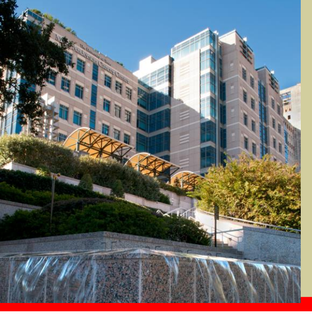

Exploring the functions of PHF20/PHF20L1 within the NSL-transcriptional complex

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Abstract

Targeted DNA is frequently observed for its characterization and role in specific protein complexes. Chromatin consists of DNA and a protein called histone. Histones support and organize the DNA by coiling and changing their shape to assist in the formation of chromosomes. Chromatin aids in processes like transcription and DNA repair by its ability to condense and uncondense DNA. The ChIP assay allows for selection of protein bound to DNA through antibody specificity which allows for quantification of bound DNA through qPCR. Previous studies have shown the NSL complex expressing PHF20 gene which is related to non-small cell lung cancer and has a paralog PHF20L1. The presence or absence of proteins can repress or enhance the process of transcription.

To better understand the role on the molecular mechanism of the NSL complex a knockdown of PHF20 and PHF20L1 were created in U2OS cells. Protein complexes can be studied through crosslinking, immunoprecipitation, and western blotting. PHF20 and PHF20L1 single knockdown efficiency was assessed through western blot. Single knockdown for PHF20 and PHF20L1 cell lines were cross linked binding the DNA to protein using formaldehyde. Sonication testing sheered the chromatins and a comparison of non-sonicated and sonicated fraction was obtained to compare DNA levels. Phenol chloroform extraction continued the purification of DNA. Western blot analysis displayed a knockdown of PHF20L1 and PHF20 demonstrating the effectiveness of these mutants. The cross-linkage of a single knockdown in PHF20 demonstrated at the NSL3 complex the amount of purified DNA in PHF20-4 increased drastically compared to the empty vector.

Through ChIP assays, we prepared these mutants for chromatin immunoprecipitation and ran a qPCR that expressed the PHF20-4 cell line with an increased amount of DNA compared to the empty vector. PHF20 and PHF20L1 proved successful single knockdowns as observed through the amount of protein signal in the western blots. Studying molecular mechanisms can help progress or change targeted cancer therapies. Creating knockdowns of PHF20 and PHF20L1 and performing ChIP assays will help us understand these cells lines specific role within the NSL complex.

Methods

- IgG antibodies and sepharose beads were utilized during chromatin immunoprecipitation (ChIP) for DNA clean up. Washing and eluting of the beads bound to the antibodies removed non-specific proteins and other unrelated cellular material.
- Quantification of DNA was observed by qPCR.
- Western blot

Aim

To understand the role of PHF20 within NSL complex we needed to generate single knockdowns of this cell line and its paralog PHF20L1 in U2OS cells. Detecting specified proteins within this complex will allow us to observe the role between the interplay of these genes. Preparing this mutant for ChIP will ensure the purification of the DNA is achieved to detect the amount of DNA concentration through qPCR analysis. Retrieving consistent measurements of DNA is critical for molecular biological studies in order to observe essential processes of the cell.

Results

Figure 1: NSL complex expressing PHF20

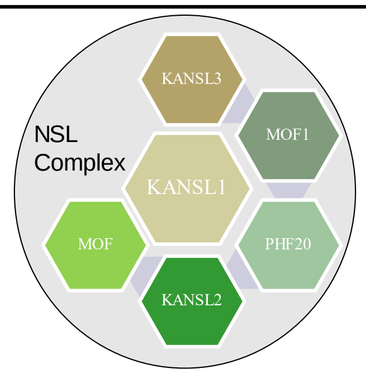


Figure 3: Analyzing Single knockdown efficiency in PHF20L1

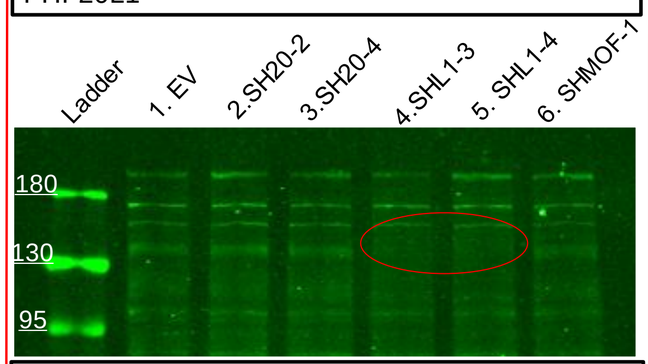


Figure 5: PHF20 and PHF20L1 protein domains

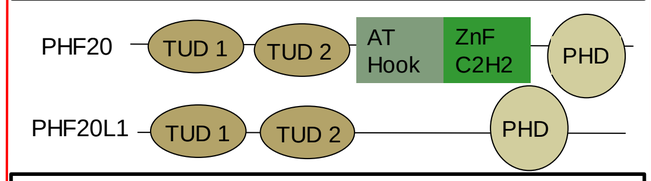


Figure 6: 2KD/1KD sonication testing of PHF20/PHF20L1

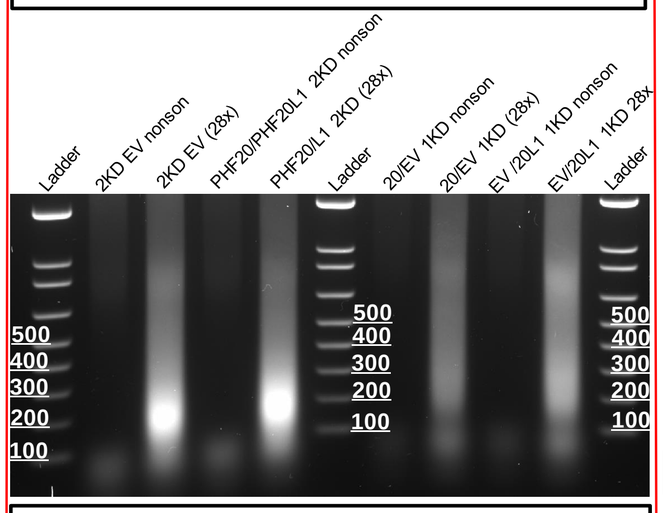


Figure 8: Sonication testing of PHF20

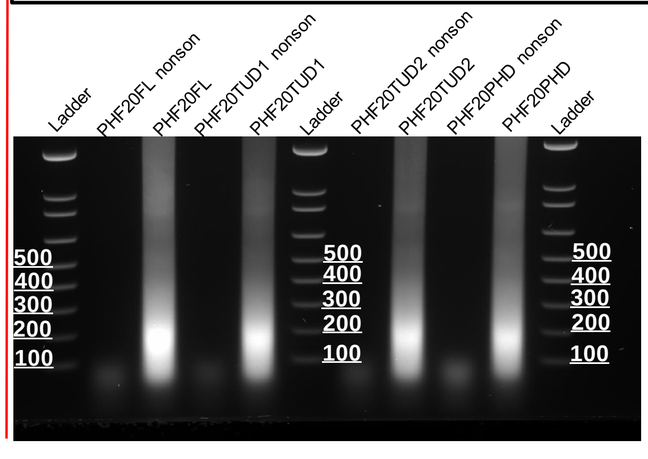


Figure 2: Generating single knockdown of PHF20/PHF20L1 in U2OS cells

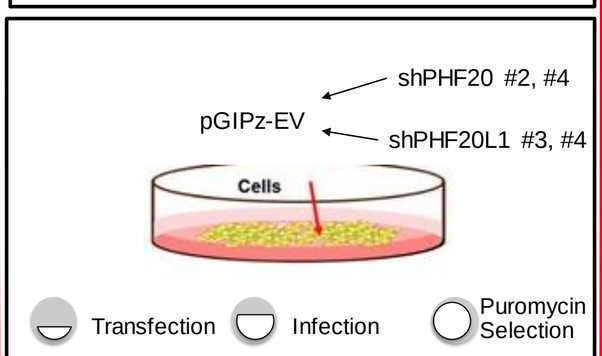


Figure 4: Analyzing Single knockdown efficiency in PHF20

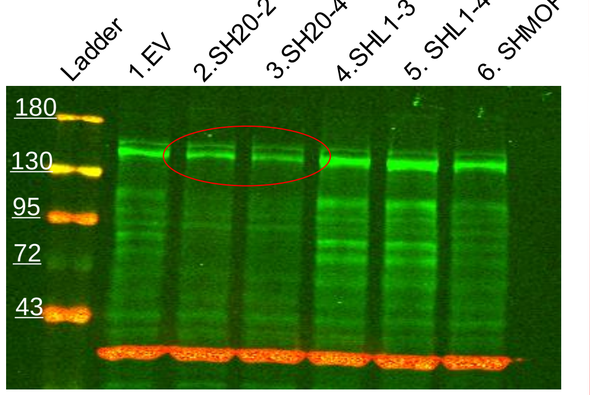


Figure 7: 1KD resonication testing of PHF20/PHF20L1

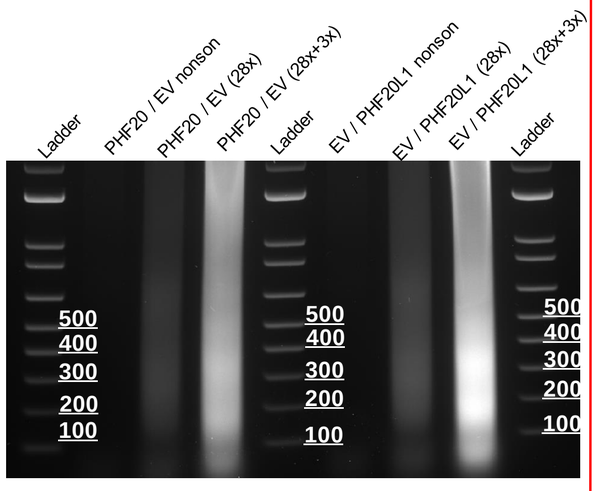


Figure 9: Sonication testing of PHF20L1

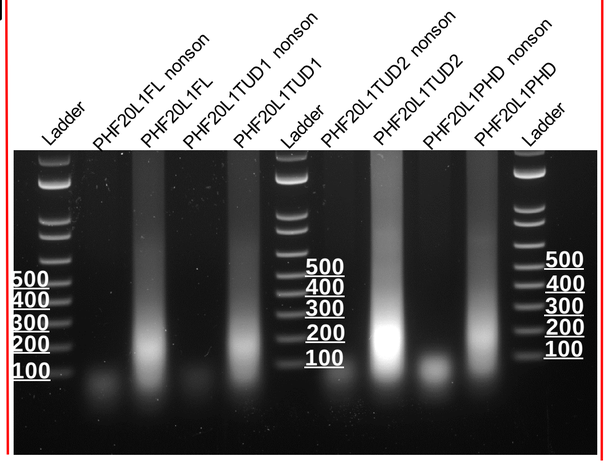
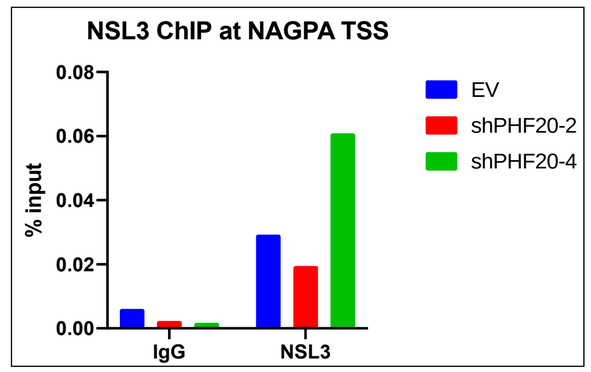
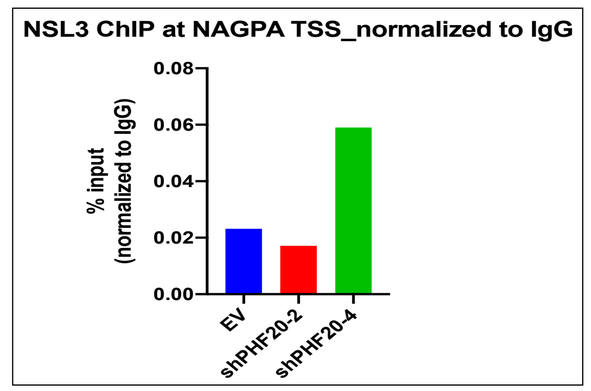


Figure 10: Amount of DNA observed through qPCR





Future Studies

Run ChIP replicates of these PHF20 knockdown cell lines to see what data is repeated. Observe a double knockdown of PHF20 and PHF20L1 in these cells will provide data on how repressing both proteins will impact the overall function of the complex. Perform ChIP on PHF20L1 knockdown cell lines and compare to PHF20 knockdown cell lines.

Conclusion

Through our western blot analysis we were able to observe the efficiency of the single knockdown in PHF20/PHF20L1. Understanding the role of PHF20/PHF20L1 within the KANSL3 complex will provide information on the molecular mechanisms within these cancer cells. The analysis of PHF20/PHF20L1 within the NSL complex through ChIP assays will demonstrate if these specific proteins bind to a specific DNA sequence and if there role is dependent on each other.

References

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