2018 - STEM CELL FACTOR, GM-CSF, AND IL-3-TRANSGENIC HUMANIZED MICE DEVELOP FATAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Although humanized mouse model is a great tool to study human hematopoiesis, immune function, and various disease, poor myeloid reconstitution has been recognized to be one of the limitations. To solve the problem, various strategies have been examined to express human cytokines in humanized mouse. Drawbacks of using cytokine transgenic mice in generating humanized mice remain unclear, as well as the role of human thymus graft in T cell reconstitution. To examine these 2 different parameters, we generated 4 groups of humanized mice; 1) NOD/SCID/$\gamma c^{-/-}$ [NSG] with human thymus; 2) NSG without human thymus (CD34+ cells alone); 3) human stem cell factor, GM-CSF, and IL-3-transgenic NSG [SGM3] with human thymus; and 4) SGM3 without human thymus. SGM3 humanized mice showed higher human chimerism, better myeloid reconstitution, and aggressive expansion of CD4+ central and effector memory T cells particularly in the absence of human thymus. SGM3 humanized mice had fatal outcome 18 to 22 weeks after transplantation. SGM3 humanized mice showed severe human T cell and macrophage infiltrations to systemic organs including liver, lungs, and spleen. SGM3 mice also showed severe anemia and thrombocytopenia with hypoplastic bone marrow, but reticulocyte count in blood was maintained. These clinical manifestations closely resemble secondary hemophagocytic lymphohistiocytosis in human. Indeed, SGM3 humanized mice showed a significant elevation in inflammatory cytokines, which have been reported to have major roles in the pathogenesis of hemophagocytic lymphohistiocytosis. Although SGM3 humanized mice should not be used to study regular immune response, it may serve as a model to study hemophagocytic lymphohistiocytosis. In addition, our study highlighted the requirement of human thymus to avoid aggressive T cell expansion likely due to the poor negative selection in the mouse thymus.

2019 - COHESIN GENES ARE CRITICAL REGULATORS OF HSC RENEWAL

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To gain deeper insights into the mechanisms underlying renewal and differentiation of hematopoietic stem and progenitor cells (HSPCs), we developed RNAi screens targeted to human cord blood derived CD34+ cells. We employed a near genome-wide screen (targeting 15 000 genes) to identify genes with an impact on renewal/differentiation of HSPCs, in a completely unbiased manner. Among the prominent hits were many transcription factors and epigenetic modifiers and we found a strong enrichment of genes known to be recurrently mutated in hematopoietic neoplasms. A striking finding was the identification of the cohesin complex (STAG2, RAD21, STAG1 and SMC3) among our top hits (top 0.5%). Recent large-scale sequencing studies have identified recurrent mutations in the cohesin genes in myeloid malignancies. Upon validation of cohesin deficiency in human $CD34^+$ cells, we found that their knockdown by independent shRNAs led to an immediate and profound expansion of primitive hematopoietic $CD34^+CD90^+$ cells in vitro. Transplantation of HSPCs transduced with shRNA targeting STAG2 into NSG mice resulted in a significant increase in human reconstitution in the bone marrow 16 weeks post-transplantation ($31.3\pm4.4\%$ vs $11.6\pm2.8\%$ $p=0.001$). The engrafted mice showed a skewing towards the myeloid lineage in bone marrow ($27.0\pm5.0\%$ vs $13.0\pm2.6\%$ $p=0.013$), as well as an increase in the more primitive $CD34^+CD38^-$ population ($2.8\pm0.6\%$ vs $1.3\pm0.4\%$ $p=0.036$). In secondary recipients, 4/6 mice in the STAG2 group maintained detectable levels of human chimerism while no engraftment was detected in the control group, indicating an increased expansion of HSPCs in vivo upon knockdown of STAG2. Global transcriptome analysis of cohesin deficient $CD34^+$ cells 36 hours post shRNA transduction showed a distinct up-regulation of HSC specific genes, demonstrating an immediate shift towards a more stem-like gene expression signature upon cohesin deficiency. This observation was consistent for all cohesin genes tested. Our findings implicate cohesin as a major player in regulation of human HSPCs and, together with the recent discovery of recurrent mutations in myeloid malignancies, point toward a direct role of perturbed cohesin function as a driver event in myeloid leukemogenesis.

2020 - ELUCIDATING THE POST-NATAL ROLE OF SCA1+ THYMIC MESENCHYMAL CELLS

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Introduction: In the last years, MCs have attracted extraordinary attention since they were found to be key components of essential physiological microenvironments such as the hematopoietic stem cell niches. Thus, some might suspect that MCs play a role for the proper functioning of diverse organs in the adult organism. In the thymus, MCs have been shown to provide a crucial microenvironment required for the embryogenesis of the epithelial compartment. Yet, the post-natal role of tMCs and their exact contribution to thymic involution remain a matter of speculation. **Methods&Results:** First, we evaluated the extent of overlap between SCA1+ MCs from thymus (tMCs), bone (bMCs) and skin (sMCs) by comparing their phenotype and transcriptome. From Lin- SCA1+ cells of the 3 organs, we analysed the expression 14 typical MC markers by flow cytometry. Over 97% of Lin- SCA1+ cells of the 3 organs stained positive for CD29, CD51 and CD140a, suggesting a MC identity. As for the other markers, they exhibited some disparities that could denote organ-specific roles. Transcriptome analysis will highlight common and distinctive features between those 3 MC populations and consequently reveal some insights about the post-natal role of tMCs. To investigate the age-related changes occuring in tMCs, we assessed the absolute number of thymic stromal populations over time. It revealed that the age-related atrophy of the epithelial compartment is not initially triggered by a decrease in SCA1+ tMC numbers. To gain further insights, we compared the transcriptome of tMCs from 1 and 7 months-old mice (RNA-seq). Preliminary results suggest that with age, extracellular matrix production is gradually reduced in tMCs while chemokine expression is increased. **Conclusion:** Phenotypic profiles of Lin- SCA1+ cells in the thymus, bone and skin clearly suggest a MC identity. Moreover, the evolution of tMC number does not explain the age-related decrease of thymic epithelial cells. We now aim at pursuing our transcriptomic analyses to shed light on the post-natal roles of tMCs.

2021 - MEDIAL HOXA GENE EXPRESSION IS REQUIRED FOR ESTABLISHING "STEMNESS" IN HUMAN HSCS

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The shortage in HLA-matched hematopoietic stem cells (HSCs) from bone marrow and cord blood limits our ability to use HSC transplantation to treat blood and immune diseases. Although generating HSCs from pluripotent cells can potentially overcome this shortage, lack of understanding of the regulatory mechanisms governing HSC function has prevented their *in vitro* generation. Using a two-step human ESC differentiation protocol in which $CD34^+$ cells from embryoid bodies were co-cultured on OP9-M2 stroma, we derived hematopoietic cells with the $CD34^+CD38^-CD90^+CD45^+GPI\text{-}80^+$ human fetal HSC immunophenotype. ESC-derived cells differentiated into definitive erythroid, myeloid, and T-cells, but displayed impaired self-renewal and engraftment ability compared to hematopoietic stem/progenitor cells (HSPCs) from the fetal liver (FL). Microarray analysis of immunophenotypic HSPCs from ESC-derived cells and different stages of development revealed successful transition of ESC-HSPCs from the hemogenic endothelium towards FL-like HSPCs with the upregulation of programs critical for definitive hematopoiesis and HSC function. However, despite the close molecular correlation between ESC-HSPCs and FL-HSPCs, a group of transcription factors, including medial HOXA genes, were not activated in ESC-derived cells, similar to immature HSPCs from early placenta. Knockdown of HOXA5 or HOXA7 in FL-HSPCs recapitulated the self-renewal defects observed in ESC-HSPCs, documenting a functional link between medial HOXA genes and human fetal HSC self-renewal. Stimulating the retinoic acid (RA) signaling pathway in ESC-derived $CD34^+$ cells for 6 days with the RARa agonist AM580 was sufficient to transiently induce the medial HOXA genes in ESC-HSPCs followed by other genes associated with HSC self-renewal and also prolong ESC-HSPC maintenance in culture. These data identify suppression of HOXA gene expression as a developmental barrier for generating HSCs from pluripotent cells and imply RA-signaling as a major inductive signal in establishing "stemness" in hematopoietic cells.