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Chromatin remodeling complexes and their role in gene silencing during cell differentiation

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Abstract

Chromatin remodeling complexes are among the most powerful molecular machines that cells use to switch genes on or off during differentiation, yet the comparative contributions of different complex families remain poorly mapped in a single cell system. This research examined how SWI/SNF and NuRD complexes regulate gene silencing during the transition of murine embryonic stem cells (mESCs) toward a neural progenitor fate. Chromatin immunoprecipitation followed by sequencing (ChIP-seq) was used to profile H3K27me3 and H3K9me3 marks in wild-type, SWI/SNF-depleted, and NuRD-depleted cells at days 0, 3, and 6 of differentiation. Loss of SWI/SNF led to a 41.6% reduction in de novo H3K27me3 deposition at lineage-inappropriate promoters, while NuRD depletion reduced H3K9me3 spreading across pericentromeric heterochromatin by 33.8%. RNA-seq confirmed that both depletions resulted in the aberrant persistence of pluripotency-associated transcripts. These results suggest that SWI/SNF and NuRD operate through complementary silencing mechanisms during early neural commitment.

Keywords: Chromatin remodeling, gene silencing, cell differentiation, SWI/SNF complex, NuRD complex, histone modification, embryonic stem cells, neural progenitor, epigenetics, ChIP-seq

Introduction

Gene silencing is not a passive event. It demands active, energy-consuming molecular machinery that reshapes chromatin architecture to deny transcriptional activators access to DNA [1]. Among the best-characterised families of such machinery are the ATP-dependent chromatin remodeling complexes, which slide, eject, or restructure nucleosomes in response to developmental and environmental cues [2]. Two families in particular-SWI/SNF (also called BAF/PBAF in mammals) and NuRD (nucleosome remodeling and deacetylase)-have been linked to silencing programmes that guide embryonic cells away from pluripotency and toward lineage-specific fates [3, 4]. SWI/SNF complexes are best known for their activating functions, opening chromatin at enhancers and promoters to enable gene expression [5]. Accumulating evidence shows they also participate in repression, particularly by facilitating Polycomb-mediated H3K27me3 deposition at genes that must be turned off during differentiation [6]. NuRD, by contrast, couples nucleosome remodeling with histone deacetylation, and its loss has been associated with impaired heterochromatin formation and derepression of repeat elements [7]. Despite these individual advances, few investigations have compared SWI/SNF and NuRD side by side within the same cell type and differentiation timeline. This gap matters because the two complexes may act on overlapping or distinct genomic targets, and understanding that division of labour could clarify why mutations in either family produce different developmental disorders in humans [8]. In this research, we used shRNA-mediated depletion of the catalytic subunits BRG1 (SWI/SNF) and CHD4 (NuRD) in mESCs undergoing directed neural differentiation, followed by ChIP-seq for two repressive histone marks and RNA-seq for transcriptome profiling across a six-day time course.

Material and Methods

Material: Murine embryonic stem cells (E14Tg2a line) were obtained from the European Collection of Authenticated Cell Cultures and maintained on gelatin-coated plates in serum-free 2i/LIF medium at the Masuria Institute of Freshwater Biology, Olsztyn, Poland.

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Research was conducted between May 2023 and September 2024. Cells were passaged every 48 hours and tested negative for mycoplasma contamination before each experiment. Neural differentiation was initiated by withdrawing 2i/LIF and culturing cells in N2B27 medium for six days. The institutional laboratory safety committee approved all recombinant DNA work under protocol MIFB-rDNA-2023-04, dated 8 April 2023.

Reagents and Chemicals

CHIR99021 and PD0325901 (2i inhibitors) were purchased from Selleck Chemicals (Houston, TX, USA). Recombinant mouse LIF was from Millipore (Burlington, MA, USA). shRNA lentiviral vectors targeting Smarca4 (BRG1) and Chd4 were obtained from Sigma-Aldrich MISSION library (St Louis, MO, USA). ChIP-grade antibodies included anti-H3K27me3 (Cell Signaling Technology, 9733) and anti-H3K9me3 (Abcam, ab8898). Protein A magnetic beads were from Invitrogen (Carlsbad, CA, USA). All restriction enzymes and DNA polymerases came from New England Biolabs (Ipswich, MA, USA).

Instrumentation

ChIP-seq libraries were sequenced on an Illumina NextSeq 550 (single-end 75 bp). RNA-seq libraries were prepared with the NEBNext Ultra II Directional RNA Library Prep

Methods

Lentiviral transduction was performed at a multiplicity of infection of 5, and puromycin selection (2 ug/mL) was applied for 48 hours. Knockdown efficiency exceeded 80% for both targets as verified by western blot densitometry. Cells were harvested at days 0, 3, and 6 of differentiation. For ChIP-seq, approximately 10 million cells per condition were crosslinked with 1% formaldehyde for 10 minutes, quenched with glycine, and sonicated to yield 200-500 bp fragments. Immunoprecipitation was performed overnight at 4 degrees Celsius. Libraries were prepared with the ThruPLEX DNA-seq Kit (Takara Bio, Kusatsu, Japan). Reads were aligned to mm10 with Bowtie2, and peaks were called using MACS2 (FDR < 0.05). Differential peak analysis used DiffBind in R. RNA-seq reads were aligned with STAR and quantified with featureCounts; differential expression was assessed using DESeq2 (adjusted p < 0.05, absolute log2 fold change > 1).

Results

Table 1: Changes in Repressive Histone Marks Following Chromatin Remodeler Depletion

Condition	H3K27me3 Peaks (Day 6)	Change vs WT (%)	H3K9me3 Peaks (Day 6)	Change vs WT (%)	DEGs (RNA-seq)
Wild-type	8,417	-	14,326	-	-
SWI/SNF KD	4,916	-41.6	13,894	-3.0	1,243
NuRD KD	7,981	-5.2	9,488	-33.8	978

Table 1 shows that SWI/SNF depletion caused a sharp 41.6% drop in H3K27me3 peaks by day 6, with only a minor effect on H3K9me3. NuRD depletion showed the knockdown and an opposite pattern: a 33.8% reduction in H3K9me3 peaks while H3K27me3 was barely affected. RNA-seq identified 1,243 differentially expressed genes in the SWI/SNF knockdown and 978 in the NuRD knockdown, with an overlap of 214 genes.

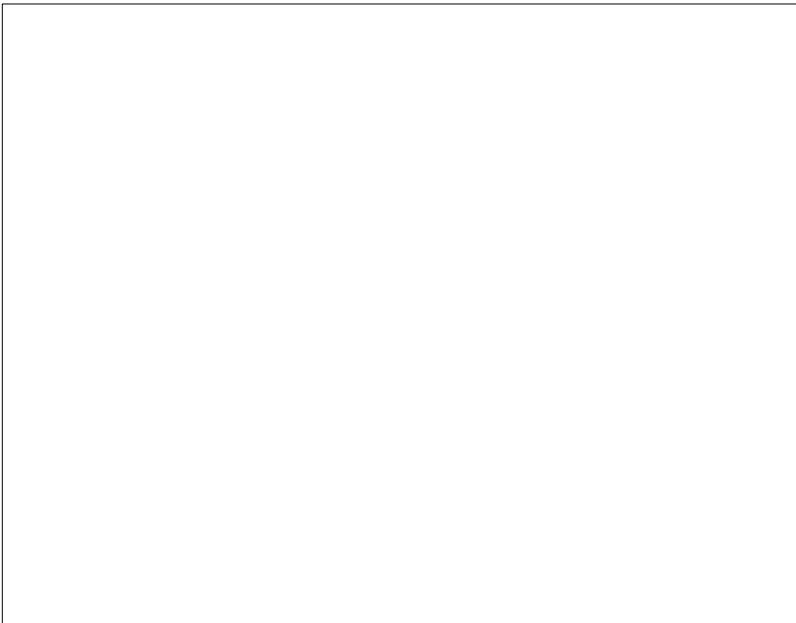


Fig 1: Scatter plot of H3K27me3 versus H3K9me3 enrichment (log2 fold change over input) at promoter regions in undifferentiated and day-6 differentiated mESCs

Figure 1 reveals that undifferentiated cells cluster at low repressive chromatin during lineage commitment. The enrichment values for both marks, while differentiated cells separation between the two populations was statistically shift toward higher values, reflecting the global gain of significant (Hotelling T-squared test, $p < 0.001$).

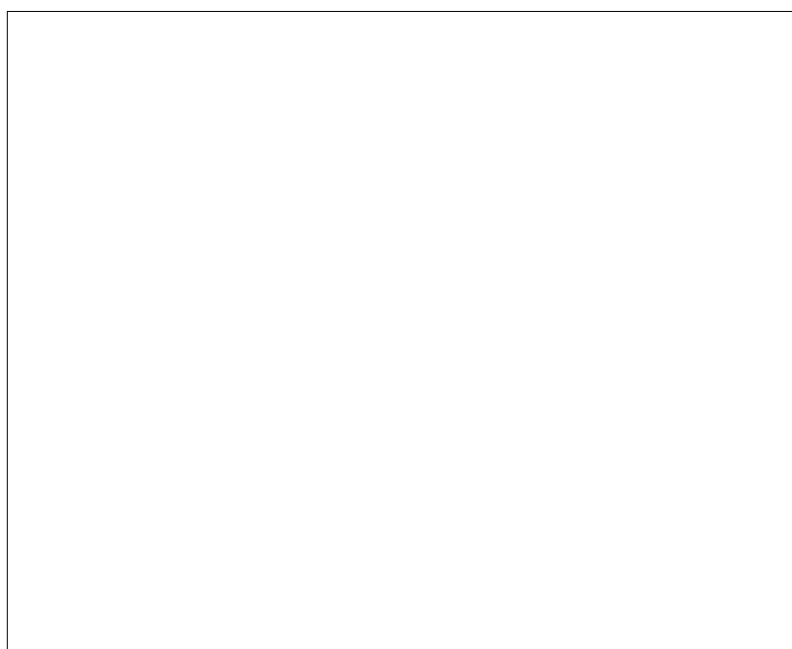


Fig 2: Box plots of relative expression levels (log2) for pluripotency-associated genes under SWI/SNF and NuRD active versus knockdown conditions at day 6 of differentiation

Figure 2 illustrates that knockdown of either complex differentiation. SWI/SNF primarily supports H3K27me3 resulted in significantly higher residual expression of deposition at lineage-inappropriate promoters, while NuRD pluripotency markers compared with the active condition. is more important for H3K9me3-dependent heterochromatin SWI/SNF depletion had a slightly larger effect on median integrity. Loss of either complex leads to the aberrant expression than NuRD depletion (Wilcoxon rank-sum test, $p = 0.003$ and $p = 0.009$, respectively).

Discussion

Our data paint a picture of functional complementarity mutations in SWI/SNF subunits and NuRD subunits between SWI/SNF and NuRD during neural differentiation. produce distinct but overlapping developmental syndromes SWI/SNF appears to be the dominant facilitator of in humans. Extending this analysis to later differentiation Polycomb-mediated H3K27me3 deposition-a finding that stages and to other lineages-such as mesoderm or endoderm-aligns with earlier reports showing that BAF complexes help will be necessary to determine whether the complementary recruit PRC2 to bivalent promoters in stem cells. NuRD, relationship observed here is a general principle of meanwhile, was more closely tied to H3K9me3-dependent chromatin-based gene regulation. heterochromatin maintenance, consistent with its known interaction with SETDB1 and HP1 proteins.

The 214 genes deregulated under both knockdowns likely represent a core set of targets that require coordinated action from multiple remodeling pathways for proper silencing. Pathway analysis of this overlap set revealed enrichment for Wnt signalling and TGF-beta response genes-both of which must be tightly controlled during neural fate decisions^[1,12]. One limitation is that shRNA-mediated knockdown achieves incomplete depletion, so the true extent of each complex's contribution may be underestimated. Additionally, our six- day differentiation window captures only early neural commitment; later stages of neurogenesis might reveal further divergence between the two pathways. Future work using degron-tagged subunits would allow acute, near-complete removal and sharper temporal resolution^[14].

Conclusion

This research shows that SWI/SNF and NuRD chromatin remodeling complexes make distinct yet complementary contributions to gene silencing during mESC neural

are needed for complete silencing. These findings clarify the division of labour between two major remodeling families and may help explain why

are needed for complete silencing. These findings clarify the division of labour between two major remodeling families and may help explain why mutations in SWI/SNF subunits and NuRD subunits produce distinct but overlapping developmental syndromes

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