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POSTER ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

Targeting Endothelial PERK-DLL4 Axis to Enhance Hematopoietic Stem Cell and Lymphoid Progenitor Regeneration

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Despite our current information on hundreds of genes implicated in hematopoietic stem cells (HSC) biology, major gaps still exist in our understanding of HSC physiology and their regeneration mechanism under disease and stress conditions, hindering the development of strategies to enhance HSC regeneration. HSCs reside in a tightly controlled bone marrow HSC niche that regulates and maintains hematopoietic homeostasis. HSC niche is composed of a heterogeneous population of specialized stroma cells. Among these, the endothelial cells and the perivascular stroma cells provide critical pro-hematopoietic factors to support HSC pool and committed lineage progenitors particularly lymphoid progenitors. In addition, evidence suggests that endothelium-expressing Notch ligand may promote Notch-dependent long-term HSC proliferation. Recent single cell transcriptome profiling revealed that Delta-like 4 (DLL4) is expressed by the vascular endothelial cells at a higher level than other Notch ligands while its expression down-regulates in response to myeloablation. However, the molecular mechanism regulating the dynamic DLL4 expression during hematopoietic regeneration in response to myeloablative stimuli is unknown. Unfolded protein response (UPR) (also known as endoplasmic reticulum stress, ER stress) has been increasingly recognized as crucial for HSC maintenance. During hematopoietic regeneration, UPR is critical for meeting the increased demand for protein synthesis required for rapid cellular proliferation and for adapting to pathological conditions such as increased levels of reactive oxygen species following irradiation or chemotherapy. Unlike HSCs, how HSC niche endothelial cells deal with redox imbalance and higher requirements of vessel regeneration to regulate stress hematopoietic regeneration is not clear.
In this study, using genetically engineered mouse models, we examined the dynamic expression and function of PERK, one arm of UPR, and its potential downstream target, Dll4, during the stress hematopoiesis triggered by transplantation. We found that PERK is activated in the mouse bone marrow endothelial cells following irradiation. Ablation of endothelial *Perk* in mice (referred as *Perk* $^{i\Delta EC}$ mice) promoted post-transplantation immediate recovery of blood cells derived from wild type donor mice and enhanced HSC and lymphoid progenitor regeneration with the expansion of B lineage progenitors including pre-proB, pro-B, and pre-B cells. In addition, by whole-mount immunostaining and intravital microscopy, we found that endothelial *Perk* deletion restrained disorganized angiogenesis and vascular leakage provoked by irradiation. Although irradiation itself had no effect on endothelial Dll4, *Perk* ablation markedly increased endothelial Dll4 expression. We found that Dll4 is essential for the post-irradiation HSC and lymphoid progenitor rejuvenation as ablation of endothelial *Dll4* in mice (referred as *Dll4* $^{i\Delta EC}$ mice) led to impaired short-term and long-term hematopoietic regeneration and markedly decreased wild type donor derived peripheral B cell and lymphoid progenitor recovery. We found that endothelial Dll4 is required for HSC quiescence maintenance, HSC niche retention and HSC self-renewal. Further, ablation of endothelial *Dll4* led to disorganized bone marrow angiogenesis and increased marrow vascular leakage. Finally, we showed that deletion of *Dll4* in *Perk* $^{i\Delta EC}$ mice completely abrogated the increased numbers of HSC and lymphoid progenitors mediated by *Perk* deletion, suggesting that up-regulation of endothelial Dll4 is likely responsible for *Perk* ablation-mediated enhanced HSC and lymphoid progenitor regeneration.
Taken together, our results revealed a novel regulatory mechanism by which endothelial Dll4 expression is suppressed by PERK of UPR during transplantation while ablating *Perk* increases Dll4 expression. Endothelial DLL4 in the bone marrow endothelial cells is critical for supporting vascular integrity and myeloablation-induced HSC and lymphoid progenitor regeneration. Our findings suggest that targeting endothelial PERK-DLL4 axis could be a viable option to improve post-irradiation regeneration of HSC and lymphoid progenitors.

Disclosures No relevant conflicts of interest to declare.

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