EUROPEAN HEMATOLOGY ASSOCIATION

EHA2021 VIRTUAL

S198

IDENTIFICATION AND PHENOTYPIC CHARACTERIZATION OF DISEASE-INITIATING CD34+/CD38− STEM CELLS IN BCR-ABL1-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

Topic: 15. Myeloproliferative neoplasms - Biology & Translational Research

Keywords: Essential Thrombocytemia, Myelofibrosis, Polycythemia vera, Stem cell marker

Daniel Ivanov[1,2], Jelena Milosevic Feenstra[2], Alexandra Keller[1,2], Gregor Eisenwort[1,2], Irina Sadovnik[1,2], Harald Herrmann[2,3], Barbara Peter[1,2], Michael Willmann[2,4], Georg Greiner[2,5,6], Gabriele Stefanzl[1,2], Katharina Slavnitsch[2,7], Gregor Hoermann[2,8], Emir Hadzijusufovic[1,2,4], Wolfgang R. Sperr[1,2], Peter Bettelheim[9], Klaus Geissler[10], Elisabeth Koller[11], Michael Fillitz[11], Thamer Sliwa[11], Michael Pfeilstöcker[2,11], Felix Keil[2,11], Thomas Rülicke[2,7], Robert Kralovics[5,12], Peter Valent[1,2]

[1] Department of Medicine I, Division of Hematology and Hemostaseology, Medical University of Vienna, Vienna, Austria
[2] Ludwig Boltzmann Institute for Hematology and Oncology, Medical University of Vienna, Vienna, Austria
[3] Department of Radiation Oncology, Medical University of Vienna, Vienna, Austria
[4] Department for Companion Animals & Horses, Clinic for Internal Medicine and Infectious Diseases, University of Veterinary Medicine Vienna, Vienna, Austria
[5] Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria
[6] Ihr Labor, Medical Diagnostic Laboratories, Vienna, Austria
[7] Institute of Laboratory Animal Science, University of Veterinary Medicine Vienna, Vienna, Austria
[8] MLL Munich Leukemia Laboratory, Munich, Germany
[9] Elisabethinen Hospital, Linz, Austria
[10] Medical School, Sigmund Freud University, Vienna, Austria
[11] Third Medical Department for Hematology and Oncology, Hanusch Hospital, Vienna, Austria
[12] CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Vienna, Austria

Background: The classical Philadelphia chromosome-negative myeloproliferative neoplasms (MPN) are characterized by uncontrolled expansion of myeloid progenitor cells, elevated blood counts, phenotype-driver mutations in certain target-genes (*JAK2*, *CALR*, *MPL*) and an increased risk of leukemic transformation. Despite being considered stem-cell disorders, the phenotype of the disease-initiating neoplastic stem cells (NSC) in MPN remains unknown.

Aims: The aim of the study is to identify MPN-initiating NSC and characterize their phenotype in order to facilitate their enrichment and develop novel treatment strategies.

Methods: As assessed by multicolor flow cytometry and a panel of monoclonal antibodies (n=50), we were able to define target expression profiles of putative $CD34^+/CD38^-$ NSC and $CD34^+/CD38^+$ progenitor cells in patients with chronic phase MPN (n=93) and post-MPN secondary acute myeloid leukemia (sAML, n=11). To confirm the disease-initiating ability of primary MPN NSC, xenotransplantation experiments with NSGS mice were performed.

Results: In almost all patients, the MPN NSC expressed several receptors involved in NSC-niche interaction, including CD97, CD99, CD117 and CD184. In contrast, NSC expressed CD26 only in a subset of patients with primary myelofibrosis (PMF). MPN NSC expressed significantly higher levels of CD184 compared to stem cells in healthy controls ($p<0.0001$) or sAML ($p<0.01$). Among the cytokine receptors tested, CD25 was identified on NSC in most patients with PMF and sAML, and in a few with essential thrombocythemia (ET), but not in patients with polycythemia vera (PV). PMF NSC also displayed higher levels of CD25 than the PMF progenitor cells ($p<0.01$). MPN NSC did not exhibit substantial amounts of IL-1RAP and erythropoietin receptor. We next examined the expression of various immunological targets and resistance-mediating immune checkpoints. In all patients tested, NSC were found to express CD33. We also found that $CD34^+/CD38^+$ progenitor cells in ET display higher levels of CD33 than NSC ($p<0.01$). However, neither NSC nor the progenitor cells in MPN expressed higher levels of CD33 compared to normal stem cells or normal progenitor cells. MPN NSC and sAML stem cells consistently expressed

the “don’t eat me” checkpoint CD47 and the classical checkpoint CD274 (PD-L1). MPN NSC displayed significantly higher levels of CD274 compared to normal stem cells ($p<0.001$) or stem cells in sAML ($p<0.05$). No difference in the expression of surface antigens on MPN NSC were observed between patients harboring mutations in the *JAK2* and *CALR* genes. Engraftment of human $CD45^+CD33^+$ cells in the bone marrow of NSGS mice was found in 15/15 mice injected with bulk mononuclear cells (MNC) containing $CD34^+$ cells and in 0/15 NSGS mice injected with CD34-depleted MNC after 28 weeks. Whereas the $CD38^-$ sub-fraction of MPN MNC produced leukemic engraftment in 10/14 mice (71.4%) only 2/13 mice (15.4%) engrafted when injected with the $CD38^+$ sub-fractions of MPN NSC. Engraftment of clonal MPN cells was confirmed by demonstrating the presence of *JAK2*-V617F mutation in engrafted human cells in mouse BM samples.

Summary/Conclusion: Together, we show that MPN NSC reside in a $CD34^+/CD38^-$ fraction of the malignant clone and display a unique phenotype, including immune checkpoint molecules, cytokine receptors and other target antigens. Our results should support the isolation of MPN NSC and the development of NSC-eradicating therapies in MPN.

Abstract Book Citations: Authors, Title, HemaSphere, 2021;5:(S2):pages. Abstract Book, DOI: http://dx.doi.org/10.1097/HS9.0000000000000566