Growth kinetics of HSJD-DIPG-07 cells

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Aims

To record the growth kinetics of the patient derived HSJD-DIPG-07 cell line so that in future experiments cells can be reliably seeded at a density ensuring their health for the duration of the experiment. This will be repeated in future with other DIPG cell lines to tailor seeding conditions to their individual growth kinetics.

Growth media

Media compositions were made up as previously described (Grasso et al., 2015), as follows:

Tumour stem medium – base (TSM-B)
- 250mL Neurobasal-A Medium (ThermoFisher)
- 250mL DMEM/F-12 (ThermoFisher)
- 5mL HEPES buffer solution (ThermoFisher)
- 5mL Sodium pyruvate MEM 100mM (ThermoFisher)
- 5mL MEM non-essential amino acids solution 10mM (100x) (ThermoFisher)
- 5mL GlutaMAX-I supplement (ThermoFisher)
- 5mL Antibiotic-antimycotic (100x) (ThermoFisher)

Tumour stem medium – complete (TSM-C)
Immediately before use, add the following to 50mL of TSM-B :
- 1mL B-27 supplement, minus Vitamin A (50x) (ThermoFisher)
- 50µL of 20µg/mL recombinant human FGF-basic (Preprotech)
- 50uL of 20ug/mL recombinant human EGF (Preprotech)
- 50uL of 2mg/mL Heparin solution (0.2%) (Preprotech)
- 25uL of 20ug/mL H-PDGF-AA (Preprotech)
- 25uL of 20ug/mL H-PDGF-BB (Preprotech)

(All growth factors are diluted in TSM-B)

Experimental details

DIPG-HSJD-07 cells growing in TSM-C were harvested into 15mL centrifuge tubes, spun at 400g for 4min and resuspended in 1mL of TrypLE™ Express Enzyme, without phenol red (ThermoFisher). Trypsinisation was carried out for 2min at 37°C, before quenching with 9mL of TSM-B (9x volume). Cells were spun at 400g for 4min and resuspended in 10mL TSM-C for counting.

Cells were seeded at densities of 12000, 6000, 3000 and 1500 cells/cm^2 (equivalent to a total of 300 000, 150 000, 75 000 and 37 500 cells) in 5mL of TSM-C in Greiner Bio-One

25cm^2 cell culture flasks (cat. no. 690175) at n=3. Flasks were incubated at 37°C , 5% CO_2 and 100% humidity. At 48h, 96h and 168h cells were harvested as previously described, stained with trypan blue and manually counted in a haemocytometer.

Every 3 days cells were fed as follows: Half the medium (with tumour spheres in suspension) was removed and spun at 400g for 4min. The old medium is removed and cells are resuspended in an equal volume of fresh TSM-C.

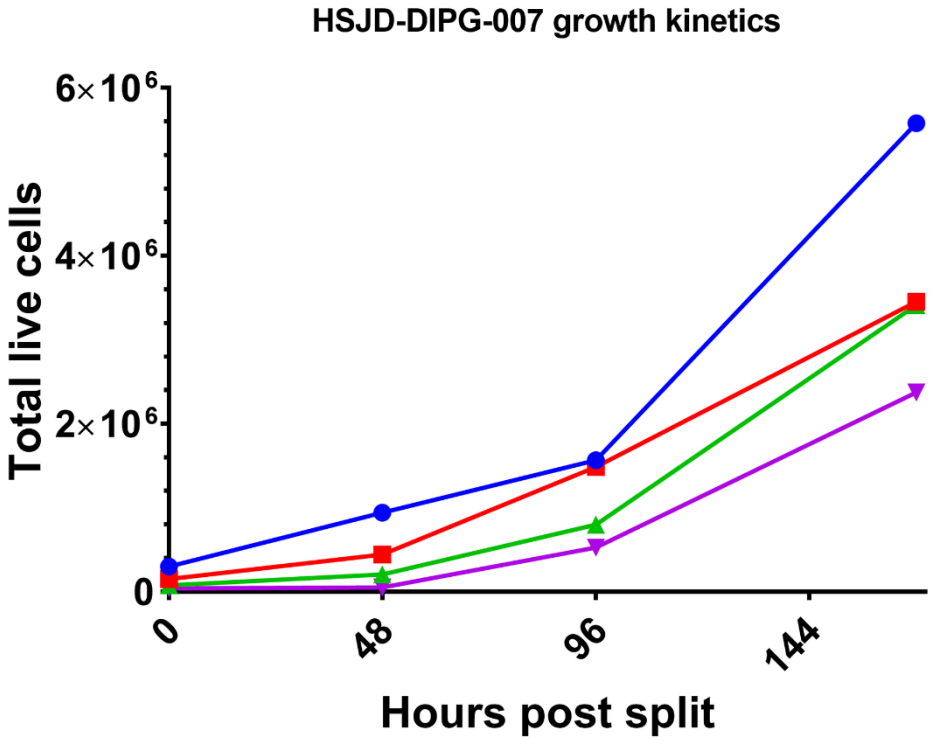

Figure 1 *– Graph illustrating the growth of HSJD-DIPG-07 cells following seeding at densities of 12000 (blue), 6000 (red), 3000 (green) and 1500 (purple) cells/cm^2.*

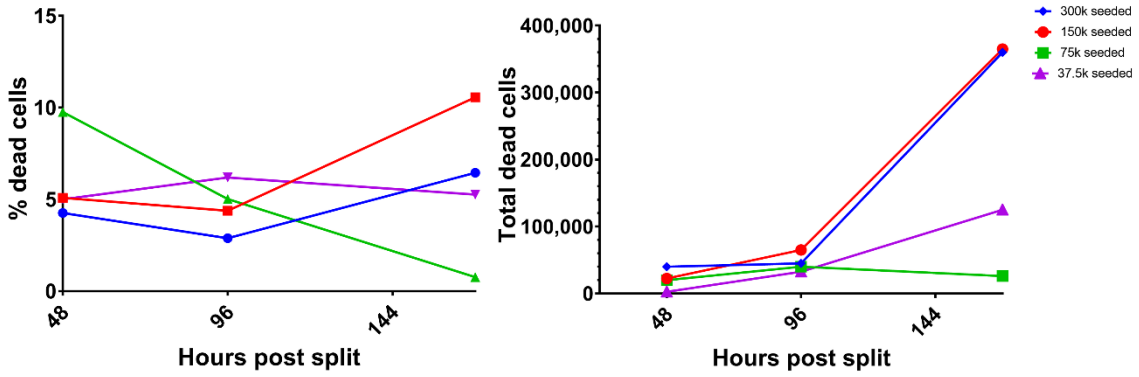

Figure 2 *– Graphs illustrating the percentage (left) and total number (right) of dead HSJD-DIPG-07 cells as determined by trypan blue exclusion, following seeding at densities of 12000, 6000, 3000 and 1500 cells/cm^2.*

Observations

During the time period observed the cells did not reach a lag phase of growth (Fig. 1), however at around 40,000 cells/cm^2 the medium became yellow within three days of feeding (the point at which previous researchers have noted the cells should be split), and above 80,000 cells/cm^2 a number of spheres became to adhere to the bottom of the flask and cell death began to rise (Fig. 2). This indicates that very high concentrations should be avoided when performing experiments, and in general culture of the cell line.

References

Grasso, C. S., Tang, Y., Truffaux, N., Berlow, N. E., Liu, L., Debily, M.-A., … Monje, M. (2015). Functionally defined therapeutic targets in diffuse intrinsic pontine glioma. *Nature Medicine*, *21*(6), 555–559. https://doi.org/10.1038/nm.3855