Inhibiting S100β and Troubleshooting Cell Growth

Department of Biology, DePauw University
Animesh Dali, Suhana Basnyat, Robert Passarelli, Dr. Nipun Chopra

Introduction

S100β is a calcium-binding protein of the S100 protein family that is found in adipose tissue and cardiac/skeletal muscles and can be expressed by many astrocytes. When damaged, astrocytes release S100B into the extracellular space. Studies have shown S100β levels increase in patients during the acute phase of brain damage. Recently, S100β has emerged as a biomarker for neural injury as S100β levels are elevated after traumatic brain injury (TBI) and other neurodegenerative diseases.

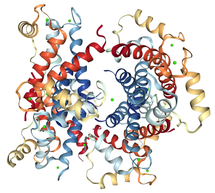

Figure 1:
X-ray structure of human Ca2+ loaded S100β

When extracellular S100β increases, RAGE receptors have been recorded to be over activated. This leads cascades of pro-apoptotic pathways in neuronal cells being activated. Therefore, there is a need to regulate S100β levels and thereby reduce S100β's apoptotic induction in neuronal cells.

Micro RNA (miRNA) are small single-stranded non-coding RNA molecules that function in RNA silencing and post-transcriptional regulation of gene expression. Mature miRNA are comprised of ~22 nucleotides and regulate RNA and gene expression via base-pairing with complementary sequences within mRNA molecules in the 3' untranslated region (UTR). Once paired with the 3' UTR on the mRNA, the miRNA can regulate translation by three methods; cleaving the mRNA strand, destabilizing the mRNA, and reducing efficiency of mRNA translation by inhibiting ribosomes. Currently, we have identified roughly 2500 effective human miRNA. Previous work in the Chopra lab utilized in silico methods and identified miR-4705 as a potential regulator of S100β.

Methods

Since we had previously identified miRNAs that could target S100β, our objective was to assess miR-4705 regulates S100β in-vitro.

Cell Lines and Culture

SK-Mel-28: Human melanoma cell line that express S100β established from patient-derived tumor sample. Cells were purchased from ATCC and grown for 8 passages. Cells were cultured with EMEM media with 10% FBS at 36C with 5% CO2 .

A375: Cell line expressing S100β that was isolated from the skin of a patient with malignant melanoma. Cells were purchased from ATCC and grown for 3 passages. Cells were grown in DMEM media with 10% FBS at 36C with 5% CO2.

Dealing with Contamination & Troubleshooting Cell Growth

`Contamination was a potent problem within the cell culture, resulting in the loss of cell culture. After contamination was detected in our lab, the incubator was decontaminated with a Stericycle. Media was treated with 0.7% Pen Strep. NIH-3T3 cells were obtained from Dr. Henning Schneider to test cell viability. T25 flasks were used instead of T75 flasks in attempt to test if cell contact would affect cell viability. A different batch of FBS was also used.

Cell Transfection

To test if S100β would be downregulated by siRNA, A375 cells were transfected on a 24 well plate with 6 repeats each of the conditions:

Untreated	Mock	NcSiRNA	siS100β	siActin
500µL Media only	450µL Media + 50µL Transfection Complex (RNAiMax, water, Opti-MEM)	450µL Media + 50µL Transfection Complex (RNAiMax, NcSiRNA, Opti-MEM)	450µL Media + 50µL Transfection Complex (RNAiMax, siS100β, Opti-MEM)	450µL Media + 50µL Transfection Complex (RNAiMax, siActin, Opti-MEM)

Cells were then lysed; protein estimates were found using the Bradford Assay and Western Blot was conducted to detect proteins.

Results and Conclusions

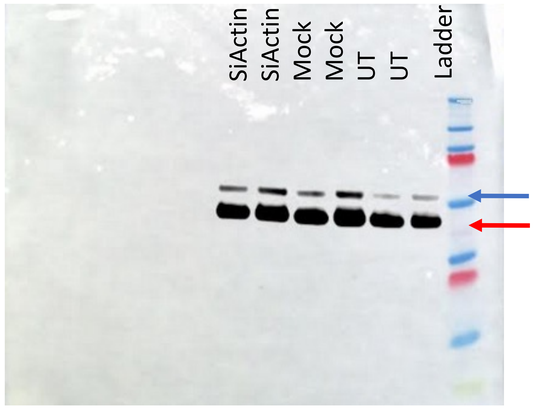

Figure 2:
Western Blot with SiActin, Mock and UT compared to Ladder.
The lanes with SiActin did not show decrease in actin concentrations, which suggests the transfection did not work as intended.

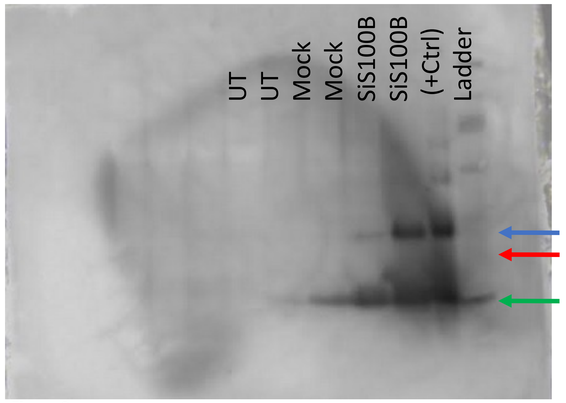

Figure 3:
Western Blot with +ve control, SiS100B, Mock and UT compared to Ladder.
The lanes with SiS100β gave bands for S100β suggesting that the transfection did not work as intended.

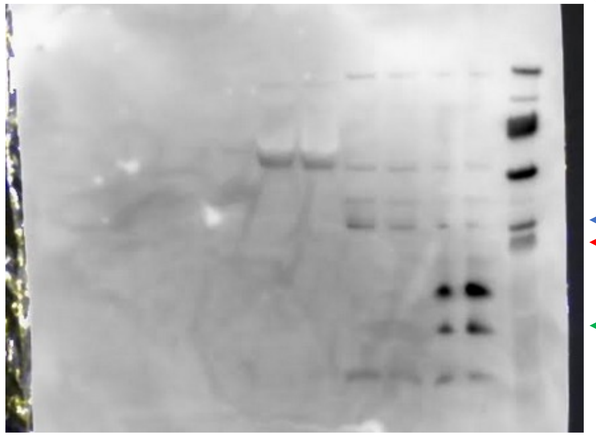

Figure 4:
Western Blot with varied pattern was conducted to ensure the bands we saw on SiS100β were not due to leakage from the +ve Control.

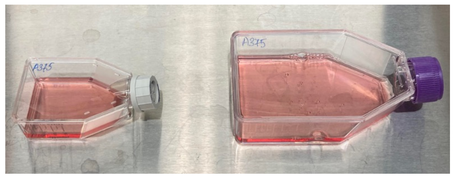

Figure 5:
Replacing T75 with T25
As our cells were not surviving in the incubator, we found in the literature that the issue could be a lack of cell contact initiating growth factors. By using T25 flasks, we hoped cell density would increase and cells would be able to initiate growth factors.

We had hoped to move onto the miRNA after testing S100β inhibition with the siRNA. Our transfection with siActin and siS100β did not appear to work. Following this, our Sk-Mel-28 and A375 cell lines started dying a few days post plating. We shifted our focus trying to investigate why our cells were dying. Contamination was found in the flasks, which we attempted to control by treating the media with Pen Strip. T75 flasks were also swapped for T25 flasks. When a new batch of FBS was used to treat the media, cell viability grew positively. miRNA inhibition of S100β still remains a pressing question and further investigation is recommended.

Acknowledgements

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We would also like to thank Dr. Henning Schneider for providing us with NIH 3T3 cells from his lab so that we could conduct experiments to troubleshoot out cell growth.