STAT5 is required for long-term maintenance of normal and leukemic human stem/progenitor cells

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Day 1

- Isolate 1.5 x 10^6 AML $CD34^+$ cells with MACS column or sort by FACS and incubate (0.5 10^6 /ml) in RPMI1640 supplemented with 10% FCS, IL-3, G-CSF and TPO (each 20 ng/ml) for 4 hours, 37ºC, 5% CO_2

- Coat 3 wells from a 12-wells-plate with retronectin: Dilute with PBS to 50 µg/ml, 0.5 ml per well of a non-tissue culture 12-well plate. Incubate at room temperature for 2 hrs, then remove retronectin and block with 2% BSA/PBS for 30 min. Wash plate twice with PBS and keep in fridge until use.

- After 4 hours split AML $CD34^+$ cells in 3 groups (at least 1.5 x 10^5 cells per group, but not more than 5 x 10^5 in 500 µl) and apply each group to one coated well:

  Group 1: No virus control (for setting FACS gates)
  Group 2: Transduce with Renilla RNAi control vector
  Group 3: Transduce with STAT5 RNAi vector

- Add 500 µl HPGM to group 1
  Add 500 µl Renilla RNAi lentivirus supernatant to group 2
  Add 500 µl STAT5 RNAi lentivirus supernatant to group 3

- Add to each well to final volume/concentration:
  10% FCS
  20 ng/ml hIL-3
  20 ng/ml TPO
  20 ng/ml GCSF
  4 µg/ml polybrene

- Incubate overnight at 37ºC, 5% CO_2 (round 1)

Furthermore:
Culture MS5 stromal cells in T75 in α-MEM supplemented with 10% FCS, so that 80% confluence is reached at day 2

Day 2

- Repeat transduction procedure in morning (round 2). Make virus supernatants (or HPGM) with 10% FCS and growth factors (each 20 ng/ml) and polybrene (4 ng/ml) (again in 500 µl) as above.
- Add 500 µl to each well (Washing of cells is not necessary).
- Incubate for 8 hrs
- Repeat this procedure in the evening (round 3)

Furthermore:

- Coat 3x T25 with 0.1 % gelatin for 2 hrs at room temperature
- Trypsinize T75 with MS5, resuspend in 15 ml α-MEM with 10% FCS and plate 5 ml in gelatin-coated T25s (these MS5 cultures will reach confluency on day 3)

Day 3

- Wash AML $CD34^+$ cells from each group 3 times with PBS and resuspend in 1.5 ml of Long Term Culture (LTC) medium (αMEM supplemented with heat-inactivated 12.5% FCS, heat-inactivated 12.5% Horse serum, penicillin and streptomycin, 200 mM Glutamine, 57.2 μM β-mercaptoethanol and 1 μM hydrocortisone) supplemented with IL-3, G-CSF and TPO (each 20 ng/ml).
- Take a 50 μl aliquot for FACS to assess transduction efficiency (use group 1 cells to set the gates).
- Plate equal amounts of AML $CD34^+$ cells on MS5 in LTC medium (at least 1.5×10^5 cells per T25, no more than 5×10^5). Sorting of YFP/GFP^+ cells is not necessary. The untranduced GFP/YFP^- cells within each culture serve as an internal control for each culture.

Every week

- Remove weekly half of the suspension (2.5 ml) from the cultures and add 2.5 ml fresh LTC medium supplemented with IL-3, G-CSF and TPO (each 20 ng/ml) (demipopulate). The removed half can be used for FACS analysis, cell count, cytospins etc.

Addendum:

1. The GFP/YFP percentage was usually determined after 2 days expansion on MS-5 stromal layer rather than directly after transduction. Then, GFP/YFP expression will have increased until steady levels and a small sample can be analyzed for proper transduction efficiency.

2. Depending upon the expansion and growth of the AML cells, the MS5 stromal cell layer may be exhausted after 4 to 5 weeks of culture. By isolating all the cells from the culture (including the trypsined adherent fraction, consisting of MS5 and AML CAFCs) and replating 10% of these cells into a new T25 (freshly coated with new MS5 cells), new cultures can be initiated and make the investigation of long term AML cultures possible.