Generation of fluorescently labelled OP9 stromal cells for growing patient leukemic cells

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Rational:

Our experiments use cells from patients with leukemia. These primary cells are notoriously difficult to culture, especially long term. To overcome this, we are using a co-culture system where we grow the leukemia cells with mouse stromal cells (OP9) to try to mimic some aspects of the bone marrow environment present in leukemia [1]. Although essential for maintenance of leukemia cells, the stromal cells can complicate many of the analyses that are required to establish the effective targeting of the leukemia cells. To get around this, we introduced fluorescent markers in the OP9 cells to easily distinguish the stromal cells from the patient cells in various applications such as microscopy and flow cytometry.

Transduction of cells:

Lentivirus vector based of the vectors made by the Naldini lab (Addgene 12252), modified to express GFP from EF1 promoter was packaged by standard methods. OP9 cells at a density of 0.1 million cells in a 10cm plate were transduced with 600µL of either EF1-mCherry or EF1-GFP virus along with 10µL of 1000X concentrated polybrene. Cells were grown at 37°C/5% CO_2 overnight. After overnight incubation, media was removed and cells were washed with PBS (Wisent). A total of 10mL of media was added to cells. OP9 media comprises of α-MEM media with GlutaMAX (Gibco) without nucleosides containing 20% FBS (Wisent), 55µM β-mercaptoethanol (Gibco) and 100U/100ug/mL penicillin/streptomycin (Gibco).

Determining expression and cell sort:

After one day, expression of fluorescent proteins was checked using the IncuCyte live cell imager (Sartorius). Cells positively expressing the fluorescent proteins were seen. Cells were then split 1:3 to prepare to sort the cells using a cell sorter at the SickKids-UHN Flow Facility. Four days later, the cells were trypsinized (1mL 0.25% Trypsin-EDTA; Gibco) and collected in PBS containing 1% FBS. Sytox Blue (Life Technologies) viability dye was added to the cells at 0.2µM. Cells were sorted by flow staff with profiles provided.

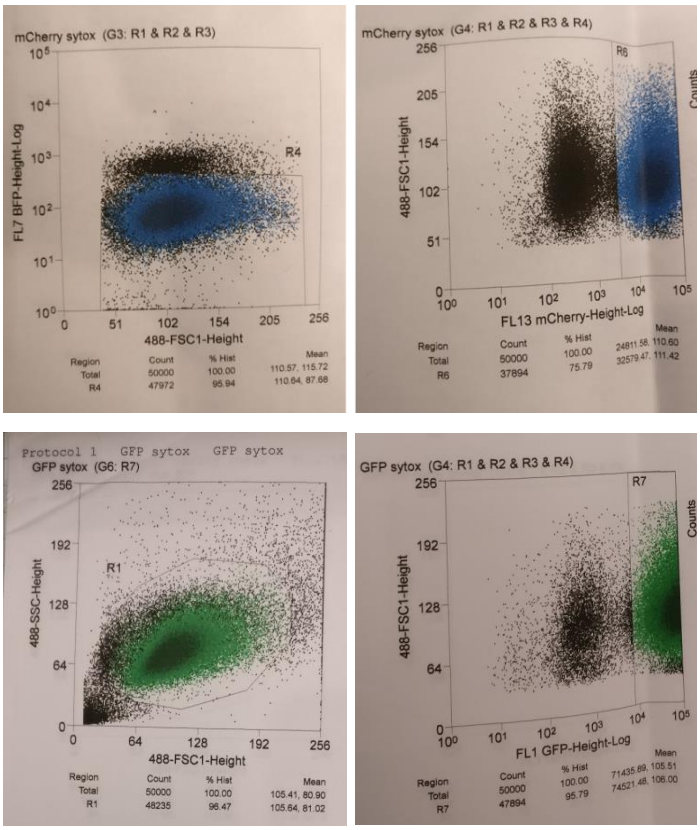

Figure 1: mCherry sort with blue dots representing cells that are collected. The first plot shows the viable cells (blue dots; sytox blue negative) and second plot, the blue dots represent the viable cells that are positive for mCherry and were collected for culturing. The same was done for GFP, but the cells selected are green dots.

Sorted cells were plated in 10cm dishes and allowed to grow for further experiments and imaged to determine fluorescence expression and purity of sorted cells.

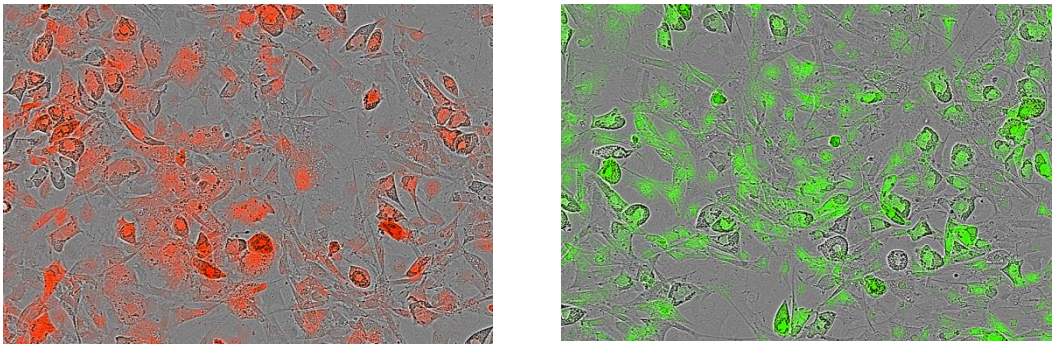

Figure 2: mCherry and GFP labelled OP9 cells. Image generated using IncuCyte live cell imager.

1. Hartwell, K.A., et al., *Niche-based screening identifies small-molecule inhibitors of leukemia stem cells.* Nat Chem Biol, 2013. **9**(12): p. 840-848.