Interaction between Chromatin Modifying Complexes and Cohesin

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Abstract

Dysregulation of chromatin organization results in an array of developmental diseases and multiple different forms of cancer. Three-dimensional genome organization is primarily mediated by the cohesin complex and cohesin-interacting proteins to form both transcriptional neighborhoods through CTCF-cohesin interactions and enhancer-promoter loops through unknown mechanisms[1,2]. Cohesin is a pleiotropic regulator of the genome and there is limited understanding of how cohesin-interacting proteins may regulate cohesin function. A comprehensive cohesin interactome has not been defined and may be key for understanding the molecular mechanisms by which cohesin regulates chromosome structure and gene expression. We utilized the TurboID biotin proximity labeling method to establish a cohesin interactome. Within the cohesin interactome, an interaction between cohesin and chromatin modifying complexes (CMCs) was revealed, including the SWI/SNF, ISWI, NuRD, MLL, NuA4, Polycomb Group (PcG), and Sin3A complexes. Nearly all the interactions detected by TurboID-Mass Spec that were selected for validation by co-immunoprecipitation and western blot were confirmed. This finding suggests potential crosstalk between the histone-mediated chromatin landscape and cohesin-mediated 3D genome organization via physical interactions. To assess the role of chromatin modifying complexes in cohesin regulation of the genome, the stability of the cohesin-SWI/SNF complex interaction was investigated. Mouse embryonic stem cells (mESCs) harboring a cohesin cancer mutation (SMC1A^{R586W}) exhibited reduced cohesin localization to enhancer and promoter regions, but unchanged cohesin levels at CTCF genomic sites[3]. This mutation did not affect the interaction of SWI/SNF and cohesin, despite reduced cohesin localization at enhancer and promoter regions. The decreased localization of cohesin coupled with maintained interaction of cohesin-SWI/SNF may indicate that cohesin mutations may cause an alteration of the stability of chromatin modifying complexes on the genome, contributing to the development of disease.

CMC-cohesin Interaction

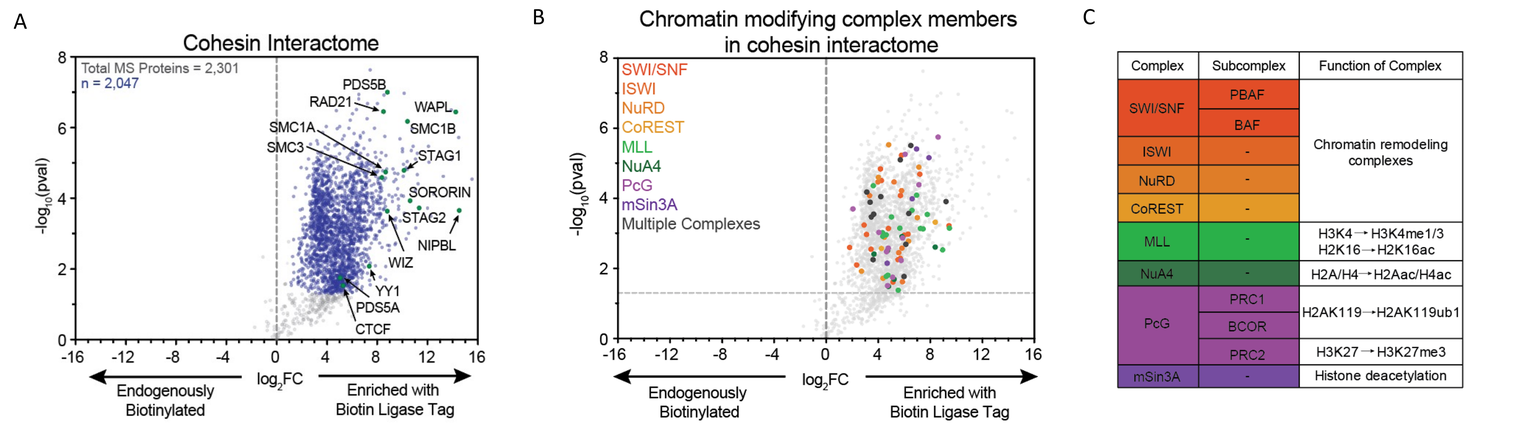

Figure 1. Protein complexes detected during biotin proximity ligase. (A)A volcano plot of biotinylated proteins in RAD21- TurboID tagged NIH-3T3 mouse fibroblasts versus NIH-3T3 cells lacking a biotin ligase reveal approximately 2,000 significant cohesin interactors. Pval <0.05; log2GC>(B) Chromatin modifying complex proteins which were identified among the 2,047 cohesin interacting proteins. (C) Table showing functions of chromatin modifying complexes identified as potential cohesin interactors.

Validation of CMC-cohesin Interaction

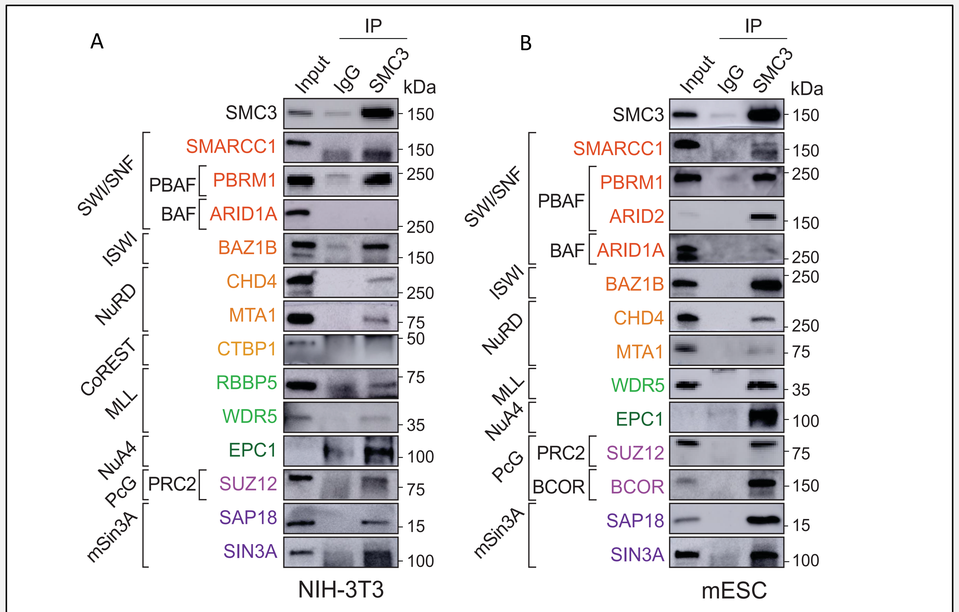

Figure 2. Western blot images of potential protein interactors of cohesin in NIH-3T3 mouse fibroblast cells (A) and mouse embryonic stem cells (B). A 2% input and IgG are used as controls for enrichment and non-specific pulldowns respectively. Co-immunopercipitations are done under low stringency conditions (75mM NaCl)

SMC1A^{R586W} Mutant Cell Line

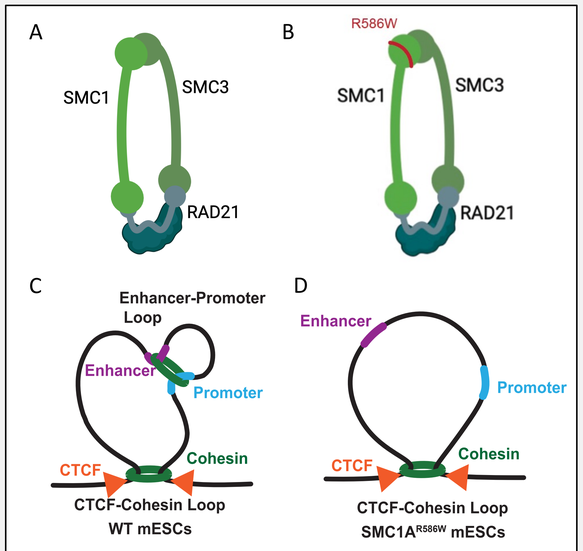

Figure 3. Mutant cohesin results in decreased localization to enhancer-promoter regions (A) Diagram of the three main cohesin subunits forming the full cohesin complex in a WT mESC. (B) SMC1A^{R586W} mutant on hinge domain of SMC1A subunit. (C) Cohesin forms loop domains through interactions with CTCF and an enhancer-promotor loop is formed by cohesin bringing two distal loci into contact. (D) Cohesin localization in SMC1A^{R586W} mutant cells which displays decreased localization of cohesin to enhancers and promoters, while maintaining expression at CTCF mediated loops.

SWI/SNF and cohesin Interaction in SMC1A^{R586W} mESCs

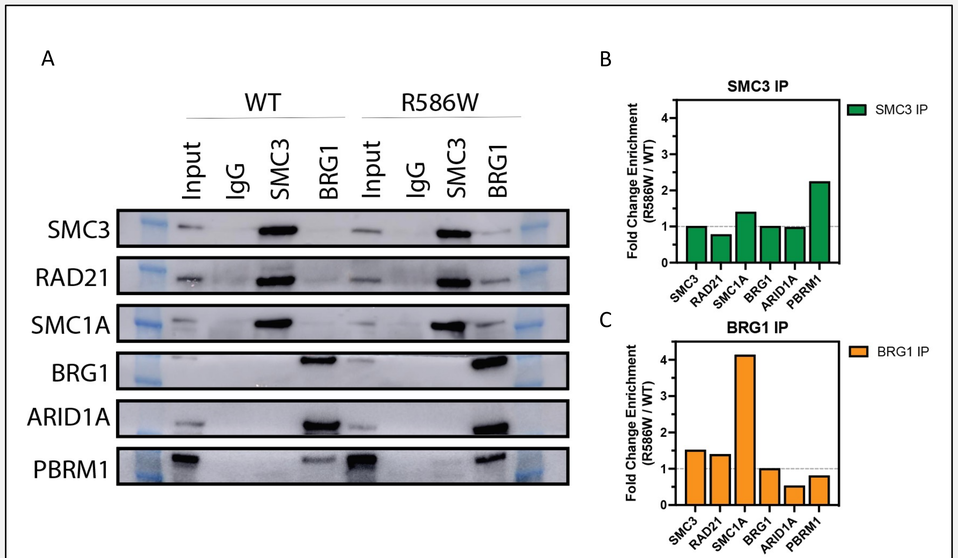

Figure 4. Investigating the Interaction between SWI/SNF and cohesin in WT and SMC1A^{R586W} mESCs (A) Co-IP assay with anti-IgG, SMC3, and BRG1 antibodies was performed in both WT mESCs and SMC1AR586W mutant cells. Co-IP assay performed under high stringency conditions (150mM NaCl and 1% detergent).(B)Bar graph showing the fold change of SMC1AR586W mESCs over WT mESCs in an SMC3 immunoprecipitation (green) (C) and a BRG1 pulldown (orange). Fold change is calculated by first dividing signal detected by signal from the IP performed to generate either an R586W normalized signal or a WT normalized signal. The R586W normalized signal is then divided by the WT normalized signal, generating a fold change of R586W signal in comparison to WT.

Future Directions

- Repeat validations under low stringency conditions
- Perform BRG1 ChIP-seq in both WT mESCs and SMC1A^{R586W} mESCs
- Chromatin fractionation to understand SWI/SNF stability on genome with cohesin mutations

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References

Graphical figures created with BioRender.com and Adobe Illustrator.

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