Abstract: P1396

Title: DEFICIENCY HIF-1A EXPRESSION IN BONE MARROW ENDOTHELIAL PROGENITOR CELLS PROMOTES ACUTE GRAFT-VERSUS-HOST-DISEASE AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Abstract Type: Poster Presentation

Topic: Hematopoiesis, stem cells and microenvironment

Background:

Acute Graft-Versus-Host Disease (aGVHD) frequently occurs after allogeneic hematopoietic stem cell transplantation (allo-HSCT), significantly affecting patient prognosis. A key factor in this process is the role of Bone Marrow (BM) endothelial progenitor cells (EPCs), which are essential for maintaining hematopoietic stem cells (HSCs) homeostasis. Our previous research demonstrated that in aGVHD patients, BM EPCs function is impaired, and there is an increase in reactive oxygen species (ROS) levels. HIF-1α, intimately linked to ROS production, is vital for EPC survival in the hypoxic bone marrow environment.

Aims:

We aims to further explore the role of HIF-1α in aGVHD progression following allo-HSCT.

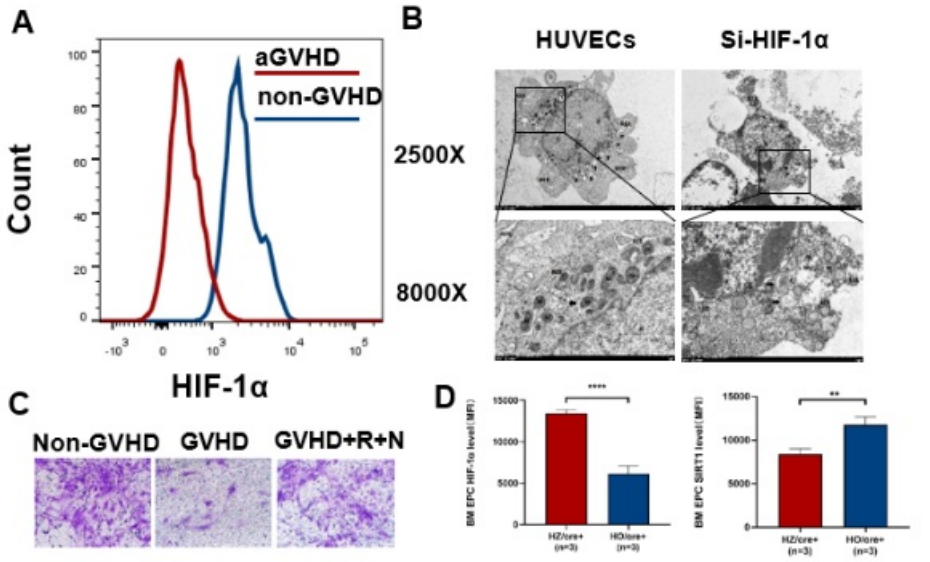
Methods:

Our clinical case-matched study compared 20 patients with aGVHD to 20 patients without aGVHD. We flow-sorted BM EPCs using CD34+, CD133+, and CD309+ biomarkers. In human umbilical vein endothelial cells (HUVECs), HIF-1α was knocked down using SiRNA transfection. Endothelial cell-specific knockout of HIF-1α in C57BL/6J is achieved by the Cre-loxP recombinase system.We determined the gene expression levels in endothelial cells (ECs) using Western blot and RT-qPCR. The functionality of ECs was evaluated through angiogenesis, Transwell cell migration, CCK-8 cell proliferation assays, and JC-1 mitochondrial staining. To assess the interaction with hematopoietic stem cells (HSCs), ECs were co-cultured with CD34+ HSCs for seven days, followed by evaluating the cellular functions and colony-forming ability of HSCs. Additionally, NAD+ and NADH levels in ECs were measured using a specific kit.

Results:

Flow cytometry revealed that the expression of HIF-1α and the autophagy-related proteins Beclin-1 and LC3 was reduced in BM EPCs from aGVHD patients compared to those from non-aGVHD patients（Figure A）. Correspondingly, cell proliferation, migration, and angiogenesis were impaired in BM EPCs from aGVHD patients. We created a low HIF-1α level EC model by downregulating HIF-1α in HUVECs. Co-culturing these HUVECs and BM EPCs from aGVHD patients with HSCs led to increased apoptosis, higher ROS levels, and reduced colony forming ability in HSCs. Moreover, inhibiting HIF-1α in HUVECs triggered abnormal activation of NAD+ depletion proteins SIRT1 and PARP1, causing decreased NAD+ levels and mitochondrial abnormalities（Figure B）. However, supplementing with β-nicotinamide mononucleotide (NMN), a NAD+ precursor, effectively raised NAD+ levels in ECs and restored cellular functions. Additionally, activating HIF-1α with Roxadustat improved cellular functions and hematopoiesis support by up-regulating HIF-1α（Figure C）. In mice with endothelial cell-specific HIF-1α knockout, low HIF-1α expression correlated with reduced autophagy and overactivated SIRT1 levels（Figure D）.

Image:



Summary/Conclusion:

The data indicate that autophagy and mitochondrial dysfunction in BM EPCs from aGVHD patients are regulated by reduced HIF-1α expression. In both HUVECs and mice models with endothelial cell-specific HIF-1α knockouts, HIF-1α deficiency leads to abnormal activation of SIRT1 and PARP1, potentially affecting NAD+ levels and mitochondrial function, thereby impairing cellular functions. These molecular alterations could be key factors in aGVHD pathogenesis post-allo-HSCT. Additionally, our findings reveal that Roxadustat and NMN can restore cellular functions and hematopoietic support through different mechanisms, suggesting a promising therapeutic strategy for aGVHD prevention and treatment following allo-HSCT.

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Keywords: Graft-versus-host disease (GVHD)