Below the NK-cell surface: PIP2

Marco Colonna WASHINGTON UNIVERSITY SCHOOL OF MEDICINE AT ST LOUIS

In this issue Blood, Micucci and colleagues demonstrate that PIP2 plays an essential role in NK–cell cytotoxicity by controlling the release of lytic mediators at the NK cell–target interface.

Membrane phospholipids are essential for a variety of cellular functions. They act as second messengers that couple engagement of cell-surface receptors with recruitment and activation of downstream intracellular molecules, and are also involved in cell adhesion, motility, actin cytoskeleton dynamics, phagocytosis, vesicle trafficking, endocytosis, and exocytosis.1-4 Despite their established physiological relevance, membrane phospholipids have generally been a rather obscure topic in the context of NK–cell function. Now, Micucci and colleagues bring phosphatidylinositol 4,5-bisphosphate (PIP2) to the forefront of NK-cell biology in this issue of Blood.

PIP2 is the precursor of 2 second messengers that are indispensable for cellular signaling—inositol 1,4,5-trisphosphate (IP3), which mobilizes Ca2+ from intracellular stores, and diacylglycerol (DAG), which activates protein kinase C (PKC). Cleavage of PIP2 into IP3 and DAG is mediated by members of the phospholipase C (PLC) family.5 PIP2 also interacts with the actin-binding proteins gelsolin and profilin, as well as with many other signaling molecules that contain pleckstrin homology (PH) domains.1-4 To visualize PIP2, Micucci et al transfected a human NK-cell line with a lentivirus encoding a chimeric PLCγ1-GFP reporter to the forefront of NK-cell biology in this issue of Blood.

To corroborate the assumption that PIP2 is required for NK cell–mediated cytotoxicity, Micucci and colleagues analyzed the effect of blocking PIP2 synthesis. PIP2 is generated from phosphatidylinositol 4-phosphate (PIP4P) by PI4P-5 kinases.6 Of the 3 PI4P-5 kinase isoforms that have been identified, NK cells preferentially express PI4P-5K1α and PI4P-5K1β. Therefore, to effectively prevent generation of PIP2, Micucci et al inhibited expression of PI4P-5K1α or PI4P-5K1β by short hairpin RNA (shRNA). Downregulation of either enzyme impaired lysis of tumor targets by NK cells, whereas secretion of IFNγ was unaffected. NK cell–mediated cytotoxicity is a sequential process involving conjugation of NK cells with the target cell, polarization of lytic granules toward the target cell, and exocytosis of the lytic mediators. Micucci and colleagues found that PIP2 is selectively required for exocytosis of lytic granules. In a previous study, the same group demonstrated that NK cell–mediated cytotoxicity requires the small GTPase ARF6,7 which is restricted function within the cell. Thus, just beneath the NK-cell surface, we begin to see the murky world of membrane phospholipids coalesce into functionally meaningful patterns.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES

AML1 and Evi1: coconspirators in MDS/AML?

Timothy Graubert WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

In this issue of Blood, Watanabe-Okochi and colleagues use a mouse bone marrow transplantation model to demonstrate that mutant alleles of AML1 (RUNXI) can initiate a myelodysplastic syndrome (MDS) that progresses to acute myelogenous leukemia (AML) in association with overexpression of Evi1.

Comment on Watanabe-Okochi et al, page 4297
The molecular pathogenesis of MDS and the mechanism of its transformation to AML are not well understood. Although several mouse models of MDS have been developed recently, most fail to recapitulate key features of the human disease. In one of the best genetically defined systems, transplantation of bone marrow cells retrovirally transduced with \(Evi1\) results in bone marrow failure, erythroid dysplasia, and evidence of increased apoptosis.\(^1\) Despite reproducing these hallmarks of MDS, the mice do not develop AML, suggesting that additional genetic events are required for full transformation.

The \(AML1\) gene is a frequent target of mutations and rearrangements in human leukemia. Although point mutations of \(AML1\) are rare in most AML French-American-British (FAB) subtypes, they are relatively common in M0 AML (12\%-33\%), MDS (23\%), and in therapy-related and radiation-associated MDS/AML (38\%-46\%).\(^2\) In this issue of Blood, Watanabe-Okochi and colleagues use a murine retroviral transduction/bone marrow transplantation model to characterize 2 mutant \(AML1\) alleles previously identified by their group: a D171N missense mutation in the C-terminal transactivation domain, and a frame shift after S291 that results in truncation of the \(Evi1\) gene.\(^3,4\) Although the mechanism of \(Evi1\) activation is artificial in this model (retrovirus-mediated insertional mutagenesis), the observations by Watanabe-Okochi and colleagues have important clinical relevance. \(EVI1\) is deregulated by the \(t(3;3)(q21;q26)\) and \(inv(3)(q21;q26)\) rearrangements in MDS and AML.\(^3,4\) Several lines of evidence have also directly linked \(AML1\) and \(EVI1\) in human leukemia biology. First, the genes are fused by the \(t(3;21)(q26;q22)\) rearrangement in blast-crisis CML.\(^5\) In addition, the proteins physically interact, leading to reduced \(AML1\) DNA binding and transcriptional activity.\(^6\)

This leads to the prediction, not tested in the current study, that \(EVI1\) and the \(AML\)D171N allele cooperate in vivo because they conspire to reduce \(AML1\) activity. If further studies support this model, there would be a rational basis for developing therapies that target this protein–protein interaction.

**REFERENCES**


---

**Osteopoietic stem cells: transplantable, but regeneratively limited**

**Susie Nilsson** AUSTRALIAN STEM CELL CENTRE

Questions about the transplantability of mesenchymal stem cells, their ability to engraft within the bone marrow of recipients, and hence their clinical usefulness have been hotly debated for several decades. In this issue of Blood, Domini and colleagues demonstrate robust serial osteopoietic engraftment, but highlight that osteopoietic chimerism declines to negligible levels after 6 months.

Due to the presence of hematopoietic stem cells in the bone marrow, bone marrow transplantation has been successfully used to treat hematological diseases for many decades. Based on the concept that bone marrow also contains microenvironmental or mesenchymal stem cells (MSCs) with osteopoietic potential, the clinical use of bone marrow transplantation to treat bone disorders is also very appealing. Although initial studies suggested that MSCs cannot be transplanted intravenously, more recently documented animal models and preclinical studies have demonstrated that donor MSCs engraft in the bone marrow and give rise to bone and muscle.\(^1,4\) Furthermore, a series of clinical trials performed by this group,\(^2,6\) involving children with severe osteogenesis imperfecta, demonstrated that transplanting unmanipulated bone marrow resulted in a marked improvement in total body mineral content, accelerated linear growth, and decreased fracture rates. Similar results were evident following transplantation of purified MSCs. In all of these studies, however, the observed accelerated growth diminished over time.
AML1 and Evi1: coconspirators in MDS/AML?

Timothy Graubert