Effect of lithium exposure on USP4 levels and subcellular localization in HeLa cells

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Introduction

Cancer patients with primary tumours in the lungs ultimately develop metastases in the brain 65% of the time. 85% of patients with lung cancer are of the non-small cell type, and their prognosis once they present with metastasis to the brain is very poor, with a median survival expectancy of 1-3 months. USP4, a deubiquitinating enzyme, is a known contributor to the development of brain metastases from primary lung cancer cells. The role of USP4 is the stabilization of substrates within the Wnt/β-catenin pathway. GSK-3 β has an already established regulatory role in this pathway, but USP4 may be an unknown substrate of the kinase. The principal goal of this study is to determine whether the USP4's subcellular localization will be influenced when a specific motif is phosphorylated by GSK-3 β. This will be explored by inhibiting the kinase with lithium, a known GSK-3 β inhibitor.

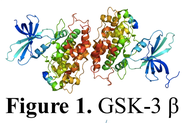


Figure 1. GSK-3 β

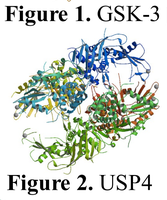


Figure 2. USP4

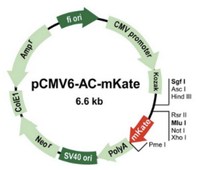
β–catenin IHSGATSTAPSLS

USP4 ex7 SLQSKSSTAPSRN

Figure 3. USP4's exon 7 has proposed GSK-3 β phosphorylation sites on the serines that occur in very similar sequence to β-catenin, which is known to be inhibited by GSK-3 β.

Methodology

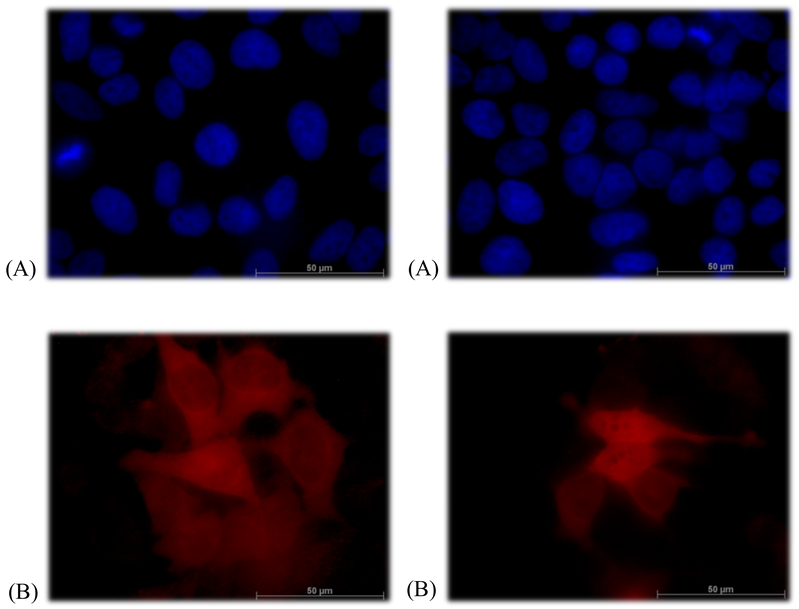
- HeLa cells were cultured, split, and grown at 37°C until they were ~80% confluent.
- 3 µL of the transfection reagent, GeneJuice (Millipore Sigma), was incubated in 100 µL DMEM for 10 min., then 1 µL DNA was added, and the mixture was applied to the HeLa cells.
- The cells were transfected with red fluorescent protein, mKate, which has the USP4 cDNA inserted into the SgfI/MluI sites of the vector (Origene).



- The cells were exposed to 10 mM LiCl inhibitor and 10 mM KCl control.
- After 24 hours of exposure, the cells were rinsed with PBS, incubated in 4% PFA for 10 min., and the cells were mounted on slides.
- The slides were observed through fluorescent microscopy, then scanned and processed with image analysis to determine the cytoplasmic to nuclear DNA ratio.

Results

Comparing and contrasting cytoplasmic and nuclear cells



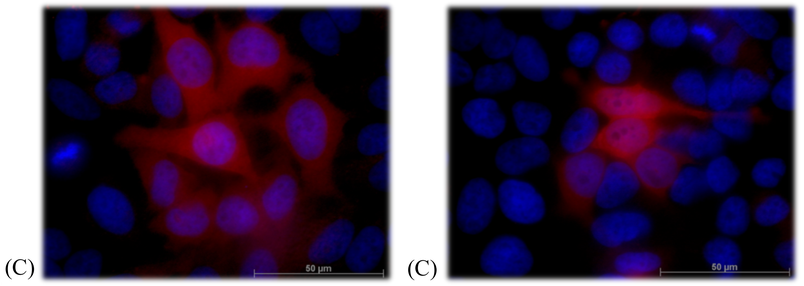


Figure 4. Example of cytoplasmic HeLa cell from KCl-induced sample.

Figure 5. Example of nuclear HeLa cell from LiCl-induced sample.

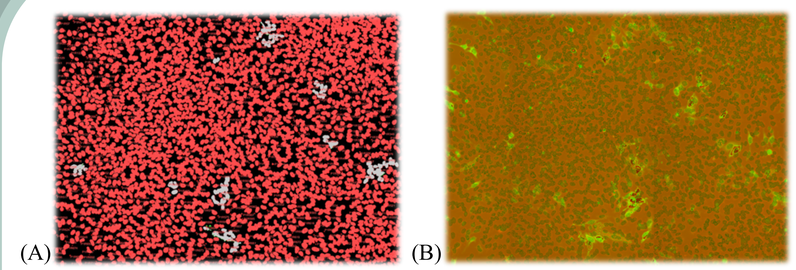


Figure 6. Sample software analysis of colocalization. KCl-induced HeLa cell slide scan, DAPI and mKate staining. An area of 48,800 µm² was assayed to determine the effects of potassium and lithium on the localization of USP4 within the cell. In (A), white regions indicate nuclear localization.

2 slides containing KCl-induced cells and 2 slides containing LiCl-induced cells were examined for a sample space 48,800µm² of cells per slide. The cells were given a colocalization score as a percent value, indicating the quantity of nuclear-localized cells within the region.

Table 1. Average percent scores of colocalized cells. A sample of 48,000 µm² on each slide was scored for colocalization as a percent, and averaged.

Condition	Colocalization Score (%)
Lithium Induction	13.5
Potassium Induction	8.5

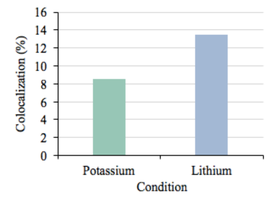


Figure 7. Average percent scores of colocalized cells. Histogram of % colocalization values.

Discussion

According to the software's image analysis results, there is evidence to indicate that in presence of lithium, the subcellular localization of USP4 within the HeLa cell occurs 37% more frequently in the nucleus as opposed to the control, potassium condition, in which the more cells are found with USP4 localized to the cytoplasm. The lithium condition also showed greater levels of transfected cells than the potassium condition. These results imply that there is a change in the presence of Li, the inhibition of GSK-3, which in turn fails to phosphorylate USP4, and allowing it to be more localized to the nucleus. In next steps, the use of a stronger inhibitor of GSK-3 β would likely yield more drastic differences.

Conclusion

Based on the subset of data evaluated, the effect of lithium on subcellular localization is an increased ratio of nuclear USP4 localization to cytoplasmic, and an increased level of HeLa cells transfected overall than the control potassium-induced condition.

References and Acknowledgements

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