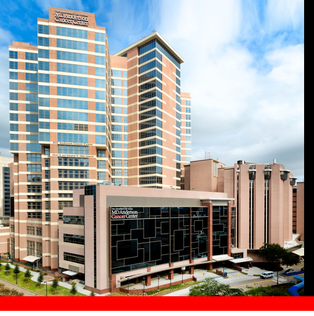

The Role of Ube2S in Transcriptional Inhibition at Double Stranded DNA Break Sites

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Introduction

- Double stranded DNA breaks (DSBs) are a highly lethal form of DNA damage that can lead to genomic instability, an enabling hallmark of cancer.[1]
- DSBs can be repaired through an ATM-mediated signaling cascade for repair proteins initiated by phosphorylated H2AX. Previously, this pathway was characterized by the conjugation of Lys63-linked ubiquitin chains on damaged chromatin via RNF8 and Ubc13.
- However, recent research has shown that RFN8 works with another conjugating enzyme, Ube2S, to add Lys11-linked ubiquitin chains at sites of DNA damage, resulting in generalized transcriptional inhibition.[2]
- Since the mechanisms by which this method of ubiquitination contributes to the DNA damage response at DSB sites are still unknown, we aimed to investigate the role of Ube2S in regulating transcription of genes near specific DSB sites.

Methods

1. DSBs were induced at specific sites in control and Ube2S knockdown DiVA cells by treatment with 4-hydrotamoxifen (4-OHT).
2. Following 4 hours of treatment, RNA was extracted from cells and reverse transcribed into cDNA (Figure 1).
3. Transcription of specific genes near damage sites (Table 1) in treated and untreated cells was evaluated using qPCR with SYBR Green reagent and quantified through the following equation: Fold change = $2^{-\Delta\Delta Ct}$.[3]
4. Ube2S knockdown was performed twice using different siRNAs and success confirmed through Western blot.

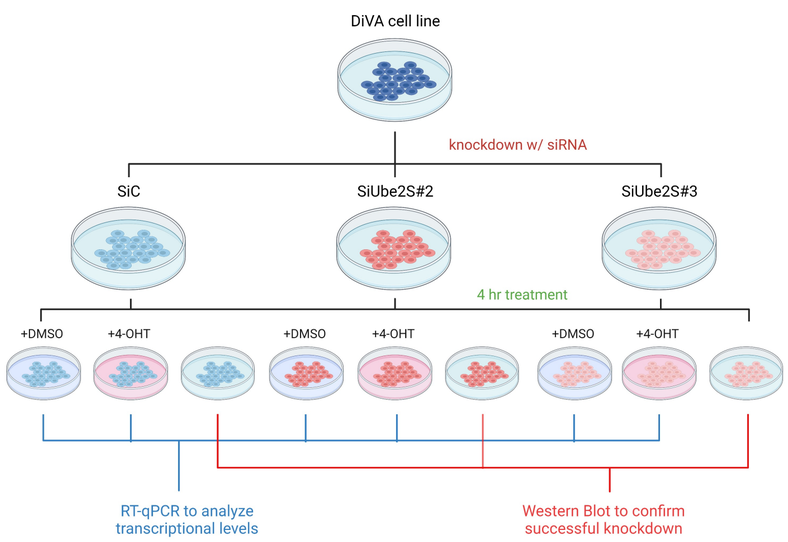

Figure 1. Experimental layout. Dishes with no label received no treatment and were instead utilized for protein extraction and Western blot analysis.

MIS12	RBMXL1	KLF7	HUNK	RPLP0
GNE	KLF6	TRIM37	CYB56D1	B2M

Table 1. Genes chosen for analysis of transcription. Genes in blue were used as housekeeper genes.

Results

- Treatment with 4-OHT resulted in lower levels of transcription of genes at DSB sites in control knockdown cells.
- Conversely, treated Ube2S knockdown cells demonstrated higher levels of transcription (Figures 2 and 4).
- These results suggest lower levels of transcriptional inhibition taking place at DSB sites in these cells.

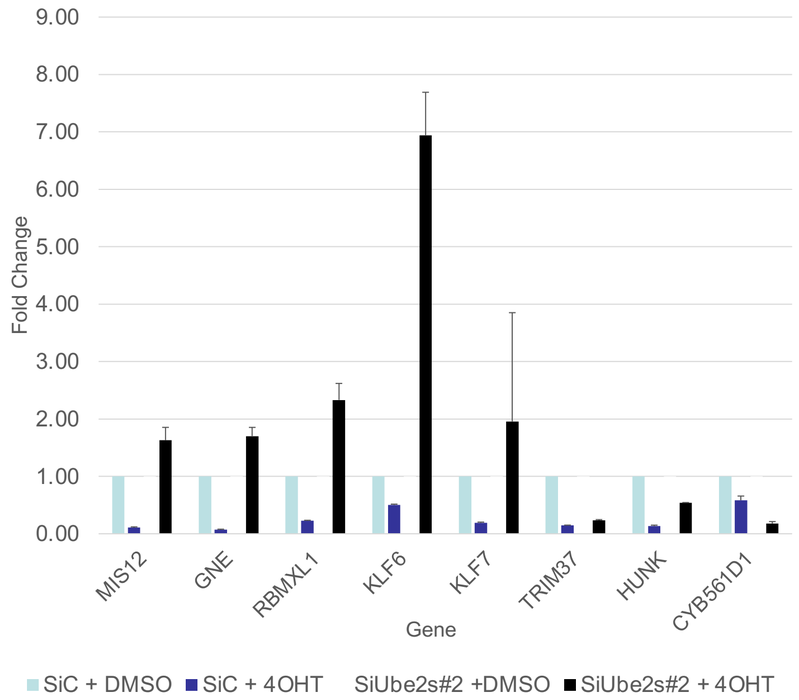

Figure 2. Relative transcription levels for control and cells knocked down with siUbe2s#2. RPLP0 was used as housekeeper gene for comparison.

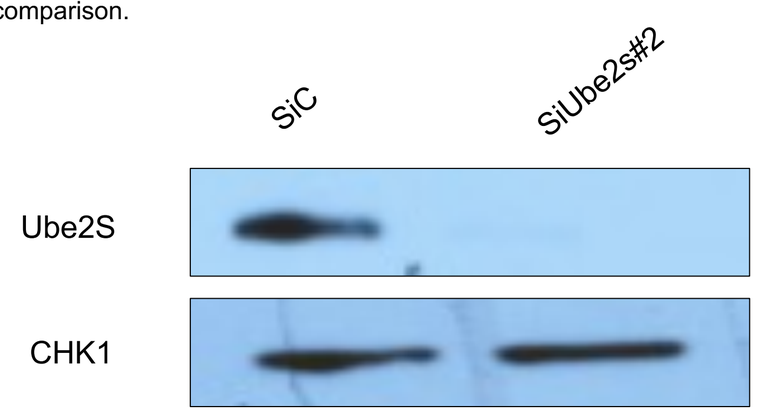

Figure 3. Western blot confirming Ube2S knockdown by siUbe2s#2. CHK1 was used as control.

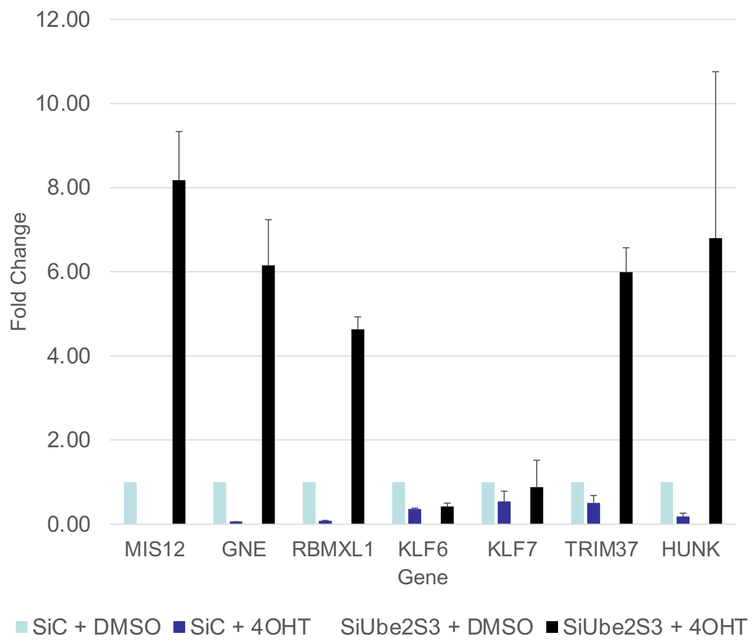

Figure 4. Relative transcription levels for control and cells knocked down with siUbe2s#3. RPLP0 was used as housekeeper gene for comparison.

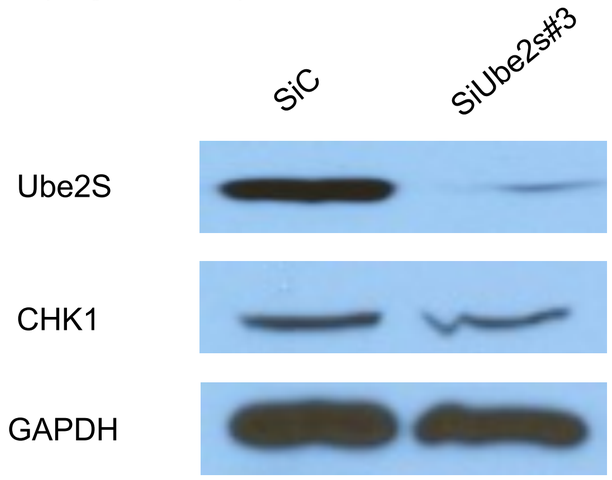

Figure 5. Western blot confirming Ube2S knockdown by siUbe2s#3. CHK1 and GAPDH were used as controls.

Conclusions/Future Work

- Ube2S aids in transcriptional inhibition at DSB sites following DNA damage, providing more insight into the role of Lys11-polyubiquitination in the DNA damage response.
- Future investigations should examine the role of other key proteins involved in conjugating Lys11-linked ubiquitin chains.

References

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