P030

Optimization of methods for hypothermic preservation of primary human Hematopoietic Stem Cells in culture

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Introduction

Biogenic amines (dopamine and serotonin) protect cells against hypothermia-induced damage (1). The newly developed compound ROKEPIE®-S01 (Sulfateq BV) has been demonstrated to be successful in hypothermic storage and transportation of several cell lines, as well as thrombocytes (2), but not of primary cells, including hematopoietic stem cells (HSCs). Hypothermic preservation of HSCs could be preferred over short-term cryopreservation, to prevent cell loss during freezing/thawing and could be particularly useful for short-term storage, e.g., during conditioning of transplant patients or transport of HSC transplants. Here, we assessed optimal doses of this compound and culture conditions to preserve CD34+ umbilical cord blood (UCB) HSCs.

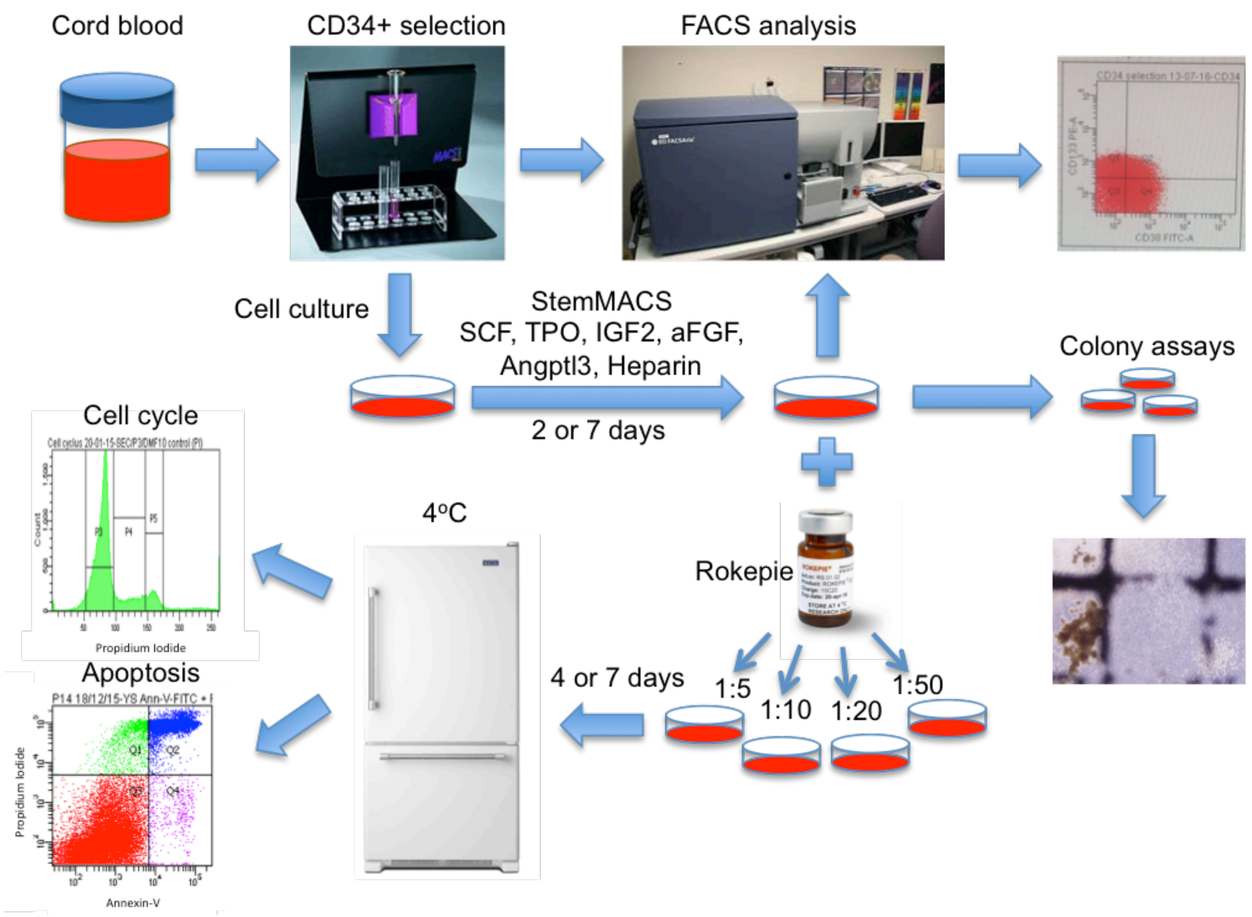

Figure 1. Prolonged hypothermic storage of Hematopoietis Stem Cells
To optimize conditions for prolonged hypothermic storage of HSCs, umbilical cord blood CD34+ cells were selected and cultured in presence of different growth factor cocktails, cultured for 2 or 7 days, supplemented without washing steps with Rokepie at dilutions of 1:5 – 1:50 and analyzed for colony forming units, CD34+ content, apoptosis (Annexin-V-FITC/Propidium Iodide) and cell cycle status.

Materials and Methods

CD34+ HSCs were isolated from freshly collected umbilical cord blood samples using magnetically labeled anti-CD34 antibodies (Miltenyi). HSCs were supplemented with TPO, TPO/SCF, TPO/SCF/Flt3L or TPO/SCF/aFGF/IGF2/Angptl3/heparin (STIFA3) for 2 days (analogous to our gene transfer method) or 7 days (analogous to our HSC expansion method). After 2 or 7 day cell culture, cell counts, colony forming assays (Methocult, Stem Cell Technologies) and CD34 cell numbers were assessed using a FACSARIA (Becton Dickinson). ROKEPIE®-S01 was then added directly to the medium, the plates closed airtight and stored for 4-7 days at 4°C. After rewarming, the frequency of apoptotic cells and cell cycle status were measured (Figure 1).

Results

Two day cultures with TPO resulted in the lowest frequency of apoptotic cells after hypothermic storage in the presence of 1:10 or 1:20 diluted ROKEPIE®S01. After 7 days of culture/hypothermic storage, cell viability was 10-30% higher in cultures containing the compound, whereas numbers of cycling cells were 10-20% lower, in particular when stimulated with STIFA3 (Figure 2).

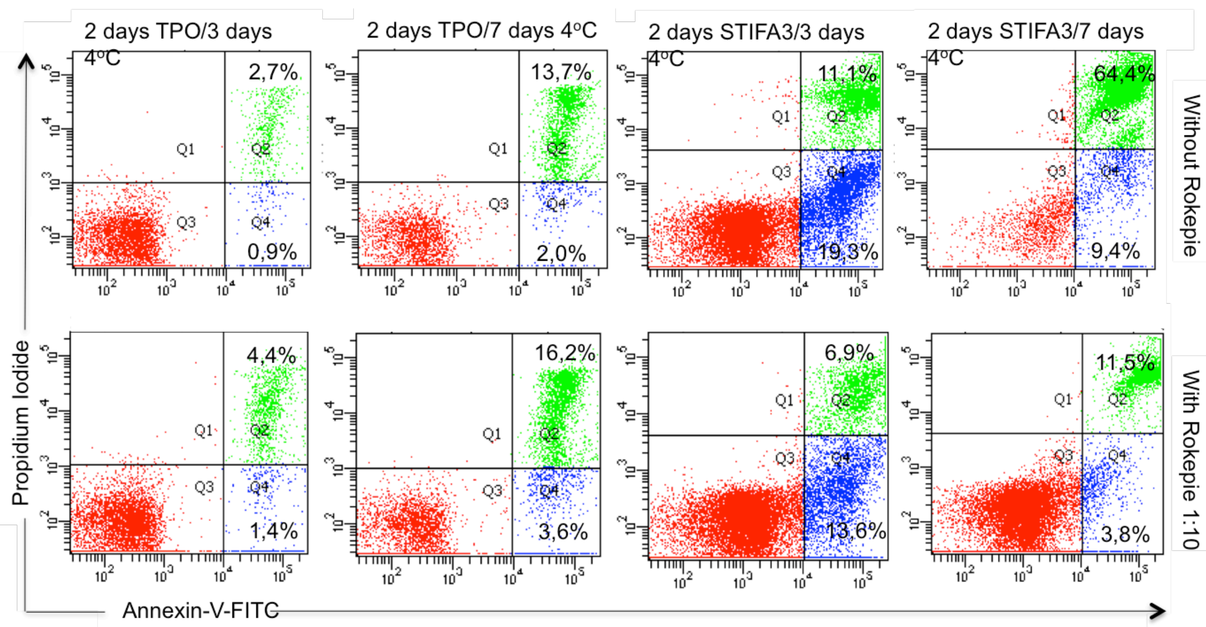

Figure 2. Apoptosis in HSC cultures after hypothermic storage
CD34+ UCB-CD34+ cells were cultured in presence of TPO for 2 days or STIFA3 for 7 days. ROKEPIE was added to the cultures and the flasks were closed air-tight and stored at 4°C for 3 or 7 days. Apoptosis was measured using Annexin-V-FITC and Propidium Iodide.

In short-term HSC cultures, followed by short-term storage at 4°C, ROKEPIE®S01 does not appear to affect cell viability or cell cycle state. Under long-term HSC expansion conditions, followed by 7 days of hypothermic storage, however, effects of the compound are significant, as evident from decreased rate of apoptosis, and increased levels of cells in G0/G1 phase (Table 1).

Table 1. Cell cycle analysis of HSC cultures after hypothermic storage

Cell cycle	G0/G1 (%)	S (%)	G2/M (%)	S+G2M (%)
2 days TPO/3 days 4°C without Rokepie	12,2	11,7	11,8	23,5
2 days TPO/3 days 4°C Rokepie	15,1	15,2	15,0	30,2
2 day TPO/7 days 4°C without Rokepie	19,9	11,5	8,5	20,0
2 day TPO/7 days 4°C Rokepie	14,5	15,9	16,4	32,3
7 days STIFA3/3 days 4°C without Rokepie	30,5	27,3	23,9	51,2
7 days STIFA3/3 days 4°C Rokepie	27,7	26,5	26,6	53,1
7 days STIFA3/7 days 4°C without Rokepie	12,7	25,1	31,4	56,5
7 days STIFA3/7 days 4°C Rokepie	24,6	26,1	24,3	50,4

Conclusions

ROKEPIE®-S01 effectively promotes cell viability of primary HSCs during hypothermic storage, and is currently assessed for its effects on long-term repopulating HSC.

Rerences

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Acknowledgements

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