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研究課題名	Investigating Putative Dendritic Cell Precursors (pre-DC) with Neutrophil Progenitor Properties	
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(Form 2-2)

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Joint Research Report (Annual/Project Completion)

Annual Report

Report: Investigating Putative Dendritic Cell Precursors (pre-DC) with Neutrophil Progenitor

Started from a potential discovery by one of Dr. Nakai's Master course students (at that time), we have tried to investigate a subtype of putative pre-DC that exhibits expressions of neutrophil progenitor. In this study, five haematopoietic datasets that use different single-cell isolation and sequencing technologies were integrated with Mutual Nearest Neighbors (Haghverdi et al., 2018). As a result, a group of rare and potentially novel subtype of bone marrow-derived pre-DCs was unveiled while studying the merged dataset.

After further investigations, the putative pre-DCs with neutrophil progenitor markers was found to be having two sub-groups. With the recent increased single cell public data resources, we were able to create a single-cell reference map and identify one of the sub-groups as $Axl^{+}Siglec6^{+}$ pre-cDCs, whereas the other sub-group is located among $CD33^{+}$ granulocyte-monocyte progenitors (GMPs). GMPs are commonly known to be homogeneous oligopotent cells. However, recent studies proposed that GMPs contain lineage-committed precursors and are therefore heterogeneous mixture of cells (Giladi et al., Nat. Cell Biol. 2018; Olsson et al., Nature 2016; Paul et al., Cell 2016; Yanez et al., Blood 2015). Furthermore, a group of early uni-potent neutrophil progenitors was revealed in GMPs (Kwok et al., Immunity 2020). This suggests the possibility of the existence of early uni-potent DC progenitors in GMPs. To verify the hypothesis of the putative pre-DCs within GMPs as early uni-potent DC progenitors, identification of cell surface markers of the putative pre-DCs are necessary to extract them for further analyses. COMET (Delaney et al., Mol Syst Biol 2019), a tool for prediction of candidate marker panels from single-cell RNA-seq data, suggests LGALS1+ITGAX and LGALS1+HAVCR2 as the potential marker panels for the putative pre-DCs within GMPs.

However, surface markers predicted from RNA-seq data may consist of delayed synthesis between mRNA and protein. Hence, we use another single-cell AbSeq bone marrow dataset and Hypergate (Becht et al., Bioinformatics 2019) to identify a gating strategy to extract the putative pre-DCs from GMPs. Hypergate suggests $CD34^{+}IgG^{int}CD123^{int}CD2^{int}CD272^{+}CD127^{lo}CD11a^{+}CD32^{+}CD11c^{+}$ with F-score of 0.6033, and $HLA.DR^{+}CD36^{-}CD4^{-}CD272^{lo}CD99^{lo}CD119^{+}CD184^{+}CD116^{+}$ with F-score of 1.0 as the potential gating strategies. The putative pre-DCs from GMPs have hallmarks of the DC lineage, including IRF8, CD74, FLT3, and MHC class II expression. We also identified the transcription factor regulons of the putative pre-DCs with SCENIC (Aibar et al., Nat Methods 2017). In December 2022, Dr. Takeuchi visited Dr, Nakai's laboratory and joined the discussion on interpreting the results of *in-silico* analyses. Dr. Takeuchi suggested to find the analogous population of putative pre-DCs from GMPs in the mouse samples. We integrated three public scRNA-seq datasets of mouse samples and we are in the process of narrowing down the counterpart of putative pre-DCs from GMPs in the mouse samples.