Determining the transfectability of different patient-derived cell lines

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Background:

Whether or not a cell line can be transfected efficiently is an important factor to consider when designing experiments. Transfection via liposome (eg: Lipofectamine 2000) is a relatively simple and direct method of delivering nucleic acids into the cells compared to electroporation or transduction via viral vectors.

Protocol:

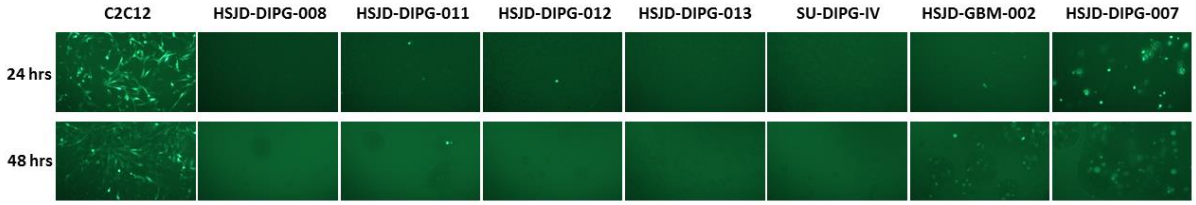
1) 1/20 of DIPG/GBM patient-derived cell lines culture were harvested and dissociated via TryPLE express treatment at 37 degree Celsius for 5 minutes, followed by P1000 trituration for 20 times. TryPLE was diluted with 9ml of TSM base and centrifuged at 400xG for 5 minutes. Cells were resuspended in complete TSM for counting.

2) $1.5x10^5$ C2C12 cells in 2ml medium were seeded into each well of 6-well plate (1 day earlier than DIPG/GBM). $1.5x10^5$ DIPG/GBM patient-derived cell lines in 500ul were seeded into each well of 24-well plate.

3) 1ug of pEGFP-C1 plasmid was diluted in 125ul OptiMEM. 10ul of Lipofectamine 2000 was diluted in 125ul of OptiMEM and left for 5 minutes before being added to the diluted plasmid. Mixture was incubated at room temperature for 20 minutes before being added to the cells in 6-well plate.
1/5 of the amount of plasmid/Lipofectamine 2000/OptiMEM was used for each well in 24-well plate.

4) Cells were checked using fluorescent microscope after 24 hours and 48 hours.

Results:



Conclusion:

Only HSJD-DIPG-007 patient-derived DIPG cell line and HSJD-GBM-002 patient-derived Glioblastoma cell line can be transfected decently using Lipofectamine 2000.