IN VITRO GENERATION OF MYELOID HEMATOPOIETIC CELLS FROM HUMAN EMBRYONIC STEM CELLS

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INTRODUCTION AND OBJECTIVES

Human embryonic stem cells (hESCs) are characterized by their unlimited capacity for self-renewal and differentiation into all the adult specialized cells. The potential for differentiation of these cells into specific cell lines makes them great promise for developing new technologies applicable to regenerative medicine and cell therapy as an alternative source of cells. Although cell culture systems have been developed for hematopoietic differentiation in vitro from hESCs, standardization to produce hematopoietic cells for potential clinical applications is the main challenge. Thus we have sought to differentiate efficiently H1-hESC in hematopoietic cells.

MATERIALS AND METHODS

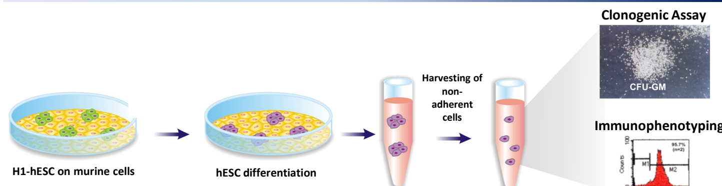

Fig. 1 – Experimental design of hCTEs differentiation in co-culture with murine cells. For hematopoietic differentiation, H1-hESCs were co-cultured with inactivated murine cells in differentiation medium supplemented with BFS and a cocktail of human hematopoietic growth factors .

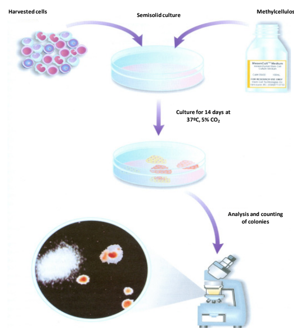

Fig. 2 – Clonogenic assay in methylcellulose

RESULTS AND DISCUSSION

We have developed an efficient method that has allowed high performance in obtaining CD45+ hematopoietic cells (greater than 90% positive cells), with viability greater than 80% for more than 50 days. We have obtained all cells of the myeloid lineage at different stages of maturation (fig. 3).

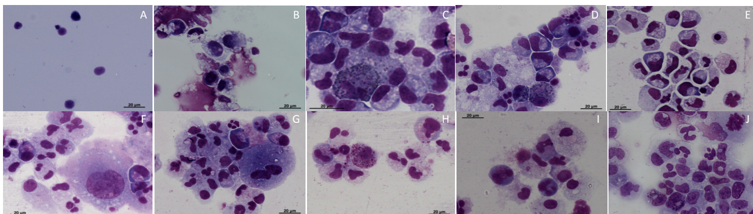

Fig. 3 - The generation of all hematopoietic cells at different stages of maturation. **A** and **B**, mature erythrocytes and erytroid progenitors. **C** and **D**, basophil progenitors and myelomonocytic progenitors. **E**, two erythroblasts and myelomocytic progenitors. **F** and **G**, two megakaryocyte, mature neutrophils and myeloid progenitors. **H** and **I**, eosinophilic progenitors, neutrophilic progenitors and macrophages. **J**, mature neutrophils and monocytes. (65x).

On day 19 of co-culture, 65% of cells were already CD45+ and when they were tested functionally in Methylcellulose (fig. 4) they were able to produce all myeloid colonies, indicating that despite the advanced stage of maturation these cells still showed a high proliferative and final differentiation potential .

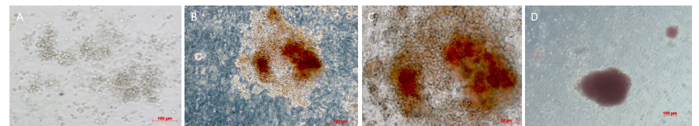

Fig. 4 – When these cells were functionally tested in methylcellulose, they were still able to generate 44,3 (±0,7) BFU-E, 44,3 (±3,7) CFU-GEMM and 48 (±3,0) CFU-GM (n= 4). **A**, CFU-GM. **B** and **C**, CFU-GEMM. **D**, BFU-E. A and B, 20X; C, 40X; D, 20X.

After 31 days of co-culture, over 91% of cells were CD45+ co-expressing the following markers: CD43, CD38, CD31, CD71, CD235a, CD14, CD15, CD16 and CD11b (fig. 5). Although 76% of cells were positive for the primitive erythroid marker CD71, CD235a expression, a specific marker for erythroid cells, was restricted just to 9% of the cell population. The highest peak of CD235a was observed on day 24, when 20% of cells expressed this marker. Despite the enrichment of CD45+ cells, the population of cells expressing CD34 did not exceed 6% during the studied period (day 19 to 31), being day 19 in which there was a greater amount of CD34+ cells. Because it is a marker whose expression precedes CD45, some analyses prior to 19 day are being carried out to verify the presence of CD34+ cells.

Fig. 5 – Immunophenotypic profile of hematopoietic cells on day 31 of differentiation.

The kinetics of differentiation by this method showed that initially were derived erythroid progenitor cells which preceded the appearance of granulocytic lineage, with subsequent predominance of monocytes and macrophages progenitors. More primitive cells have demonstrated high capacity to adhere to the feeder, an indication that these cells could have great potential for graft.

CONCLUSION

The observations of the predominance of lineage-specific precursors during differentiation opens up alternatives to select specific cell populations to direct the differentiation to specific mature hematopoietic lineages in vitro and insights into ontogeny of hematopoietic lineages, both in ES cell-derived cultures and during normal development.

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