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631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

Newly Identified Roles for PIEZO1 Mechanosensor in Controlling Normal Megakaryocyte Development and in Primary Myelofibrosis

Iris Karkempetzaki[1,2], *Vittorio Abbonante, PhD*[3], *Catherine Leon, PhD*[4], *Nasi Huang, MD*[1], *Christian Di Buduo, PhD*[3], *Daniele Cattaneo, MD*[5], *Christina Ward*[1], *Shinobu Matsuura, PhD DVM*[6], *Alessandra Iurlo, MD PhD*[7], *Alessandra Balduini, MD*[8,3], *Katya Ravid, Dsc*[6,1]

[1] Whitaker Cardiovascular Institute, Boston University Chobanian and Avedisian School of Medicine, Boston, MA
[2] Department of Internal Medicine, University of Crete, School of Medicine, Heraklion, Greece
[3] University of Pavia, Pavia, ITA
[4] Université de Strasbourg, Strasbourg, FRA
[5] Hematology, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milano, Italy
[6] Cardiovascular Section/Department of Medicine, Boston University Chobanian and Avedisian School of Medicine, Boston, MA
[7] Hematology Division, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
[8] Tufts University, Medford, MA

Introduction: Mechanisms through which mature megakaryocytes (Mks) and their progenitors sense the bone marrow extracellular matrix (ECM) to promote lineage differentiation in health and disease are still partially understood. We found PIEZO1, a mechanosensitive cation channel, to be expressed in Mks. Human mutations in PIEZO1 have been described to be associated with blood cell disorders. Primary Myelofibrosis (PMF) is a Chromosome Philadelphia-negative Myeloproliferative Neoplasm (MPN), characterized by altered stiffer ECM and megakaryocytosis, often driven by the gain-of-function mutation in JAK2 ^{V617F}. Here, we study a role for PIEZO1 in megakaryopoiesis and proplatelet formation under normal physiological conditions and in the context of PMF.
Methods: Bone marrow Mks from C57BL/6J control and myelofibrotic mice carrying the human JAK2 $^{V617F+}$ mutation, or Mks derived from stem cells of patients carrying the same mutation were analyzed at mRNA and protein levels for Piezo1 expression. Effects of Piezo1 activation or inhibition on Mk maturation and ploidy were assessed by flow cytometry, and proplatelet formation by fluorescent microscopy. Human peripheral blood samples from PMF patients, diagnosed according to established criteria, or healthy individuals were used to compare the two categories for Piezo1 relative expression on mRNA and protein levels and platelet biogenesis. Mk-specific double knockout mice of *Piezo1/2* were generated to compare platelet counts under normal conditions or bone marrow ablation with 5-FU challenge. Culture of mouse and human Mks in different 3D microenvironments was used to demonstrate in vitro association of Piezo1 expression levels with different ECM stiffness substrates.
Results: Mouse Mks express PIEZO1 and negligible levels of PIEZO2. Pharmacological activation of mouse PIEZO1 increases the number of immature CD41 $^{+}$ Mks, while the number of mature CD41 $^{+}$CD42 $^{+}$ Mks and Mk ploidy level are reduced. Piezo1/2 knockout mice show an increase in Mk size and platelet count, both at basal state and upon marrow regeneration. Similarly, in human samples, PIEZO1 is expressed during megakaryopoiesis. Its pharmacological activation reduces Mk size, ploidy, maturation, and proplatelet development. Resulting effects of PIEZO1 activation on Mks resemble the profile in PMF. Intriguingly, Mks derived from PMF mice bearing the *Jak2* ^{V617F} mutation show significantly elevated PIEZO1 expression, compared to wild-type controls. Pharmacological activation of PIEZO1 in these cells significantly augments the number of immature CD41 $^{+}$ Mks, and sharply reduces the number of mature CD41 $^{+}$CD42 $^{+}$ cells and ploidy level. Accordingly, Mks isolated from bone marrow aspirates of *JAK2* ^{V617F}PMF patients show increased PIEZO1 expression compared to Essential Thrombocythemia. Additionally, PIEZO1 was overexpressed in 3D culture of human and mouse Mks in stiffer microenvironments as compared to softer ones and liquid medium. Most importantly, PIEZO1 expression in bone marrow Mks is inversely correlated with patient platelet count. The ploidy, maturation, and proplatelet formation of Mks from *JAK2* ^{V617F}PMF patients are rescued upon PIEZO1 inhibition with GsMTx4.

Discussion: Our data show that PIEZO1 might serve as a break of Mk maturation and platelet formation in physiology, and its upregulation in PMF Mks might contribute to aggravating some hallmarks of the disease. Our finding that inhibition of PIEZO1 activity partially rescues the aberrant Mk phenotype associated with PMF suggests that GsMTx4 holds potential as a therapeutic strategy to mitigate the pathological effects observed in Mk development in PMF patients.

Disclosures Cattaneo: *Novartis, Pfizer, Incyte, BMS, GSK:* Honoraria. **Iurlo:** *Novartis, Pfizer, Incyte, BMS, GSK, AOP Health:* Honoraria.

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