Endothelial cell-specific overexpression of Del-1 drives expansion of hematopoietic progenitor cells in the bone marrow

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Dear Editor

Hematopoietic stem cells (HSCs) are responsible for the maintenance of hematopoiesis under normal conditions and its adaptation to hematopoietic stress (1). HSCs are localised in the bone marrow (BM) in a micro-anatomic space designated as the HSC niche (2) comprising different cellular components. While osteoblasts predominantly contribute to the restoration of hematopoiesis after transplantation, endothelial and perivascular stromal cells form a perivascular niche, which promotes the self-renewal of HSCs and their multi-lineage differentiation, and, hence the maintenance of hematopoiesis (2). Cells of the perivascular niche not only provide the mechanical barrier between the BM microenvironment and circulation, but also regulate hematopoiesis, either through adhesive interactions with HSCs (3) or by the release of paracrine factors (4,5). The adhesive interactions of HSCs with endothelial and perivascular cells mediate both the trafficking of HSCs into and out of the BM (6) and their maintenance and proliferation potential (3).

Developmental endothelial locus-1 (Del-1) is a secreted glycoprotein that interacts with integrins (7–9). We have recently shown that Del-1 is expressed by important cellular components (endothelial, mesenchymal stromal cells, and cells of the osteoblastic lineage) of the HSC niche, where it regulates hematopoietic progenitor function and their myeloid differentiation (10). This ability of Del-1 to act as a HSC niche factor was attributed to its interaction with $\beta 3$ integrin on hematopoietic progenitors (10). Here, we set out to precisely define the role of Del-1 as an endothelial cell-derived perivascular niche factor. To this end, we studied steady-state and regeneration hematopoiesis using an independent *in vivo* approach, namely mice with endothelial-specific overexpression of Del-1.

We engaged transgenic mice with endothelial cell-specific overexpression of Del-1 (EC-Del1), generated by utilizing a tie2 promoter/enhancer construct (11,12). EC-Del1 mice

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exhibited significantly elevated Del-1 gene (*Edil3*) expression in the BM (Figure 1A), as assessed by qPCR, which was performed as described (10). Relative mRNA expression levels were calculated according to the $\Delta\Delta C_t$ method upon normalization to 18s (10,13). Enhanced Del-1 expression in the BM was owing to increased expression in endothelial cells ($\text{Lin}^- \text{CD45}^- \text{CD31}^+ \text{Sca1}^+$) (Figure 1B), isolated from the BM by FACS sorting as previously described (10). On the contrary, no expression of Del-1 in hematopoietic progenitors was observed (data not shown). Endothelial cell specific overexpression of Del-1 resulted in an increase in total BM cellularity (data not shown). We then performed phenotypic analysis of hematopoietic cells in the BM by flow cytometry (10), in order to address whether Del-1 overexpression can affect the numbers of hematopoietic progenitors in the BM. Flow cytometry analysis was performed by FACS Canto II flow cytometer using the FACSDiva 6.1.3 software (BD, Heidelberg, Germany). FlowJo (Tree Star) software was used for data analysis. Antibodies used for FACS analysis are described in supplementary table 1. Consistent with a role for Del-1 as an HSC niche factor, EC-Del1 mice exhibited an expansion in the absolute cell numbers of hematopoietic progenitors (LSK; $\text{Lin}^- \text{Sca1}^+ \text{cKit}^+$), long-term (LT)-HSCs ($\text{Lin}^- \text{cKit}^+ \text{Sca1}^+ \text{CD48}^- \text{CD150}^+$) and short-term (ST)-HSCs ($\text{Lin}^- \text{cKit}^+ \text{Sca1}^+ \text{CD48}^+ \text{CD150}^-$), while the cell numbers of multipotent progenitors (MPP; $\text{Lin}^- \text{cKit}^+ \text{Sca1}^+ \text{CD48}^+ \text{CD150}^-$) were not significantly increased (Figure 1C–D). The relative abundance of LT-HSCs, ST-HSCs, MPPs and LSK cells in total bone marrow cells, or the relative abundance of LT-HSCs, ST-HSCs and MPPs within LSK cells, did not differ between EC-Del1 mice and littermate WT mice (data not shown). Moreover, the myeloid progenitor pool was expanded as a result of endothelial Del-1 overexpression, as evidenced by the increased absolute cell numbers of common myeloid progenitors (CMP; $\text{Lin}^- \text{cKit}^+ \text{Sca1}^- \text{CD16/32}^- \text{CD34}^+$), megakaryocyte erythrocyte progenitors (MEP; $\text{Lin}^- \text{cKit}^+ \text{Sca1}^- \text{CD16/32}^- \text{CD34}^-$) and granulocyte macrophage progenitors (GMP; $\text{Lin}^- \text{cKit}^+ \text{Sca1}^- \text{CD16/32}^+ \text{CD34}^+$) in adult EC-Del1 mice (Figure 1E, F). Similarly, the relative abundance of CMPs, MEPs and GMPs within the myeloid progenitors was not altered in EC-Del1 mice as compared to littermate WT mice (data not shown). Thus, endothelial-specific overexpression of Del-1 caused a symmetric expansion in the cell numbers of HSCs and myeloid progenitors without affecting their relative abundance. The increased presence of functional progenitor cells in the BM of EC-Del1 mice, compared to WT littermates, was also shown by engaging a colony forming assay (CFU assay) (Figure 1G). The MethoCult™ GF M3434 medium (Stemcell Technologies) was used for the CFU assay, following the manufacturer's instructions. Quantification of colony formation was performed using a STEMvision™ Instrument (Stemcell Technologies). Taken together, Del-1 overexpression in endothelial cells results in elevated absolute number of phenotypic and functional progenitor cells in the BM.

To functionally evaluate LT-HSCs from Del-1 overexpressing mice, we engaged a competitive repopulation assay using the CD45.1/CD45.2 congenic system (Figure 1H and Supplementary Methods), as described (10). Analysis of peripheral blood chimerism (percentage of CD45.2^+ cells) and calculation of long-term reconstitution units (R.U.) (10) was performed at week 16 after transplantation, *i.e.*, at a time when hematopoiesis restoration is mediated by LT-HSCs whereas other progenitor cell populations are already exhausted (14,15). The increased numbers of phenotypic LT-HSCs due to Del-1

overexpression were in accordance with the increased numbers of R.U. in the BM of EC-Del1 mice (Figure 1I). The increased numbers of R.U. in the BM of EC-Del1 mice was further associated with increased chimerism levels in Gr1⁺CD11b⁺ myeloid cells in peripheral blood of recipient mice (Figure 1J,K), while no alterations were observed in CD19⁺ B cells and CD3⁺ T cells (Figure 1K). In line with this observation, donor chimerism levels in BM Gr1⁺CD11b⁺ myeloid cells was enhanced also in the BM of recipients that had received donor BM cells from EC-Del1 mice, whereas no significant difference was observed in CD19⁺ B cells (Figure 1L). Therefore, Del-1 overexpression in the perivascular niche results in the expansion of long-term repopulating cells with an enhanced potential for myeloid-lineage reconstitution.

In conclusion, we show that endothelial-specific overexpression of Del-1 causes expansion of hematopoietic and myeloid progenitor cells in the BM and primes long-term repopulating cells towards myeloid lineage, as shown by the competitive repopulation assay. These findings specifically implicate endothelial cell-derived Del-1 as a regulator of the HSC niche and consolidate its function to regulate myelopoiesis at the level of early hematopoietic progenitors. Del-1, therefore, is a critical component of the perivascular niche, driving hematopoiesis through β 3-integrin dependent interactions with hematopoietic progenitors. Our observation that endothelial-specific overexpression of Del-1 results in the expansion of hematopoietic and myeloid progenitors is clinically important. Indeed, it suggests that Del-1 may be a potential therapeutic target in disorders characterised by dysfunction of hematopoietic progenitors, including acquired BM failure syndromes and iatrogenic myelosuppression. Therapeutic strategies aiming at increasing Del-1 levels in the BM could have a beneficial effect on the restoration of hematopoiesis upon chemotherapy-associated myelosuppression or in the context of HSC transplantation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Endothelial-specific overexpression of Del-1 results in increased absolute cell number of phenotypical and functional hematopoietic and myeloid progenitor cells.

Edil3 expression (A) in the BM (n=5–7 mice per group) and (B) in Lin⁻CD45⁻CD31⁺Sca1⁺ endothelial cells from EC-Del1 mice, compared to transgene-negative littermates (WT) (n=7 mice per group). The mRNA expression was normalized against 18s. (C) Representative flow cytometry plots and (D) LSK, MPP, ST-HSC, and LT-HSC cell numbers in the BM of littermate WT and EC-Del1 mice (n=5–8 mice/group). (E) Representative flow cytometry plots for the characterization of myeloid progenitors. After gating for Lin⁻cKit⁺Sca1⁻ cells, different subpopulations of myeloid cells were characterized based on the expression of CD16/32 and CD34. (F) CMP, MEP and GMP cell numbers (n=5–8 mice). (G) Colony-forming cells (CFC) in the BM of WT and EC-Del1 mice (n=5–7 mice). (H) Competitive repopulation assay. BM cells from either EC-Del1 mice or WT mice (CD45.2⁺; donor) and

CD45.1⁺ BM cells as competitors were transplanted into B6.SJL recipient mice (CD45.1⁺) (n=8 recipients for the WT donor group and 10 recipients for the EC-Del1 donor group). (I) Reconstitution units (R.U.) per femur in EC-Del1 and WT littermates. (J) Representative flow cytometry plots for the evaluation of donor chimerism in Gr1⁺CD11b⁺ myeloid cells, and (K) frequency of donor-derived cells in Gr1⁺CD11b⁺ myeloid cells, CD19⁺ B cells and CD3⁺ T cells in the peripheral blood of recipient mice at 16 weeks after transplantation. (L) Frequency of donor-derived cells in Gr1⁺CD11b⁺ myeloid cells and in CD19⁺ B cells in the BM of recipient mice at 16 weeks after transplantation. Mann-Whitney U test. Statistical analysis was performed using GraphPad Prism (GraphPad Inc., La Jolla, CA). Data presented as mean±SEM. *p<0.05, **p<0.01, ***p<0.001.