocked down via shRNA in human HSPCs, resulting in their reduced adhesion to both retronectin (mediated by ITGβ1) and ICAM1 (mediated by ITGβ2) and reduced integrin activation in vitro assays. When human HSCs deficient in CYTH1 are challenged to engraft in immunodeficient NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ (NSG) mice, they show reduced ability to home to the bone marrow, lodge within the parenchyma, and drive efficient long-term engraftment. A member of another family of guanine exchange factors and linked to the regulation of Rho GTPases, Vav1, was previously shown to contribute to murine HSPC localization in the bone marrow and subsequent engraftment, with Vav1 knockout HSPCs showing a phenotype similar to that of the human cytohesin-deficient HSPCs in the Rak et al study. Rak et al take advantage of more recently developed time-lapse intravital microscopy of engrafting HSPCs to unravel the mechanism behind the observed aberrant HSPC localization. Fluorescently labeled CYTH1-deficient HSPCs were injected in NSG recipient mice, and intravital microscopy revealed that most cells found 4 days later were unable to settle in the marrow parenchyma in the same way as control cells.

The homing and lodgment defects observed are not as severe as the engraftment reduction, and the low levels of engrafted hematopoiesis show balanced differentiation. The cell cycle profile of cytohesin-deficient cells is unaffected, suggesting that a fraction of the observed HSPCs are able to engraft. This raises the questions of whether the nonengrafting HSPCs prematurely differentiate or die, whether the few engrafting HSPCs are those showing localization and migration more similar to control cells, and what is the differentiation and functional state of both control and knockdown cells observed. Even though CYTH1 is efficiently knocked down in all HSPCs assessed through functional studies, some engraftment is obtained. This study reveals that most cells found 4 days later were unable to settle in the marrow parenchyma in the same way as control cells.

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highly defined and uniform compensatory mechanisms or is the result of stochastic and variable activation of other components of the HSPCs’ molecular motors.

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REFERENCES


PNH is a clonal hematopoietic stem cell disease associated with a complement-dependent hemolytic anemia. The disease manifests after the expansion of a multipotent hematopoietic stem cell that acquires a mutation of the PIGA gene. The PIGA gene product is required for the biosynthesis of glycoporphatidylinositol anchors, a glycolipid moiety that attaches dozens of proteins to the plasma membrane of cells. Thus, all blood cells derived from the PIGA mutant stem cell, including mature red blood cells, are missing glycoporphatidylinositol–anchor proteins. Two of these proteins, CD55 and CD59, are complement regulatory proteins. CD55 inhibits C3 convertases, and CD59 blocks formation of the membrane attack complex (MAC) by inhibiting incorporation of C9 into the MAC. The loss of CD55 and CD59 renders PNH erythrocytes susceptible to intravascular hemolysis. Eculizumab is a humanized monoclonal antibody that blocks terminal complement by binding to C5 and is the treatment of choice for PNH. Eculizumab virtually eliminates the intravascular hemolysis in PNH patients as evidenced by the rapid fall in lactate dehydrogenase levels after administration of the drug. Through steric hindrance, the antibody protects C5 from cleavage by the C5 convertase and thus inhibits terminal complement activity by decreasing the generation of C5a and C5b. It therefore inhibits terminal complement and compensates for the CD59 deficiency. Eculizumab is a first-in-class, breakthrough drug for PNH that prevents thrombosis (the leading cause of death in PNH), improves quality of life, and often eliminates the need for blood transfusions; however, most patients continue to have low level hemolysis and mild to moderate anemia. Until now, most of residual hemolysis in patients with PNH on eculizumab was attributed to extravascular hemolysis. Previous work has shown that PNH red cells in patients on eculizumab often accumulate C3 fragments because eculizumab blocks complement at C5 (upstream of CD59 but downstream of CD55). ECD55 (also known as decay accelerating factor) shortens the half-life of C3 by acting on the C3 convertases. Because PNH red cells are missing CD55, these C3 fragments accumulate and lead to opsonization and destruction of the PNH red cells in the spleen. Importantly, when patients with PNH on eculizumab acquire bacterial or viral infections, they frequently develop “breakthrough” intravascular hemolysis accompanied by worsening anemia, a significant rise in lactate dehydrogenase levels, hemoglobinuria, and a return of their PNH symptoms. The intravascular hemolysis typically resolves with clearance of the infection. The mechanism of breakthrough hemolysis, presumed to be complement mediated, was not entirely clear.

Harder et al performed a series of elegant ex vivo experiments to model breakthrough hemolysis of PNH red cells in the setting of C5 inhibition and to improve our understanding of the mechanism of action of eculizumab. The authors demonstrate that residual lytic activity of the terminal pathway of complement depends on the strength of the complement activator and the resulting surface density of C3b, which amplifies the alternative pathway through its potent amplification loop (see figure). They also show that at high densities of C3b on red cells, C5 inhibitors such as eculizumab and the tick-derived C5 inhibitor OmcI (Coversin) reduce but do not abolish terminal complement activity. Thus, C5 inhibitors stabilize the unprimed C5 conformation, making it much less susceptible to cleavage by convertases. C5 is more susceptible to cleavage under conditions of more intense priming due to excess C3b on the red cells, even in the presence of a C5 inhibitor. Interestingly, a combination of eculizumab and Coversin, which bind to different epitopes of C5, has additive effects and together can abolish residual lytic activity even in the presence of strong activation. Similarly, combining eculizumab with drugs or conditions that inhibit the amplification loop of
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