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S100A14 Protein Expression in Prostate Cancer Cell Lines

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S100A14 Protein Expression in Prostate Cancer Cell Lines

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Introduction

KBU2046 is a small molecule designed to inhibit cell motility and invasion in vitro through binding to the cleft of HSP90β and CDC27 and affecting their downstream client proteins' phosphorylation status.[1] This has suggested effectiveness across many different types of cancers, and a wide set of prostate cancers that include PC3 cells.[1] Cell motility inhibition has been a critical step in clinical cancer treatment since its inception and is a key factor in mortality rate and disease progression.

Mass spectroscopy screens of PC3 cells treated with KBU2046 versus control have shown significant changes in expression of several key proteins.

S100A14 was one of the candidate proteins ascertained from the PC3 mass spectrometry screens; therefore, Western blots were performed to determine KBU2046's effect on the protein in primary cancer cells. S100A14's role in cancer is largely unclear, with it performing opposite functions in different cancer types.[2] This makes it an especially fascinating protein for investigation, and a potential key to understanding cancer mechanisms.

Experimental Methods

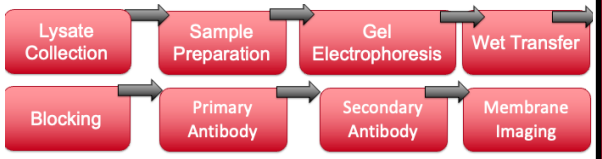

Fig. 1. Experimental workflow of protein assessment.

PC3 cells were initially treated with either DMSO, a negative control, or KBU2046, a known inhibitor of cell motility. After 72 hours of treatment, cells were harvested, with subsequent protein sample preparation afterwards. The next day, Western blotting procedure was followed to assess loading and S100A14 protein expression across cell line and treatment condition. This began with gel electrophoresis, as each individual gel tested the control and treatment conditions across a given cell line. The two cell lines within the individual were 1532NPTX, the patient's normal cell line, and 1532CPTX, the patient's cancerous cell line. After gel electrophoresis, proteins were transferred to nitrocellulose membrane via wet transfer sandwich and electrophoretic transfer. After that, membranes were incubated step-wise with blocking buffer, primary antibody to the desired protein or loading control, and secondary. Loading controls used were GAPDH, Beta-Actin, and CD9, always with the end goal of S100A14 Protein Assay. After secondary antibody incubation, membranes were imaged using Enzyme West Femto Super Signal Maximum Sensitivity Substrate across multiple exposures.

Background

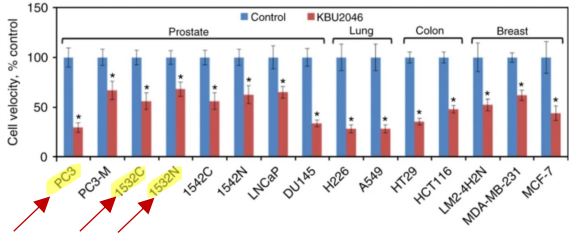

Fig. 2. KBU2046 inhibits cell migration across cancer type. Cells were treated for 72 hours with 10 μM KBU2046 or control, after which single-cell velocity was measured. Data are mean ± SEM, N=24, * indicates P<0.05.[1]

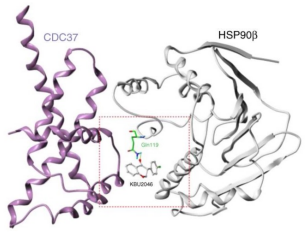

Fig. 3. *In silico* model of CDC37 (purple) and HSP90Beta (gray) depicting KBU2046 hydrogen bonding with Gln119 of HSP90Beta.[1]

Results: Cancer Cell Line 1532C

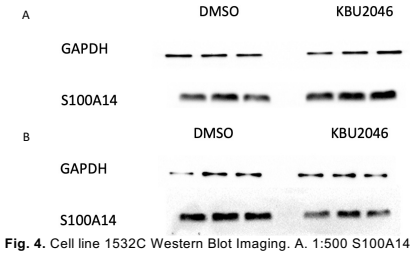

Fig. 4. Cell line 1532C Western Blot Imaging. A. 1:500 S100A14 Primary Antibody Dilution, 80 Protein Loading Concentration. B. 1:2000 S100A14 Primary Antibody Dilution, 40 Protein Loading Concentration.

Results: Normal Cell Line 1532N

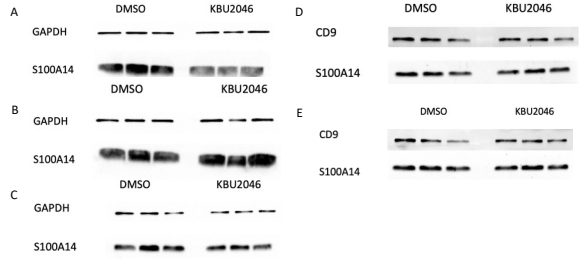

Fig. 5. Cell line 1532N Western Blot Imaging. A. 1:500 S100A14 Primary Antibody Dilution, 150 Protein Loading Concentration. B. 1:500 S100A14 Primary Antibody Dilution, 150 Protein Loading Concentration. C.1:1000 S100A14 Primary Antibody Dilution, 80 Protein Loading Concentration. D. 1:5000 S100A14 Primary Antibody Dilution, 40 Protein Loading Concentration. E. 1:5000 S100A14 Primary Antibody Dilution, 40 Protein Loading Concentration.

Conclusion and Future Directions

1. Gained experience in a laboratory setting that tackles real-world problems.
2. Learned technical procedure of Western blot and Cell Lysate Collection.
3. Investigated an unanswered question relating to disease mechanism.
4. Experienced the complexities of the research process.
5. Initial evidence suggests that KBU2046 increases S100A14 expression in 1532CPTX cells.
6. Results for 1532NPTX cells were inconclusive. Potential reasons include technical error, imaging error, variable cell behavior during culture stage.

References

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2. Basnet, S., Sharma, S., Costea, D. E., & Sapkota, D. (2019). Expression profile and functional role of S100A14 in human cancer. Oncotarget, 10(31), 2996–3012. https://doi.org/10.18632/oncotarget.26861