Hayden Shinn

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Professor Quan Wan

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Group 3 Final Project Individual Write-up

In the study, *BRG1 HSA domain interactions with BCL7 proteins are critical for remodeling and gene expression*, Dietrich et al. investigate the role of the BRG1 HSA domain and its relationship with BCL7 proteins. They explore how these interactions influence BRG1 functionality in chromatin remodeling, a process crucial for gene expression. The SWItch/Sucrose Non-Fermentable (SWI/SNF) complex are "multi-subunit, ATP-dependent molecular machines" that play a key role in controlling chromatin accessibility and gene transcription regulation (Chen et al., 2023). Specifically, SWI/SNF alters the contacts between histones and DNA in chromatin, which either allows or blocks the access to genetic material for transcription and gene expression (Dietrich et al., 2023). The Brahma related gene 1 (BRG1) and Brahma (BRM) are critical subunits of this complex as they bind to chromatin and interact with various transcription factors (Dietrich et al., 2023). Within BRG1, the helicase/SANT-associated (HSA) domain has been found to further mediate vital protein interactions as a binding partner within the SWI/SNF (Trotter et al., 2008). Because several cancer-associated mutations have been identified in the HSA domain, Dietrich et. Al found it necessary to further investigate this domain and understand the mechanisms at play regarding cancer and chromatin remodeling (Sankareswaran et al. 2018). One HSA domain interaction of key interest is that of the B-cell CLL/lymphoma 7 protein family (BCL7). BCL7 members A, B and C, also part of the SWI/SNF complex, "bind to BRG1 and are associated with cancer incidence and progression" (Dietrich et. Al 2023). The lack of investigation into the BCL7 protein family and its role in chromatin remodeling served largely as motivation for Dietrich et al. to further expand and characterize the role of BCL7 in the SWI/SNF complex and how irregularities in its function can lead to cancer.

The first prediction of this paper is that the deletion of the HSA domain in BRG1 would hinder BRG1-driven gene expression (Dietrich et. al, 2023). To test this hypothesis, SW-13 cell lines were constructed that express wild-type BRG1 (iBRG1) or BRG1 with the HSA domain deleted (iΔHSA) upon induction with doxycycline. When analyzing the transcriptomes of both treatment groups, 256 differentially expressed genes (DEGs) were identified when iBRG1 cells were compared with control cells while only 68 DEGs were identified with the expression comparison between iΔHSA and control cells. Similarly, as

shown by the volcano plots in Figure 1, our group was able to identify 157 DEGs when iBRG1 was expressed but only 21 DEGs for iΔHSA. These results were able to support the prediction that iΔHSA had a reduced ability to modify the gene expression of target genes (Dietrich et al., 2023).

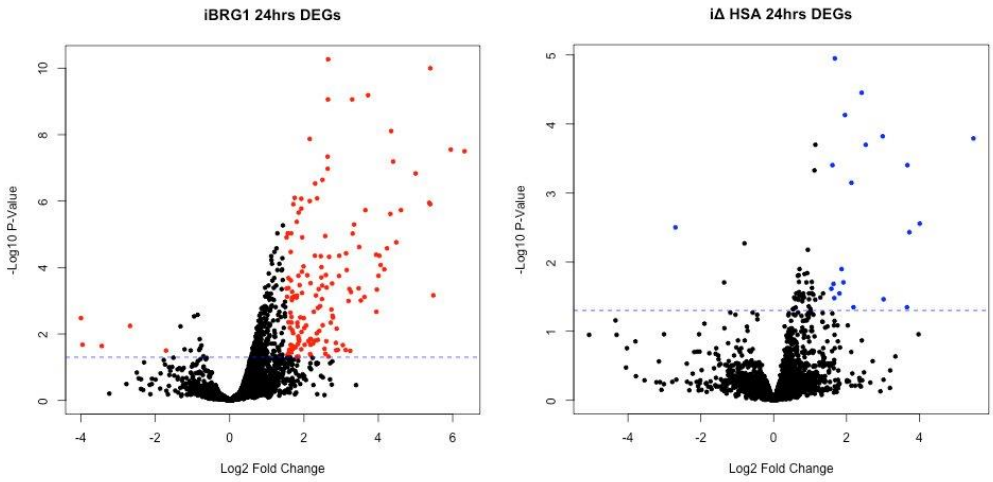

Figure 1: Volcano plots showing differentially expressed genes for iBRG1 cells (red) and iΔHSA cells (blue) after 24 hours of treatment with doxycycline compared to control cells.

In terms of downstream effects, the authors identified multiple pathways implicated in cancer progression. Similarly, through our enrichment analysis we identified multiple pathways that involved various types of cancer. For example, non-small cell lung cancer, endometrial cancer and melanoma were identified in both cluster networks (Figure 2) we generated for iBRG1 and iΔHSA. The iΔHSA cluster network also included prostate cancer, pancreatic cancer, glioma and bladder cancer which could indicate the detrimental effects of the deletion of the HSA domain in BRG1. We also identified certain target genes in Figure 3 such as SMARCA4 that had consistent expression across both iBRG1 and iΔHSA meaning it was largely unaffected. However, other genes such as SLC15A3 and COL26A1 show a decrease in the magnitude of expression which could have serious effects as SLC15A3 for example is involved in the transport of amino acids and oligopeptides (Weizmann Institute of Science, 2024). From these results we can determine that HSA is required to drive a full transcriptional program (Dietrich et al., 2023).

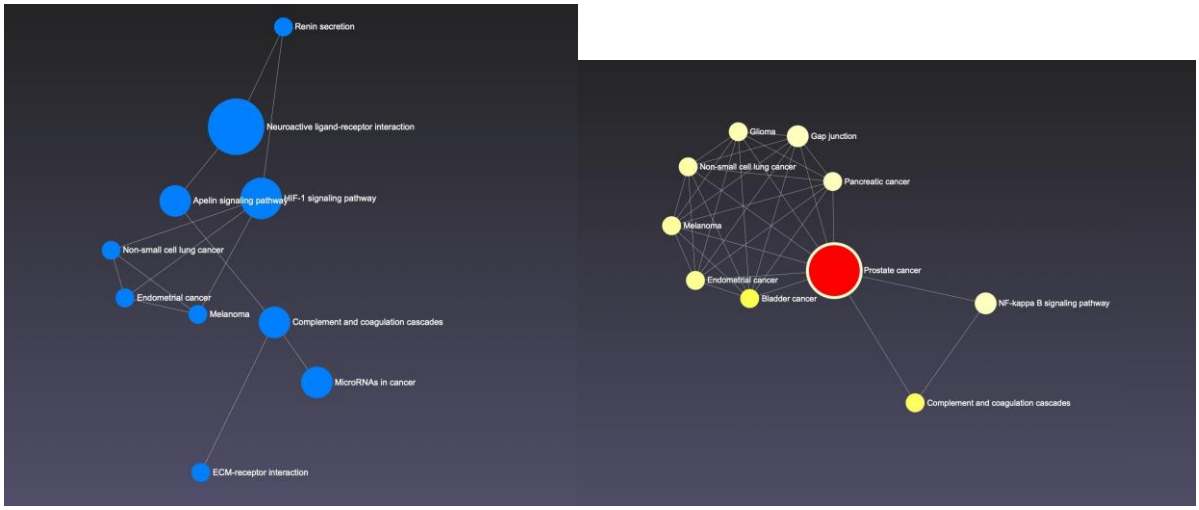

Figure 2 Cluster networks for iBRG1 (left) and iΔHSA (right) in 24-hour DEGs.

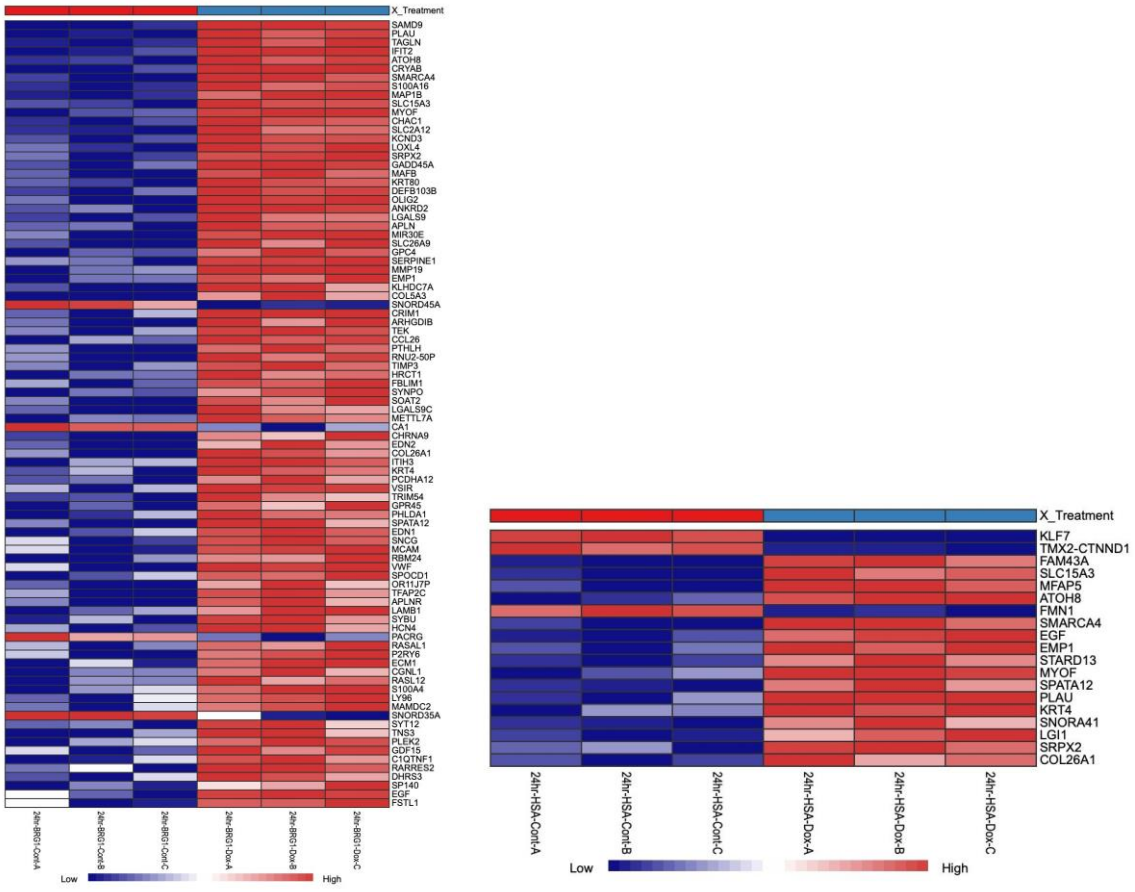

Figure 3: Heatmaps for iBRG1 and iΔHSA 24-hour DEGs.

The authors also wanted to investigate long term treatment of iBRG1 and iΔHSA so they treated the cells with doxycycline for 14 days to see if there were any prolonged effects or cancerous implications. Through transcriptome analysis, the authors identified 2622 DEGs in iBRG1 cells compared with control and 214 DEGs in iΔHSA compared to the control cells. There was a large increase in transcriptional changes in both groups. Our group was able to identify 2603 DEGs in iBRG1 and 219 DEGs in iΔHSA as shown in Figure 4 which also suggests a significant change in transcription.

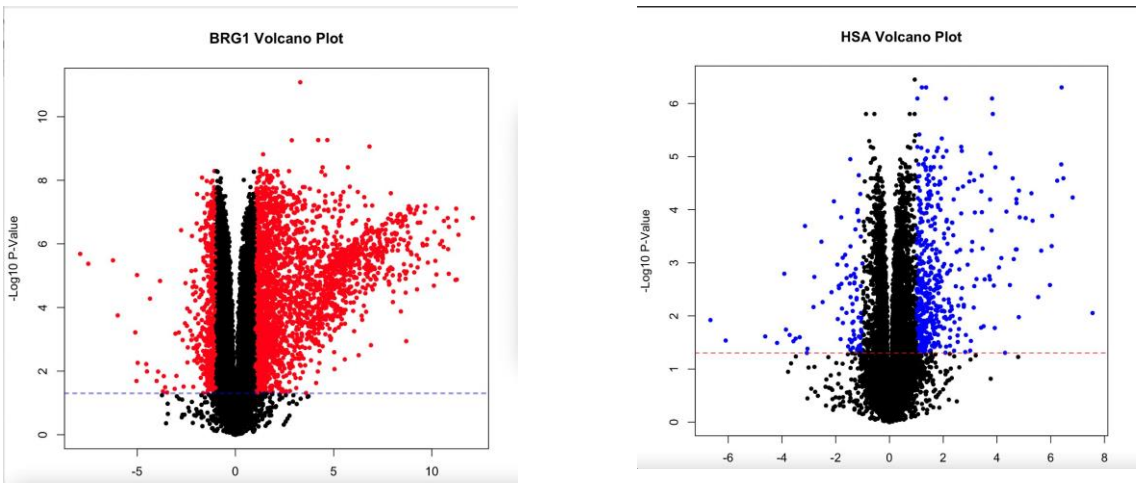

Figure 4: Volcano plots for long term DEGs in iBRG1 (red) and iΔHSA (blue).

Our heatmaps also show a strong upregulation of BRG1-driven genes in the iBRG1 samples while the iΔHSA samples show a less pronounced change in gene expression (Figure 5). This further suggests that the HSA domain plays a significant role in SWI/SNF driven changes in gene expression (Dietrich et al., 2023).

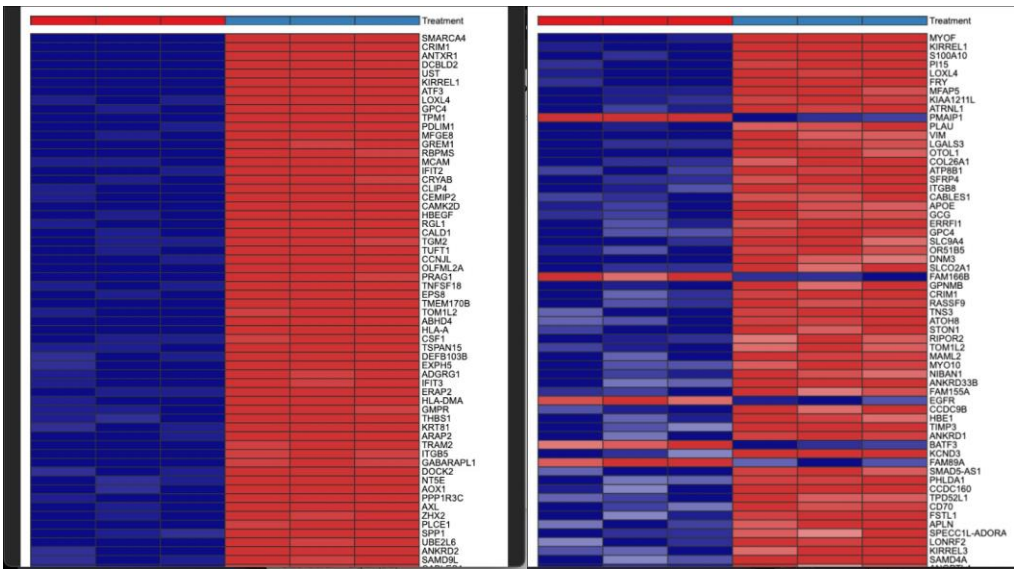

Figure 5: Heatmaps of long term DEGs in iBRG1 (left) and iΔHSA (right).

One mechanism affecting gene expression that the paper investigated was the ability to bind to chromatin with and without the HSA domain present. To this they generated sequencing data using CUT&RUN which allowed the binding sites of iBRG1 and iΔHSA to be determined. The paper found that the loss of the HSA domain does not affect the ability of BRG1 to localize transcription start sites (Dietrich et al., 2023). As shown in Figure 6, we were also able to come to the same conclusion that there was significant enrichment of the signal for both iBRG1 and iΔHSA at a comparable level meaning the HSA domain was not essential for transcription start site binding.

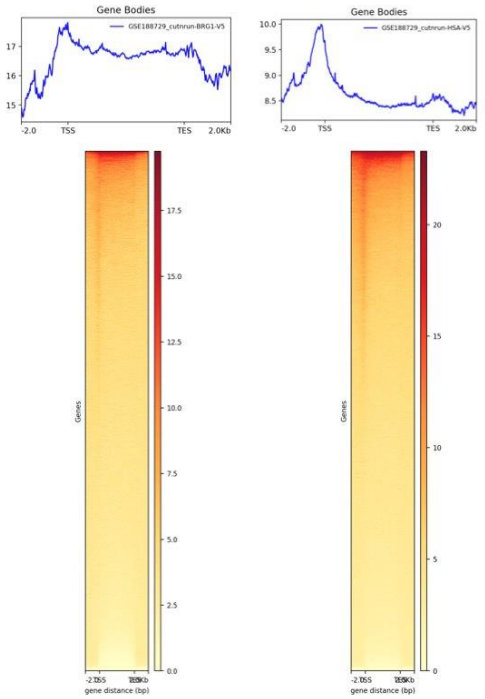

Figure 6: Heatmaps and metaplots of iBRG1 or iΔHSA CUT&RUN signal in a 6-kb window around all transcriptional start sites, and they are scaled to represent all gene bodies.

Additionally, peaks were called for both datasets alike to the authors, we determined that there were a similar number of peaks and coverage for both iBRG1 and iΔHSA as shown in Figure 7. While there is a slight variation in the coverage and peaks, this difference is not significant enough to imply that the HSA domain is responsible for these discrepancies. This further proves that the HSA domain does not play a role in DNA site binding specification and some other protein or domain is responsible for binding specification (Dietrich et al., 2023).

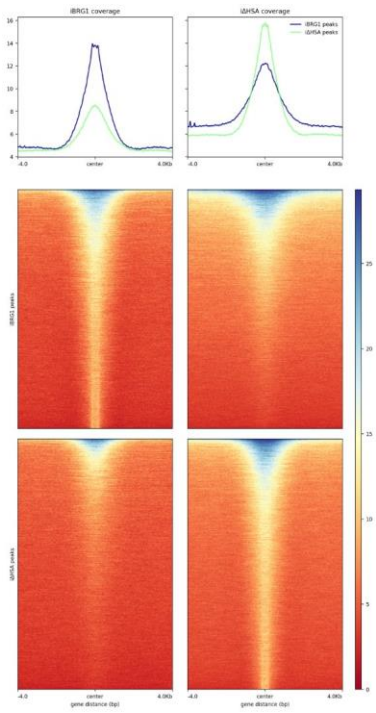

Figure 7: Heatmaps and metaplots of iBRG1 or iΔHSA CUT&RUN signal at called iBRG1 or iΔHSA CUT&RUN peaks

Another significant prediction made by the authors was that BCL7 protein interactions are critical for BRG1 functionality, and the absence of this interaction drives the phenotypes observed when the HSA domain is deleted from BRG1 (Dietrich et al., 2023). To test this, siRNAs were used to target BCL7 proteins in combination with the induced expression of BRG1 (Dietrich et al., 2023). A strong knockdown of all BCL7 proteins via siRNA transfection. As a result, many of the BRG1 target genes were not expressed in knockdown samples demonstrating that the BCL7 proteins are required for changes in gene expression driven by BRG1 (Dietrich et al., 2023). Similar to the paper's examination of iΔHSA driven transcription, our group observed a reduced change in gene expression for the BLC7A, B and C knockdown as demonstrated in Figure 8. They identified 61 BRG1-dependent DEGs in siABC cells while we observed 13 DEGs for the same group (Dietrich et al., 2023). Furthermore, we also observed that BRG1 serves primarily to upregulate genes and that "HSA domain is essential for the interaction of BRG1 with the BCL7 proteins, and this interaction is required for BRG1-driven SWI/SNF function" (Dietrich et al., 2023).

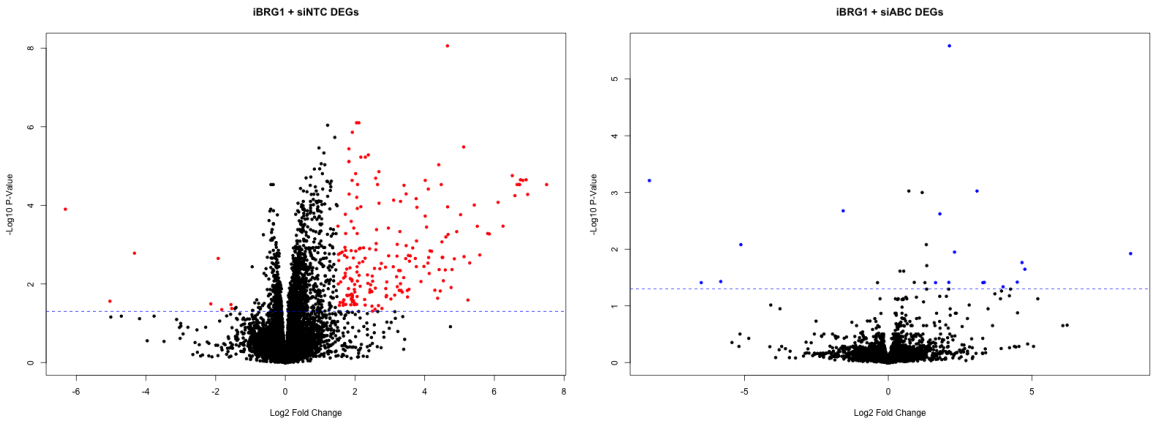

Figure 8: Volcano plots of DEGs identified by RNA-seq in iBRG1 cells after knockdown of a non-template control (left, red) or of BCL7A + BCL7B + BCL7C (right, blue).

In terms of the validity of the methods and results, the authors used suitable methods to analyze the changes in expression via RNA sequencing techniques as well as changes in chromatin accessibility through the utilization of CUT&RUN. The methods utilized by the paper were described in a manner such that they could be reproduced as proven by our analysis. They were able to generate results consistent with prior findings and used appropriate data analysis methods to generate charts and figures. Any biases or discrepancies in the data were explained thoroughly. One drawback identified in the paper was the choice to use CUT&RUN over another sequencing method such as ChIP-seq to identify and analyze chromatin accessibility and resulting expression as it is considered the gold standard in this realm. CUT&RUN is favored for analyzing open regions of chromatin which could limit the insights it is able to draw. On the other hand, ChIP-seq can identify a wider range of patterns in chromatin accessibility, even in closed regions (Simmonds et al., 2017). Dietrich et al. determined that HSA does not play a role in DNA site binding specification, but it would be interesting to see if that conclusion remained the same if ChIP-seq was used.

References

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