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POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Single Cell Analysis Identifies Expression of PD-L2 on Human Bone Marrow Hematopoietic Stem Cells

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The identification of specific and reliable markers for human hematopoietic stem cells (HSCs) is crucial to enable their prospective isolation allowing the investigation of unique physiological HSC functions within the blood cell hierarchy. Despite significant advancements in identification of surface markers for human bone marrow HSC isolation, the enriched populations remain heterogeneous. Here we applied single cell CITE-Seq (cellular indexing of transcriptomes and epitopes) of FACS-enriched hematopoietic stem and progenitor cells (HSPCs) to decipher differentiation hierarchies and to identify markers of most immature populations.

We FACS-isolated over 60.000 high-quality human bone marrow HSPCs by sorting CD34 $^{+}$ and CD34 $^{+}$CD38 $^{-}$ cells from 15 different healthy donors. These were subjected to scCITE-Seq using the BD Rhapsody system with a targeted gene panel comprising 600 most relevant genes of early HSC differentiation and 50 antibodies. To validate differentially expressed surface marker genes, we performed extensive *in vitro* and *in vivo* studies of FACS-purified subpopulations of human bone marrow and mobilized peripheral blood samples (mPB) from healthy donors.

Our multi-omics based single HSPC profiling indicated Programmed death ligand 2 (PD-L2 / CD273) as a surface marker expressed on the most immature HSCs within the hematopoietic compartment defined as CD34 $^{+}$CD38 $^{-}$CD45RA $^{-}$CD90 $^{+}$. Prospective FACS-based isolation allowed a deeper understanding of transcriptomic differences depending on PD-L2 positivity. A transcriptomic principal component analysis of CD273 ^{hi} and CD273 ^{low} expressing HSCs isolated pairwise from four healthy donors proved distinct clustering accompanied by discriminative molecular signatures. Stem cell genes as *HOPX*, *MLLT3* and *MPL* were enriched in CD273 ^{hi} HSCs, and *CDK6* was downregulated, further validating our single-cell analysis. The differential expression of stem cell genes was validated at protein level. Single cell tracking using video-microscopy confirmed a delayed entry into cell cycle in CD273 ^{hi} HSCs, suggesting deeper quiescence of these cells. Furthermore, their mitochondrial potential was reduced. *In vivo* transplantations of both populations in immunocompromised NSGs are ongoing and will reveal the long-term repopulation potential of CD273 ^{hi} HSCs. Multiple *in vitro* differentiation assays demonstrated CD273 positivity to be associated with an increased stem cell phenotype with higher expression of EPCR (CD201) and CD90 in liquid culture, a delayed differentiation and high capability of generating multipotent colonies. Interestingly, CD273 surface expression was strongly upregulated in HSCs upon *in vitro* culture.

Since PD-L2 is known as a ligand for the Programmed death receptor 1 (PD1), we investigated the immunomodulating function of PD-L2 expressing HSPCs by establishing an allogeneic lymphocyte reaction assay. In addition to their stem cell-like characteristics, PD-L2 expressing HSPCs mediated diminished T cell proliferation in allogeneic co-culture settings. Furthermore, cytokine profiling of co-culture supernatants indicated increased secretion of anti-inflammatory cytokines (IL-10 and TGF-β1), accompanied by reduced secretion of pro-inflammatory cytokines (IL-17A) and a diminished expression of the pro-inflammatory receptor CD69 on CD8 $^{+}$ T cells. These findings showed that CD34 $^{+}$ cells isolated from mobilized peripheral blood of healthy donors possess immunomodulating capabilities, and that PD-L2 expression indicates an immunocompromising HSC phenotype.

In conclusion, this study demonstrates differential surface expression of PD-L2 within the most immature human HSCs. PD-L2 expression is associated with HSC quiescence and delayed *in vitro* differentiation. Furthermore, these results provide valuable insights into the immunomodulating features of HSCs expressing PD-L2.

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