CONTENTS

1 Characterisation of differentiation

1.1 Aim

Determine the extent and markers expressed by the SY5Y cell line.

1.2 Methods

Same as for the Ren VM, just plate and change the medium, D0 is the one before the medium change, use the same markers.

2 SiRNA

2.1 Aim

Determine the effect of DYRK1A knockout on radiated and control cells (differentiation wise).

2.2 Methods

Transfect in 6 well plate: (1 MOCK + 1 SIRNA)x2 + 1 LUC and add one cover slip in each. Plate and transfect (D0). Next day change to MM and radiate??? Wait 3 more days and collect the analysis.

2.2.1 Staining

Check for differentiation markers.

2.2.2 Western Blot

Blot for DYRK1A. Check for SIRT1/P-Thr to see that it is decrease (compared to normal).

3 Drug toxicity/efficiency

3.1 Aim

Determine the specificity and toxicity of the drugs with 2 different concentrations each (0.1 and 1 uM).

3.2 Methods

3.2.1 AlamarBlue

2x6-well plate, with 1 cover slip each (second plate only for control). Plate $0.5x10^6$ cells per well, Get cell viability of each well before radiation. Then check on D1, D2, D3 (separate wells so it doesn't affect the free ROS). Then blot for SIRT1 and its phosphorylation.

Test for each drug (maybe start at D1 before radiation? Should seperate the samples so that no removing the medium for each day - maybe blot after each day??).

3.2.2 DCF-DA

For each day, check the luminosity of DCF.

3.2.3 Western Blot (hypothesis)

Check the specificity (should reduce the phosphorylation of SIRT1 if DYRK1A is inhibited).

4 Calibration curve for H2AX damage

4.1 Aim

Find the extent of double-stranded breaks in the cells following 2Gy of radiation at different times points.

4.2 Methods

Plate the cells in 3x10cm dishes with X (min 9) coverslips. Time points: D0 (twice, with only secondary Ab), 0 (before IR), 30 min, 1 hr, 2hr, 6hr, 24hr, 72hr. Plate 1: radiate. Plate 2: control (no IR).

4.2.1 Staining

For every time point, pick a coverslip of each dish and stain. Stain for H2AX (M) + TUJ1 (Rb) + NESTIN (Gt).

Ren VM: PLATES: 3x10cm dishes (1 D/NR, 1 D/R, 1 ND/R) and 1x6cm dish (1ND/NR). 1) Stain before radiation by taking one coverslip of each dish. 2) RADIATE AND WRITE DOWN THE TIME. 10:25 3) Fix 1 cs @ 30 min = 10:55 (22.06.18) 4) Fix 1 cs @ 1hr = 11:25 (22.06.18) 5) Fix 1 cs @ 2hr = 12:25 (22.06.18) 6) Fix 1 cs @ 6hr = 16:25 (22.06.18) 7) Fix 1 cs @ 24hr = 10:25 (23.06.18) 8) Fix 1 cs @ 72hr = 10:25 (25.06.18)

SY5Y: PLATES: 3x10cm dishes (1 D/NR, 1 D/R, 1 ND/R) and 1x6cm dish (1ND/NR). 1) Stain before radiation by taking one coverslip of each dish. 2) RADIATE AND WRITE DOWN THE TIME. 10:25 3) Fix 1 cs @ 30 min = 10:55 (22.06.18) 4) Fix 1 cs @ 1hr = 11:25 (22.06.18) 5) Fix 1 cs @ 2hr = 12:25 (22.06.18) 6) Fix 1 cs @ 6hr = 16:25 (22.06.18) 7) Fix 1 cs @ 24hr = 10:25 (23.06.18) 8) Fix 1 cs @ 72hr = 10:25 (25.06.18)

4.2.2 Western blot

On the remaining cells, look for DYRK1A expression? YAP?

5 Reversibility of IR-induced differentiation (clonogenic assay)

5.1 Aim

VM: determine if the degree of differentiation acquired following 2Gy of x-rays is reversible or not. SY5Y: determine

5.2 Methods

Plate the cells in 6 well plate and for (only use 4 wells). Then radiate the cells (D0) and at D1 and D3, transfer the cells to larger dish (split in 3 to get biological replicates, with 24-well plate, for each one maybe get a coverslip+well to stain later on? = 3*2*4=24!), leave for a week and then find the number of cells (just to see cells viability, can be very small number of cells!) and stain.

5.2.1 Staining

5.2.2 Western Blot

Blot for DYRK1A. Check the specificity (should reduce the phosphorylation of SIRT1 if DYRK1A is inhibited).

6 CRISPR

6.1 Aim

Check if knocking out DYRK1A leads to a decrease (or delay) in differentiation (which should be the case!).

6.2 Methods

Plate the cells in a 6 well plate, half is control, the other half is radiated. Look at the differentiation patterns with staining?

6.2.1 Western Blot

Must verify the knock out with a simple WB (due to time constraints, the gold standard would be to grow single colonies from a verified clone to get a stable knock out cell line).

7 Basic response of SY5Y to drugs

7.1 Aim

Test the toxicity of drugs in SY5Y.

7.2 Methods

Alamarblue on 96 well plate, same as before (3 days of diff, radiate). AB before, 24, 48 and 72h.

8 Kinks to iron out

8.1 Get a D0

Get a better D0 for the control of differentiated cells. Also get a secondary only antibody staining (to prove that the background isn't that high, and it is specific).

- check how well they work: do WB with different concentrations and look at SIRT1 and pThr.
- Do flow cyto ROS with SY5Y and Ren VM - would take 7 days (w/ analysis).
- determine what to do with protein samples (diff and non diff IR)