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POSTER 5: Do embryonic immune megakaryocytes exist?

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Megakaryocytes (MKs) are large multinucleated cells localized in bone marrow that produce platelets, necessary for the hemostasis of the organism. However, recently the murin platelet-forming MKs, especially those residing in lung, were described to be able to participate in several immune responses. Furthermore, a small cluster of "immune" MKs characterized by the expression of non-classical molecules (CD14, CD53) in embryonic and adult human samples were described and were classified as non-platelet forming MKs (Wang et al., 2021). Although the ability of platelet forming MKs to present antigens is quite well demonstrated, the existence of the "immune" MK cluster remains controversial.

Therefore, to better understand the existence and role of embryonic immune MKs in relation with various pathologies, the aim of this work is to characterize the "immune" MK cluster by single cell RNA analysis of MKs differentiated *in vitro* from hIPSC-derived hematopoietic progenitors.

Transcriptomic analysis revealed ITGA2B (CD41) and GP9 (CD42) RNA expression only in 64.92% of cells sorted of CD41 and CD42 markers. Five of eleven found clusters were negative for MK markers at RNA level and annotated as myelocytes (C1), neutrophils (C3), macrophages (C6), basophils (C7) and eosinophils (C8) suggesting that platelet or MK membrane were stuck on their cell surface. MK clusters (C0, C2, C5, C9 and C10) were selected by their expression of MK genes for a further re-analysis. Two of these five clusters present immune related pathways. Interestingly, MK cluster 10 (1.08% of cells), is enriched in "Neutrophil degranulation" pathway, it expresses only a low level of MK markers and an even lower level of the immune genes described by Wang et al., 2021. Projection of the different sets of differentially expressed genes (DEG) showed that up-regulated genes of this cluster were highly expressed in the neutrophilic clusters (27,8% of cells in culture, C1 and C3).

The second cluster (C2 24.6% of cells), highly expressing MK genes, expresses immune genes enriched in "Cellular response to type I interferon" and could be involved in response to viral infections. Of note,

signature DEG published in Wang et al. 2021 for immune MKs were only slightly expressed in our MK clusters but more importantly in the neutrophilic clusters.

We further focused on CD14 and CD53 defined as immune markers by Wang et al. In our sc-RNA seq analyses, we only found these markers in cluster 6 corresponding to macrophages. However, when we performed flow cytometry, we were able to identify, accordingly to the results obtained by Wang, a population high for CD14, CD53, and low for CD41 and CD42. The MGG coloration and immunofluorescence staining showed that these cells expressing CD14 were macrophages positive for Von Willebrand factor. Finally, we have sorted the myeloid cells (CD14+CD41-CD42-) and MKs (CD41+CD42+CD14-) separately, colored both populations with CFSE and violet-dye respectively, and co-cultured them. Two days later, presence of CFSE+violet-dye+ cells was detected confirming *in vitro* exchange of proteins between MKs and myeloid cells.

In conclusion, we have identified 2 embryonic MK clusters with potential immune phenotype, the minor expressing low MK markers is enriched in neutrophil genes and could reflect an exchange of RNA between neutrophils and MKs, the second one corresponds to true MKs able to respond to viral infections. Finally, the immune MKs derived from ESC/iPSC, as described by Wang et al, rather corresponds to macrophages phagocyting megakaryocytes than to a new MK population.