Effects of chronic hypo-osmotic stress on the growth and cell cycle of Nalm6 cells

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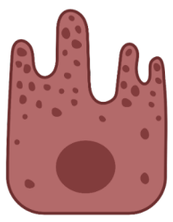
BACKGROUND

The **osmolarity of the extracellular environment** affects cellular behaviour. Changes in this osmolarity may be transient (causing acute stress) or persistent (causing chronic stress). Previous work has primarily focused only on the effects of acute stress, while chronic stress can lead to significantly different outcomes.

Human cells experience **chronic hypo-osmotic stress** (CHS) during edema, cirrhosis, hypothyroidism, several cancers, SIADH (syndrome of inappropriate anti-diuretic hormone secretion), and other conditions where chronic inflammation or fluid accumulation occurs in the body. The long-term effects of CHS on cell growth and division have not been studied previously.

Experiments carried out to test the effects of CHS on retinal pigment epithelium (**RPE**) cells demonstrated that:


RPE cell (adherent)


Nalm6 cell (suspension)

1. CHS decreases growth of RPE1 WT in a hypotonicity-dependent manner, without inducing significant cell death after 72h
2. CHS causes cell cycle arrest of RPE1 WT cells in the G1 phase after 72h
3. CHS upregulates the expression of p53 and p21 proteins in RPE1 WT after 72h

RPE cells are **substrate-adherent**. These observations may or may not generalise to **suspension** cells, which differ from adherent cells in morphology, size, composition, and interaction with the external environment.

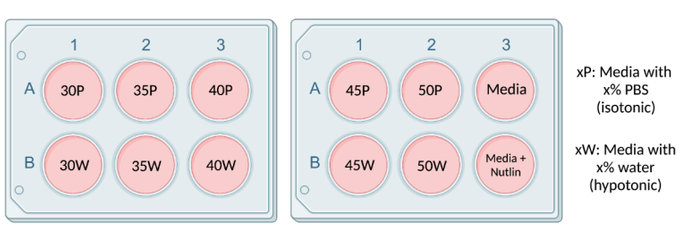
AIMS

To observe and quantify the effect of varying levels of chronic hypo-osmotic stress on **Nalm6** suspension cells with respect to:

1. cell population growth rate and viability
2. proportion of cells found in G1 phase of the cell cycle
3. up- or down-regulation of the p53 and p21 proteins

METHODS

Day 0: Seed 2e5 Nalm6 cells for each condition in pure media.
Day 1: Apply chronic hypo-osmotic stress by changing the media to appropriate dilutions of media with water, along with PBS-diluted controls, and letting the cells incubate for 72 hours.

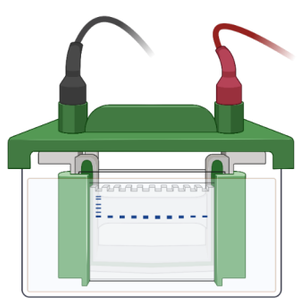


Day 4 (after 72 hours have elapsed under CHS):

1. Quantify the cell population growth rate and viability by counting the live and total cells in each condition considered.

2. Observe the proportions of cells in the G1 stage (containing 2n DNA) and post-G1 stages (containing >2n DNA) of the cell cycle, by staining the cells with Hoechst dye and using FACS to quantify the DNA present.

3. Obtain a measure of the expression of p53 and p21 (normalised by actin) in each condition via western blotting, and compare their expression in corresponding isotonic (P) and hypotonic (W) extracellular conditions.



RESULTS: DECREASE IN CELL NUMBER AND VIABILITY

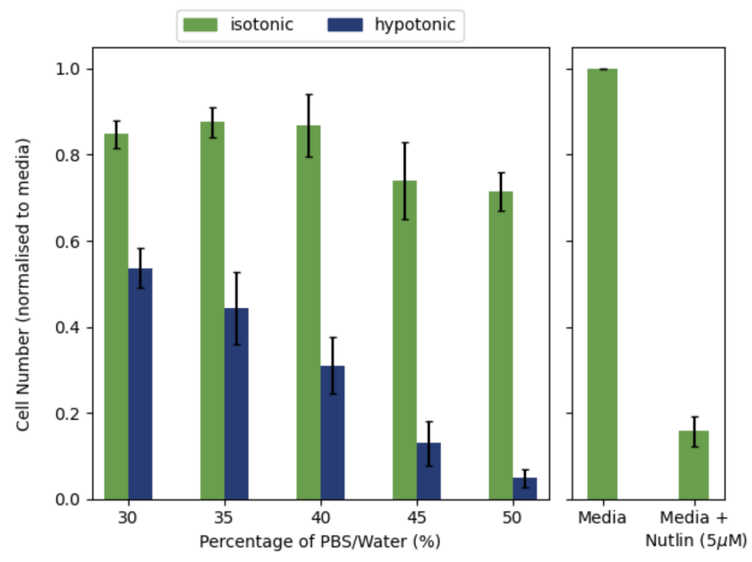


Figure 1. Cell number (normalised) after 3 days CHS. The cell count consistently falls as % of water in the media increases.

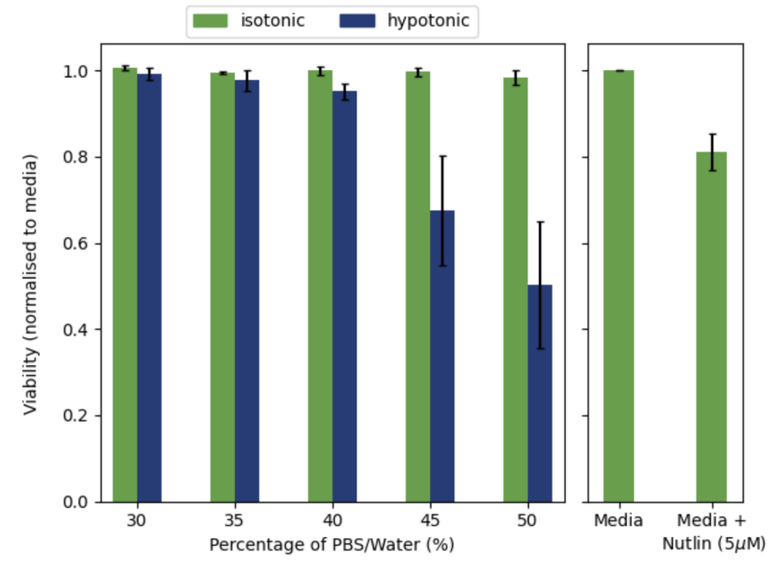


Figure 2. Population viability (normalised) after 3 days CHS. The viability decreases sharply at higher levels of hypotonicity.

RESULTS: INCREASE IN PROPORTION OF G1 CELLS

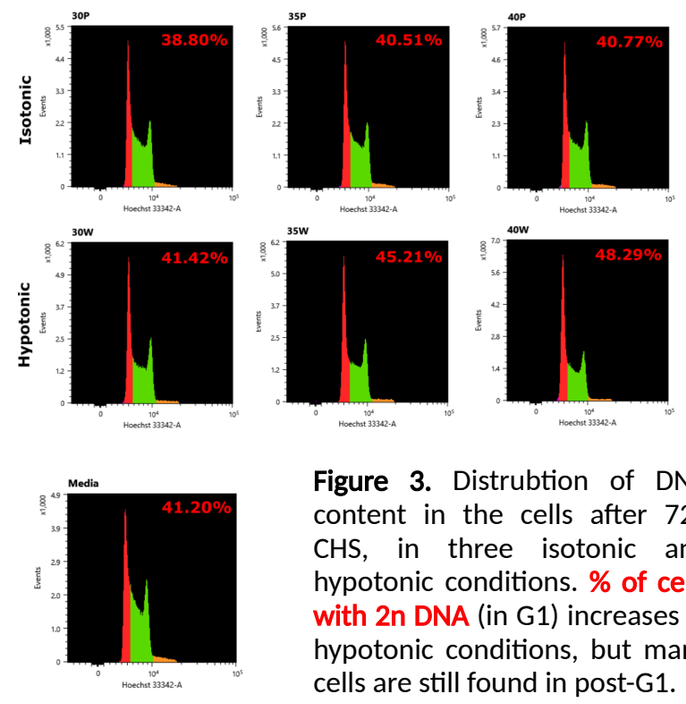


Figure 3. Distrubtion of DNA content in the cells after 72h CHS, in three isotonic and hypotonic conditions. **% of cells with 2n DNA** (in G1) increases in hypotonic conditions, but many cells are still found in post-G1.

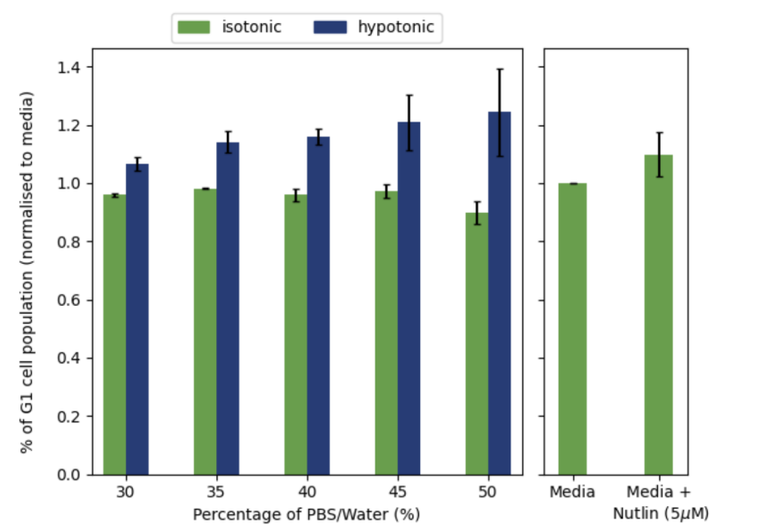


Figure 4. Percentage of the live cell population in G1 phase of the cell cycle after 3 days CHS, normalised to the media condition. As the level of hypo-osmotic stress applied to the cells increases, there is a steady rise seen in the proportion of the cells found in G1 phase.

RESULTS: UPREGULATION OF p21 AND p53

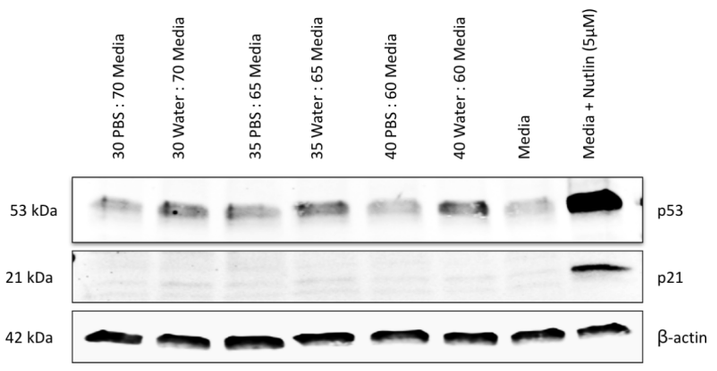


Figure 5. Western blot results for p53, p21 and actin, after 3 days incubation in isotonic and hypotonic conditions. The right-most lane is a positive control for p53 and p21.

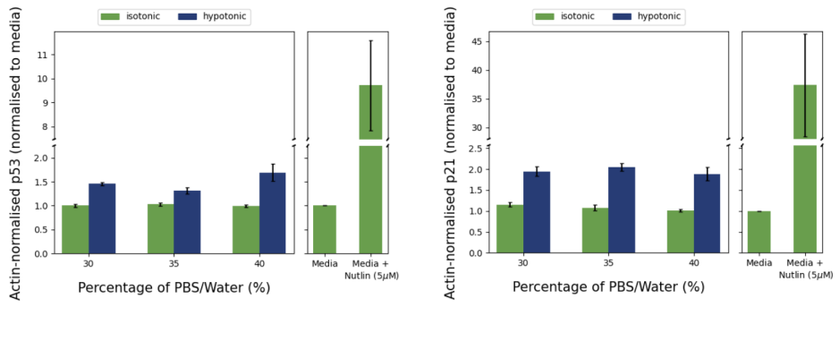


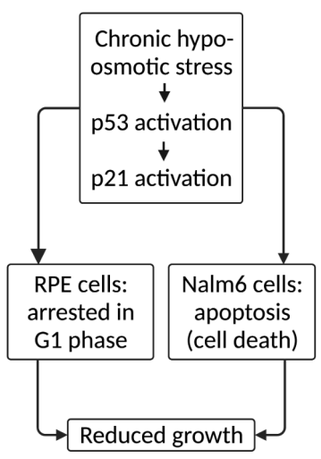
Figure 6. Observed intensities of p53 and p21 after 3 days CHS, normalised by actin. The expression of both proteins is significantly higher in the hypotonic conditions compared to the corresponding isotonic conditions. Nutlin is an activator of p53 and consequently p21, and hence Media+Nutlin acts as a positive control for both.

CONCLUSIONS

In contrast to RPE WT cells, CHS induces significant cell death in Nalm6 cells after 72h.

Though CHS increases the % of G1 cells in both RPE and Nalm6, the RPE cells undergo cell cycle arrest in G1, which is not the case for Nalm6 cells.

Upregulation of the p53 and p21 proteins is observed in both RPE and Nalm6 cells.



FUTURE DIRECTIONS

Experiments carried out in this work showed that CHS induces cell death in Nalm6 cells in a hypotonicity-dependent manner. This death may be caused by apoptosis (triggered by p53 over a longer timescale) or necrosis (instant, due to osmotic shock). Repeating these experiments with p53 knocked out may help identify which of the two is applicable in this case.

ACKNOWLEDGMENTS

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