ELSEVIER

Blood 142 (2023) 1786–1787

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

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

Role of *Lama4* Expression in Bone Marrow Niche for the Progression and Treatment Response of Chronic Myeloid Leukemia

Alma Mansson[1], *Huan Cai, PhD*[2], *Juan Pablo Medina Giménez*[1], *Leif Stenke, MDPhD*[3], *Hong Qian, PhD*[2]

[1]Center for Hematology and Regenerative Medicine (HERM), Department of Medicine, Huddinge, Karolinska Institute, Stockholm, Sweden
[2]Center for Hematology and Regenerative Medicine (HERM), Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden
[3]Department of Medicine, Solna, Karolinska Institutet, Stockholm, SWE

Although tyrosine kinase inhibitors (TKIs) have dramatically improved the treatment outcomes of chronic myeloid leukemia (CML), TKIs cannot eliminate CML-initiating stem cells (LSCs), leading to CML persistence and relapse post TKI-discontinuation. Thus, it is imperative to develop more effective therapeutic options to overcome the treatment challenges. Targeting the bone marrow (BM) niche has become one of the research focuses in drug discovery since increasing evidence suggests that BM niches protect CML LSCs. Laminin alpha4 chain (Lama4), a functional chain for several laminin isoforms, is widely expressed in bone marrow (BM). We have recently shown the critical impact of LAMA4 expression in the microenvironment for hematopoiesis regeneration including megakaryocyte maturation and acute myeloid leukemia progression in mice (Cai et al., Blood, 2022). Furthermore, we have by RNA sequencing shown downregulation of *LAMA4* mRNA in freshly isolated BM mesenchymal stem cells (MSCs) from newly diagnosed patients with CML (Dolinska, Cai, Mansson, et al., Blood, 2023). However, the functional impact of the LAMA4 expression on CML LSCs remains to be investigated.

We have here explored the role of LAMA4 in CML development and drug response by using both mouse models and primary patient BM samples. To first assess the *in vivo* impact of *LAMA4* expression in BM niches on CML development, we transplanted BM cells (CD45.2) from *Scl- tTA× TRE-BCR-ABL1 (BCR-ABL1)* mice prior or following tetracycline withdrawal into irradiated *Lama4* $^{+/+}$ and *Lama4* $^{-/-}$ CD45.1mice and analyzed CML progression and BCR-ABL1 LSC activity by flow cytometry and droplet digital PCR (ddPCR). Following transplantation of unfractionated BM cells from the *BCR-ABL1* mice with CML induced by tetracycline withdrawal, *Lama4* $^{-/-}$ recipients showed significantly increased platelet counts in blood compared to *Lama4* $^{+/+}$ recipients at 2-8 weeks post-transplantation (P=0.005 at week 2, P= 0.008, n=7-8, at week 8), which is in contrast to the impaired platelet recovery in non-transplanted *Lama4* $^{-/-}$ mice following sublethal irradiation. This suggests that *Lama4* deficient microenvironment is favorable for platelet production from BCR-ABL1 CML cells. Consistent with this, megakaryocyte-erythrocyte progenitors (MEPs) derived from *BCR-ABL1* $^{+}$ cells were significantly increased in Lama4 $^{-/-}$ BM at the endpoint (8 weeks, p=0.02, n=3). Megakaryocyte progenitors (MkPs) seemed to be increased in *Lama4* $^{-/-}$ recipient BM (P=0.05, n=3). Intriguingly, when LIN $^{-}$SCA1 $^{+}$KIT $^{+}$ CD150 $^{+}$ hematopoietic stem cells from *BCR-ABL1* mice without prior tetracycline withdrawal were transplanted, the donor-derived MEPs were significantly reduced in *Lama4* $^{-/-}$ BM (P=0.02, n=4-6), which is accompanied with a trend of reduction in MkP but no changes in platelets. ddPCR analysis indicated that the ratio of BCR-ABL1 $^{+}$ cell fraction to total CD45.2 $^{+}$ cells (detected by FACS) seemed to be lower in *Lama4* $^{-/-}$ recipients (33.6%) than that in *Lama4* $^{+/+}$ recipients (41.5%), raising a possibility that *Lama4* $^{-/-}$ niches is less suitable for maintenance of BCR-ABL1 $^{+}$ cells. Together, these data point to a possible differential regulation of CML stem and progenitor cells by the Lama4 expression. More work will be done to dissect the regulatory mechanisms through *in vitro* co-cultures, transplantation and confocal imaging. In parallel, the functional impact of LAMA4 on human CML LSC survival was evaluated by a revised long-term culture-initiating cell assay using CML patient derived MSCs. The preliminary result indicated that recombinant LAMA4 peptides appeared to inhibit CML patient BM-derived CD34 $^{+}$CD38 $^{-}$ cell growth.

In conclusion, *LAMA4* is downregulated in BM MSCs and endothelial cells of CML patients at diagnosis. *Lama4* $^{-/-}$ microenvironment might act differentially in regulating BCR-ABL1 $^{+}$ hematopoietic stem and progenitor cell activity post-transplantation. However, more work is required to validate the finding and the effect of LAMA4 peptides on the megakaryocyte differentiation and maturation.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-173844