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Molecular regulation of emerging hematopoiesis from human induced pluripotent stem cells (iPSCs)

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Our group recently reported enhanced erythroid differentiation from human iPSC by closer mimicry of physiological cell contacts in a three dimensional network, termed haematopoietic cell forming complex (HCFC). From this HCFC, CD43+ hematopoietic cells (purity >95 %) were continuously released into the culture supernatant. Characterization of the HCFC in more detail is currently limited by accompanying destruction of the complex, hindering further haematopoietic cell fate tracking. To overcome this limitation, we developed a CD43 fluorescence reporter hiPSC line that allows live-cell imaging of haematopoietic cells as they emerge in a spatiotemporal manner from the HCFC.We have successfully reprogrammed human peripheral blood (PB) CD34+ derived erythroblasts to iPSC (PEB-AL#6). Thereafter, we have successfully performed gene knock-in of a CD43 promoter region tagged with GFP using CRISPR/Cas9-mediated homology-directed repair (HDR) mechanisms in the AAVS1 safe harbor locus. Adeno-associated viral vectors (AAV) were used for efficient DNA donor delivery into hiPSCs. Following HDR-mediated donor integration, clones with on-target seamless DNA integration were isolated, which was confirmed by Sanger sequencing and in-out PCR. To confirm the functionality of the CD43 fluorescent reporter, haematopoietic and erythroid differentiation of the CD43R-iPSC was induced. GFP-expression of CD43+ hematopoietic cells inside the HCFC was monitored by fluorescence microscopy and live cell imaging. Haematopoietic nature of released GFP+ cells was confirmed by flow cytometry (CD43, CD34, CD45) and colony formation assay. The established CD43 fluorescent reporter system allows to track hematopoietic development from hiPSCs within three-dimensional structures like organoids. Therefore, the system represents an attractive tool to investigate the hematopoietic development from hiPSCs.The established system can be used for different hiPSC lines or other primary cells.