STRONG CHILDREN'S RESEARCH CENTER

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ABSTRACT

Validating the Immunophenotype of Mesenchymal Stem Cell Populations in a Murine Model of Myelodysplastic Syndrome.

Background: Myleodysplatsic Syndrome (MDS) is a disease of bone marrow that disrupts hematopoiesis. MDS has a 5-year overall survival rate of less than 50%. No new therapies have been developed for the last decade, underscoring the importance of identifying novel therapeutic agents. Mesenchymal stem cells (MSCs) are non-hematopoietic multipotent stem cells that give rise to other elements that comprise the bone marrow microenvironment (BMME). MSC's support the hematopoietic niche and, importantly, are dysfunctional in MDS. We hypothesize that certain subpopulations of MSC's are enriched for during aging and disease, therefore serving as a potential therapeutic target.

Objective: To develop a novel flow-cytometry panel to characterize the immunophenotype of MSCs and progenitor cell populations in a murine model of MDS, and further interrogate changes in these populations during aging and disease.

Methods: Bone marrow was harvested from healthy young, MDS-transformed (NHD13) and aged-matched healthy control mice. Cells were stained with fluorophore conjugated primary antibodies recognizing specific cell-surface markers. Lineage markers (CD3e, B220, Gr1, TER119, CD45 and CD31) were used to negatively select out hematopoietic and endothelial cells. CD51, SCA1, CD271, CD146, and CD106 were used to define subpopulations of interest. Cells were analyzed using fluorescence activated flow-cytometry. Biparametric analysis and gating strategies of positive selection markers were used to characterize subpopulations of MSCs.

Results: Murine MSCs are characterized by SCA1 and CD51 expression within the non-hematopoietic compartment. Our lab and collaborators at the Harvard Stem Cell Institute have shown that CD271, CD146 and CD106 are positive selection markers that enrich for subpopulations of MSC's in the human. Utilizing anti-mouse antibodies for these positive selection markers of MSC's, we illustrate that the SCA1+/CD51+ population is enriched for CD271+/CD146+/CD106- cells and the SCA1-/CD51+ population is enriched for CD271+/CD146-/CD106- cells in healthy young mice. In aged and diseased mice, both SCA1+/ CD51+ and SCA1-/CD51+ populations were predominantly enriched for CD271+/CD146-/CD106- cells. Consistent with published results, NHD13 mice displayed a higher frequency of SCA1-/CD51+ cells compared to young and aged healthy controls.

Conclusions: We successfully created a novel flow panel to further characterize MSCs in a murine model of MDS by incorporating knowledge about the immunophenotype of both murine and human defined MSC's. This provides a consistent and reproducible immunophenotype to compare subpopulations of MSCs across species, thereby facilitating our understanding of their biologic relevance. Interestingly, diseased mice and aged matched controls showed an enrichment of CD271+ /CD146- /CD106- and SCA1-/CD51+ subpopulation of MSCs whereas, healthy young control mice had a higher frequency of CD271+ /CD146+ /CD106- and SCA1+/ CD51+ population. Published and preliminary data suggest that the SCA1+/CD51+ and CD271+/CD146+ phenotypes behave most like MSCs. Our data illustrates that those immunophenotypes identify the same subpopulation of cells in healthy controls. Furthermore, our data suggests aging and disease are diminished within these populations, supporting our hypothesis that specific subpopulations with the MSC compartment change with aging and disease progression. Functional *in vitro* assays will be necessary to further interrogate the ability of these cells to differentiate into downstream progeny and assess their biologic activity.