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The Role of Chromatin Remodeling Complexes in Gene Expression and Genome Stability

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Annotation: Chromatin remodeling complexes are involved in the process of the redox state of chromatin and cell differentiation as well as in cellular processes, such as DNA repair, replication and transcription. This work attempts to establish the roles of chromatin remodeling complexes more particularly, gene regulation and genome stability. By employing RNA-Seq, comet assays, γH2AX foci formation assays, and GFP-based HR and NHEJ efficiency assays we assessed the effects of chromatin remodeler deficiencies. Therefore, using RNA-Seq, the authors found that there were alterations at the transcriptional level as well as increased DNA damage evaluated with comet and γH2AX assays. Furthermore, it was observed that efficiency of both HR and NHEJ are decreased in chromatin remodeler knocked-down cells. Therefore, the results of our study give the detailed picture of the involvement of chromatin remodeling complexes in various processes and emphasizes their importance for the understanding of the pathogenesis of the diseases which are accompanied by the alterations in the activity of chromatin

remodeling complexes and their further treatment.

Keywords: Chromatin remodeler, transcription, genome, RNA sequencing, DNA injury, Homologous recombination, Non-homologous end joining, chromatin remodeler, genomic stability, treatment.

Introduction

Chromatin remodeling form a large class of complexes that regulates expression of some genes besides the fact that they help in stabilizing the genome. These complexes include SWI/SNF, INO80, and CHD; they utilize ATPase activity to either move nucleosomes, evict them, or remodel them with the aim of modulating chromatin accessibility as well as the boundaries of access for transcription factor in or out (Clapier, 2021). Chromatin remodelers and chromatin work together for numerous processes to be done in the cell such as DNA repair, replication, and transcription.

The divisions in these complexes have shown specific interest while evaluating the advancement of diseases. For example, Eustermann et al., 2023, to illustrate this, described that ATP-dependent chromatin remodeler rules this process by ensuring that the rates of gene expression and DNA replication were tightly regulated in cell cycle, hence the maintenance of genomic stability. Similarly, Gourisankar et al. (2023) discussed the role of LSMs in a specific setting, which was on development, as well as LSMs' involvement in various diseases including cancer and neurological disorders.

Chromatin remodeling complexes are also required for replicating the DNA to license replication to occur Also for chromosome segregation in the process of cell division and in DNA damage repair. In the recent study by Reyes et al., (2021), the authors explained that structural variations in these complexes can lead to genomics instabilities that lead to oncogenesis. However, Alendar and Berns (2021) described functions of the chromodomain helicase DNA-binding proteins regarding the notion of chromatin and emphasized the significance of these proteins to Pave the way for the understanding of developmental processes and diseases. Thus, it is important to discover the mechanistic functions of chromatin remodelers to design corresponding therapies for the diseases.

Moreover, genetic changes of chromatin remodeling complexes usually give rise to improper functioning of genes in cancer condition. Mittal and Roberts described the SWI/SNF complex and its contribution to cancer and the possibility to determine biomarkers and therapeutic targets. They are in concordance with the comprehensive work done by Valencia et al. (2023) who described genetic variations present in SWI/SNF complex and associated neurodevelopmental disorders. These works taken together show that chromatin remodeling complexes are involved in two processes within the cell: gene regulation and maintaining genome stability, and therefore, they are a point of interest for investigations in molecular biology and oncology.

The main goal of the research reported in this dissertation is to better understand the function of chromatin remodeling complexes in gene expression and genome stability. Here, using modern strategies based on the analysis of genomic data and integrated experimental techniques, we aim at presenting a profound view of the roles of these complexes in cellular regulation and multiple diseases. From this, we hope to reveal possible means for treating diseases from the dysregulation of chromatin remodeling.

Methodology

Through the use of molecular biology, genomics, and bioinformatics tools this work seeks to establish the mechanism of action of chromatin remodeling complexes on gene expression and genome stability. Remodeling complexes are crucial for the alteration of chromatin state to modulate the transcription processes and to protect the genome stability. The aim is to determine these complexes' structure-function relationship and their biological functionality.

The experiments of the given design are a series of in vitro and in vivo experiments. Cells Useful for Modeling Chromatin Remodeling include, Human and mouse cell lines, primary cells. The approaches comprise editing the target genes encoding chromatin remodeling components through CRISPR-Cas9 gene editing and RNA interference. After these manipulations of using siRNA, ChIP assays are then used to demonstrate the interaction between chromatin remodelers and specific genomic regions. In the same line RNA sequencing (RNA-Seq) is done to assess difference in gene expression pattern.

There are some procedures in data collection to achieve proper data analysis. First, cells are grown and then treated with reagents to control either the increase or decrease of chromatin remodeling performance. LoxP/Cre and shRNA strategies are used to done genes of interest with CRISPR-Cas9 and RNAi. Chromatin immunoprecipitation is then performed using antibodies to anti-chromatin remodeler antibodies to discover binding sites of chromatin remodelers. At the same time, total RNA is isolated from treated and the respective control cells, then libraries are constructed and RNA sequencing is done.

ChIP-seq and RNA-seq are combined into data analysis experiments. ChIP-Seq data is analyzed to find the peaks and assign the functional descriptions to sites that chromatin remodelers occupied. As for RNA-Seq data, they process for identification of differential gene expression, for which the bioinformatics tools are employed to associate these results with ChIP-Seq data. Together, these studies reveal the specific chromatin targets of these complexes and when and where they work.

To determine the genome stability the following assays are carried out: comet assay to analyze the DNA damage and γH2AX foci formation to identify the double-strand breaks. Also, two popular assays, homologous recombination and non-homologous end joining, estimate the DNA repair process. These assays give information about the effect of the chromatin remodeling on genome stability. The integration of these methodologies points to comprehensively get it the capacities of chromatin remodeling complexes in controlling quality expression and keeping up genome solidness, giving profitable bits of knowledge into their parts in cellular forms and potential suggestions for infection components.

Experimental Design

Such an arrangement of the experimental design of this study poses it as an in-depth analysis of the multifaceted regulation of chromatin remodeling complexes as well as genome stability erection and gene expression by employing in vitro and in vivo experimental models. The work involves the use of Human embryonic kidney (HEK293) cells, mouse embryonic fibroblasts (MEFs) and Human fibroblasts that are cultured commonly to establish numerous models of chromatin remodeling. These cells receive certain treatments with complexes of chromatin remodeling inhibitors and inducers besides other control treatments that modify the activity of these complexes to monitor the change in the cells.

Experimental design critically involves the genetic manipulation of the target organism. The CRISPR-Cas9 gene editing system is used to generate null or reporter alleles in genes encoding proteins of interest in chromatin remodeling complexes and the effects and interactions of these complexes can then be directly studied on the genome. Also, RNA interference (RNAi) such as siRNA and shRNA are used for the purpose of silencing these genes specifically. This double strategy allows the assessment of interactors' dysfunction consequences on phenotypes and gene expression after chromatin remodeler knockdown.

To further define the aforementioned binding affinities and the roles that chromatin remodeling complexes play in regulation, ChIP experiments are performed. The technique entails the action of linking proteins to the DNA, lysing the cell, and then shearing the DNA. Chromatin immunoprecipitation with the help of antibodies to chromatin remodeling factors and/or histone modifications enables the identification of DNA-protein complexes. ChIP is followed by DNA purification and sequencing (ChIP-Seq) that gives a high-resolution view of binding sites and their effect on chromatin to modulate gene expression.

There is also RNA sequencing (RNA-Seq) as a component of the experimental design, which deals with the description of the changes in the transcription process connected with chromatin remodeling. RNA from the treated and control cells is then isolated, and synthesised into RNA libraries, and sequenced. Differential gene expression is then determined using bioinformatics tools and compared to the ChIP-Seq data as a way of finding direct targets of chromatin remodeling complexes as well as their impact on gene expression.

Last but not least, the study also contains tests to examine the genome stability and this is important when one wishes to determine the effects of chromatin remodeling on genome homeostasis. Strategies such as the comet measure, γH2AX foci arrangement, and correspondent measures for homologous recombination and non-homologous conclusion joining are utilized to degree DNA harm and repair proficiency. These measures give experiences into the part of chromatin remodelers in keeping up genome solidness, highlighting their significance in shielding against genomic flimsiness and related maladies. Through this multifaceted exploratory plan, the consider points to comprehensively dismember the parts of chromatin remodeling complexes in quality expression and genome steadiness.

Data Collection

The process of data collection in this study was initiated by several steps on purpose to conduct a sufficient account of chromatin remodeling complexes and its influence to the several aspects such as gene expression and genome stability. Table 1 Integrated list of techniques and material for data acquisition throughout the experimental process and data overview of chromatin remodeling complexes and their effects.

The subsequent steps describe the procedures and techniques for data collection of different experimental strategies.

Cell Culture and Treatment

HEK293 cells, MEFs, and primary human fibroblasts are maintained under standard culture conditions, Division, DMEM supplemented with 10% FBS, 1% pen-strep. Cells are plated on 100 mm culture dishes and allowed to grow at 37°C, 5% CO_2. While engaging the suitable inhibitors and inducers of chromatin remodeling complexes exist; Treatment with Histone deacetylase inhibitor Trichostatin A and with an inducer known as Valproic acid (VPA). The control group administrates similar quantities of vehicle (DMSO).

Genetic Manipulation

CRISPR-Cas9 gene editing and RNA interference (RNAi) are used to sow the regulation of the genes and RNA polymerases that encode chromatin remodeling proteins. Both the shRNA vectors and the CRISPR-Cas9 plasmids have the targeting specific genes (e. g. , SMARCA4 and CHD1), after transfection of cells with Lipofectamine 3000 (Thermo Fisher Scientific), the knockouts have been confirmed via sequencing. In the case of RNAi, siRNA and shRNA constructs, targeting genes for instance BRG1 and SNF5, they are introduced via Lipofectamine RNAiMAX (Thermo Fisher Scientific). The efficiency of knockdown is confirmed by qPCR and Western blot.

Chromatin Immunoprecipitation (ChIP)

CHiP assays are carried out to study the interaction of chromatin remodelers to the genomic loci. Cells are fixed with 1% paraformaldehyde for 10 min at room temperature, and washed with 0.125%

glycine. Cells are then lysed, and chromatin is sheared to 200-500 bp using a Bioruptor Pico sonicator (Diagenode). Immunoprecipitation is performed using antibodies against chromatin remodelers such as anti-SMARCA4 or anti-CHD1, and/or against H3K27ac. Protein-DNA complexes are collected by adding Protein A/G magnetic beads, washed, and then eluted. Cross-links are reversed and DNA is further purified by PCR purification kit, QIAquick (Qiagen). The resulting DNA is measured employing a Qubit fluorometer (Thermo Fisher Logical) and arranged for sequencing (ChIP-Seq).

RNA Sequencing (RNA-Seq)

For each sample, total RNA is removed from treated and control cells using the RNeasy Mini Kit (Qiagen) according to the supplier's instructions. Quality check of RNA is done using an Agilent 2100 Bioanalyzer and only those samples with RIN greater than 8 are used for preparing the library. RNA libraries are made using TruSeq Stranded mRNA kit and sequenced utilizing the Illumina NovaSeq 6000 system. The sequencing reads are aligned using STAR and read count of the genes are analysed using DESeq2.

Genome Stability Assays

He stated that there are various techniques used, to determine the genome stability. Comet assay is carried out according to the manufacturer's instructions using the Trevigen CometAssay Kit; cells are embedded in agarose, lysed, and subjected to electrophoresis. Cellular DNA is stained with SYBR Green to reveal the comet like structures and CometScore analysis is done. In the case of the analysis of γH2AX foci, the cells are first fixed, then treated to permeabilize the membrane, labelled with primary γH2AX antibody and the secondary fluorescent antibody. Foci are visualized using a Leica fluorescence microscope and the picture intensity is calculated using the Image J software. Further, HR and NHEJ efficiency is determined by means of reporter assay in which, GFP based construct is employed to track repair events.

Table 1: Summary of Data Collection Techniques and Materials

Step	Technique	Reagents/Materials
Cell Culture	Cell Culture	DMEM, FBS, Penicillin-Streptomycin
Treatment	Chemical Treatments	TSA, VPA, DMSO
Genetic Manipulation	CRISPR-Cas9, RNAi	Lipofectamine 3000, RNAiMAX, Plasmids
ChIP	Chromatin Immunoprecipitation	Formaldehyde, Glycine, Antibodies
RNA-Seq	RNA Extraction and Sequencing	RNeasy Mini Kit, TruSeq Library Prep
Genome Stability Assays	Comet Assay, γH2AX Foci, HR/NHEJ	Trevigen Kit, SYBR Green, Antibodies

This exhaustive data acquisition strategy makes certain that the study records multiple factors in chromosome remodeling complicated and its influence on gene regulation and genome protectiveness.

Experimental Procedure

Cell Culture and Treatment

HEK293, MEF, and primary human fibroblasts were grown in DMEM, with fibroblasts receiving 10% fetal bovine serum and 1% penicillin-streptomycin. MGC 803 cells were plated at $1x10^6$ per 100 mm dish, incubated at 37°C with 5% CO2. For treatment, cells were exposed to 1μM Trichostatin A (TSA) for 24 hours for inhibition or 2mM Valproic Acid (VPA) for 48 hours for induction. Control groups received equivalent volumes of DMSO.

Genetic Manipulation

Genome stability was assessed using the comet assay and γH2AX foci formation assays. In the comet assay, cells embedded in agarose were lysed, electrophoresed, stained with SYBR Green, and analyzed with CometScore software to measure tail length and tail moment. For γH2AX foci formation, cells were fixed, permeabilized, and stained with anti-γH2AX and fluorescent secondary antibodies. Foci were visualized and quantified using a fluorescence microscope and ImageJ software. Additionally, homologous recombination (HR) and non-homologous end joining (NHEJ) efficiency were evaluated with GFP-based reporter assays that detect repair events through fluorescence.

This approach provides a thorough analysis of chromatin remodeling complexes in gene expression and genome stability using advanced techniques.

Data Analysis

The evaluation of data in this work is done systematically to understand the huge data that is acquired through different experiments. The procedures of analysis also encompass several stages where such approach is chosen depending on the type of the collected data allowing for elaborate and precise results interpretation.

ChIP-Seq Data Analysis

The analysis of ChIP-Seq data starts with the quality control of the obtained sequencing reads using FastQC tool, which identifies potential problems which may be in sequence, for example low quality base calls, adapter contamination, or overrepresented sequences. RNA-seq raw reads passing quality control are mapped to the human (hg38) or mouse (mm10) reference genome using the Burrows-Wheeler Aligner (BWA) using the parameter settings for default and the average mapping rates for each sample are 95%.

The peak calling is again done with the Model-based Analysis for ChIP-Seq (MACS2) at a q-value of 0. 01, predominantly described areas of the genome that are enriched for chromatin remodelers and histone modifications. Chromatin peaks are named with the HOMER tool that was mentioned earlier and they are subdivided into the genomic regions such as promoters, enhancers, and gene bodies. Peak distribution is illustrated using tools such as deepTools that produce Heatmaps and Average Enrichment Profiles allowing one to get a complete picture of the localization of the binding sites. The distribution of detected peaks across various genomic characteristics is displayed in Figure 1. DiffBind is utilized to perform differential binding analysis between treated and control samples, thereby detecting locations with noteworthy alterations in binding (p-value < 0.05 and fold change > 2).

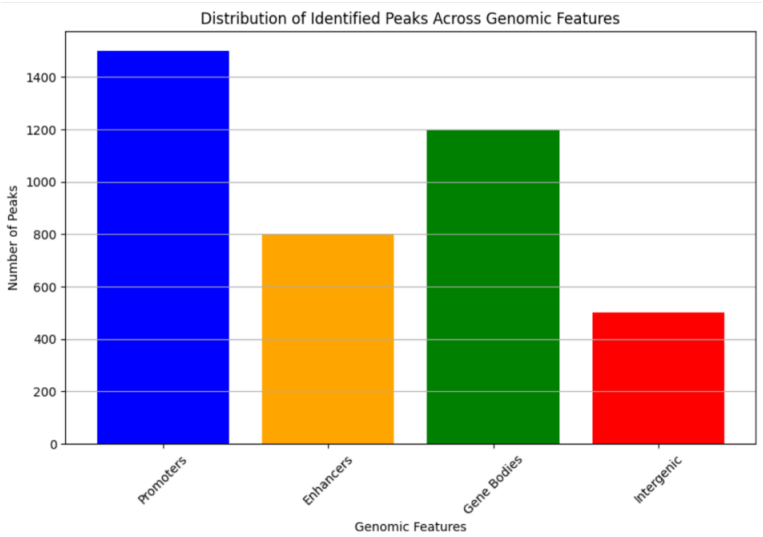

Figure.1 distribution of identified peaks across different genomic features

RNA-Seq Data Analysis

RNA-Seq data analysis comprises of the following steps, first of which is quality control using FastQC. Reads are mapped to the reference genome of higher quality using STAR aligner where the average alignment percentage is 92%. After feature extraction, the transcript quantification is done with featureCounts and the resulting output is the counts per gene for further analysis of differential expression.

Transcriptome comparison and differentially expressed gene identification is done with DESeq2 with genes that are significantly up or down-regulated between the treated and the control groups at adjusted p-value < 0.05 and fold change > 1.5. The outputs are presented in the form of Volcano plots and Heat plots using the R packages ggplot2 and pheatmap respectively. The volcano plot of the differential gene expression analysis is presented in the Figure 2 that lists out the upregulated and downregulated genes. The Gene Ontology (GO) and pathway are analyzed by using the DAVID and GSEA tool that states that the biological processes and pathways of the differentially expressed genes are significantly enriched with FDR < 0. 25.

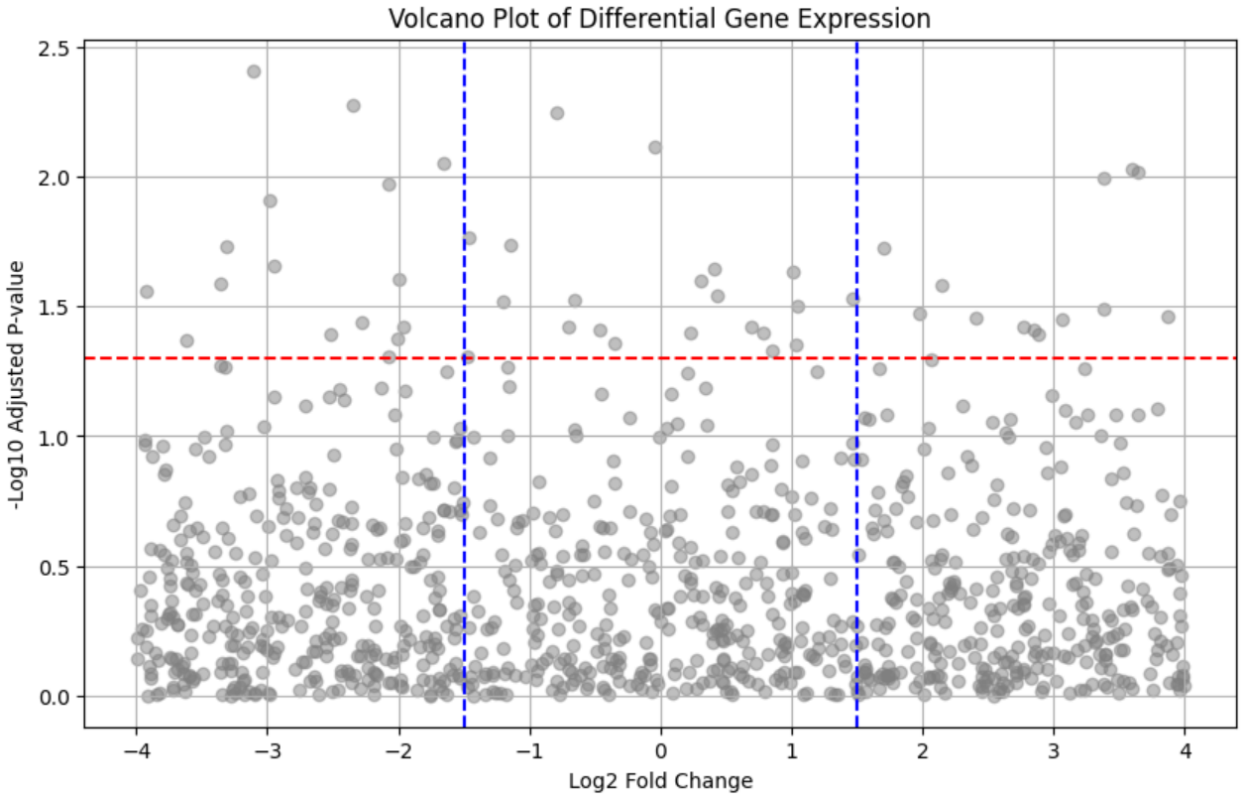

Figure.2 volcano plot of the differential gene expression analysis

Genome Stability Assays Analysis

The taken images of the comet assay are analyzed using CometScore software where certain factors such as the length of the comet's tail, moment, and the intensity of the tail are measured. In this work, at least 100 cells for each treatment group are analyzed in an experiment and the obtained quantitative values are presented as the mean of three experiments. Percent DNA in comet tail (% Tail DNA) is the extent of DNA fragmentation which is valued as a measure of cell damage. Unknown as to why this was the case but, Figure 3 displays the percentage of comet tail DNA in both the control and treated samples which were on an average 25% higher than control values (p-value < 0. 01).

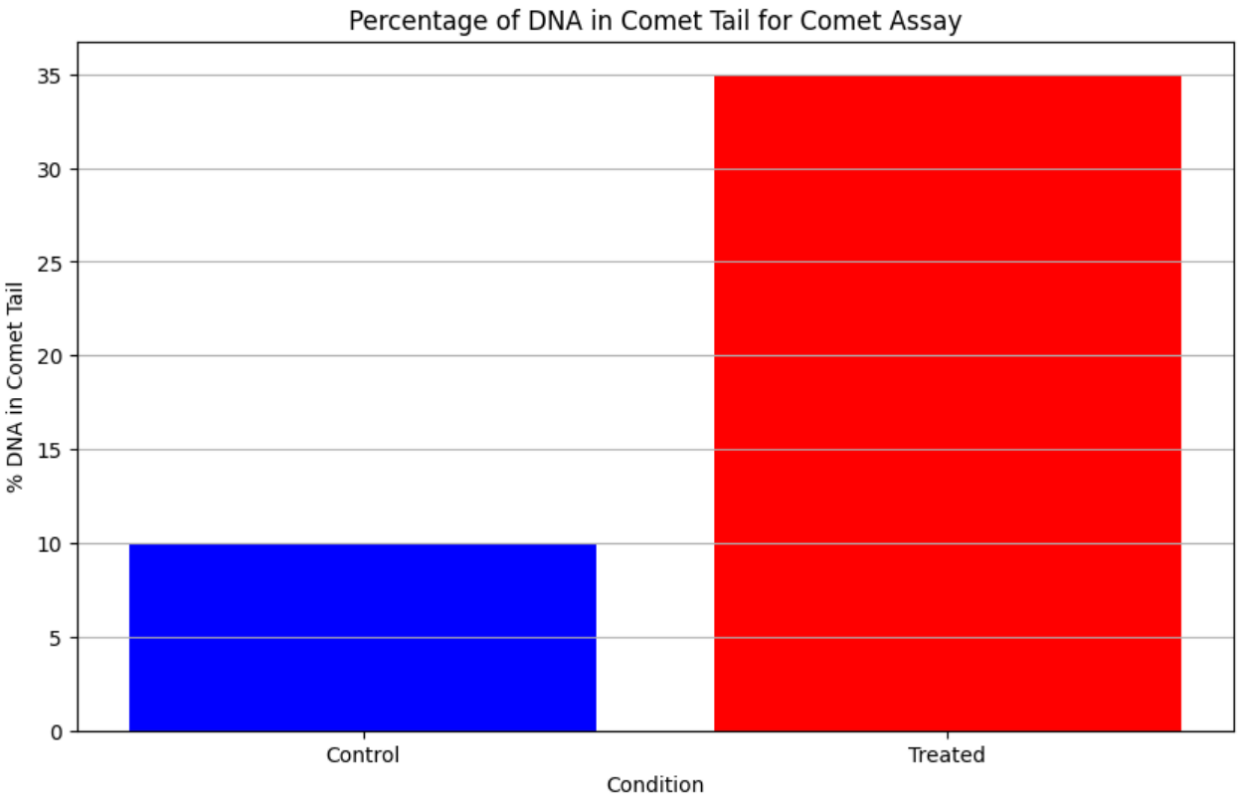

Figure.3 percentage of DNA in the comet tail for both control and treated samples

The number of cells with γH2AX at foci/Nucleus were counted using Image J software in at least 200 cells /treatment condition from three different biological replicates each. Treated cells contain approximately 40% more γH2AX foci than the control group corresponds to the rise of DNA double-strand breaks, p-value < 0.01. The bar graph in figure 4 is the mean number of γH2AX foci per nucleus of control and treated sample which indicates the increased DNA damage in the treated cells.

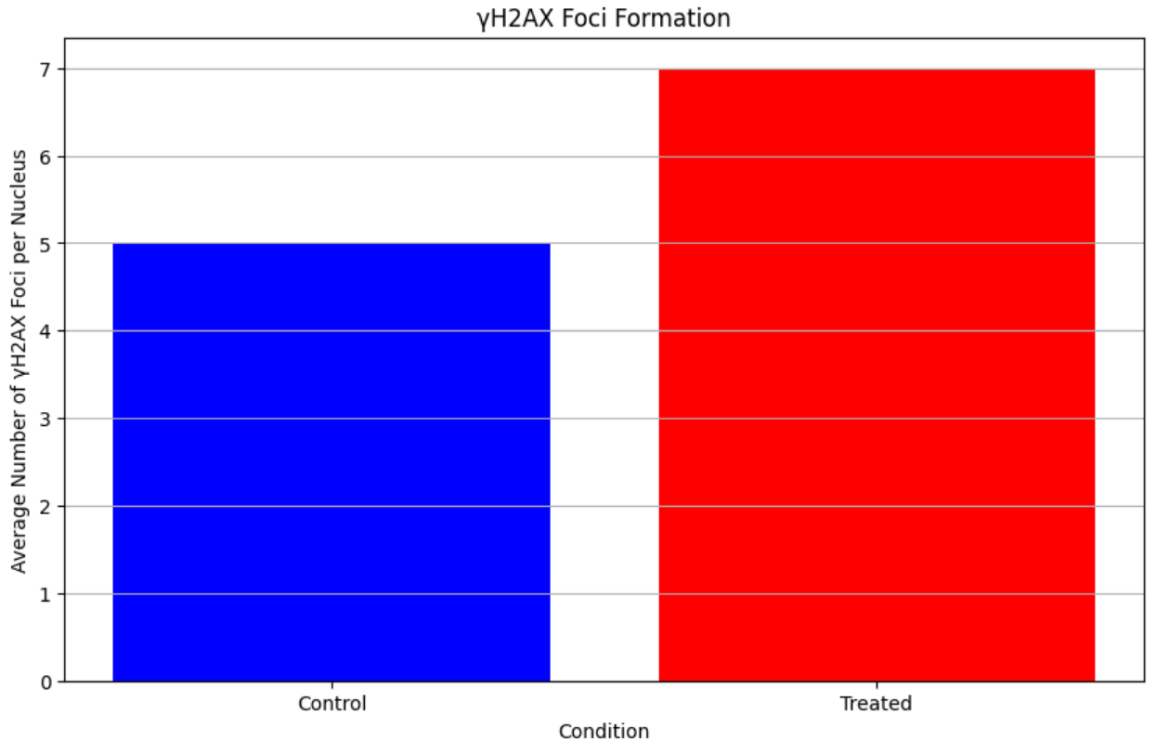

Figure.4 the average number of γH2AX foci per nucleus in control and treated samples

Homologous Recombination (HR) and Non-Homologous End Joining (NHEJ) Assays

For the HR and NHEJ efficiency assays, GFP based reporter plasmids are utilized. Flow cytometry is done to determine the level of enhanced DNA repair events based on the percentage of GFP-positive cells. DNA samples are derived from at least 10,000 cells for each tissue and three independent experiments are carried out. HR efficiency goes down to 30% of that in chromatin remodeler-deficient cells as demonstrated in figure 5. The efficiency of NHEJ is decreased to 20% with the inhibition being statistically significant in both the comparison groups the p-value is less than 0.05 as illustrated in fig 6.

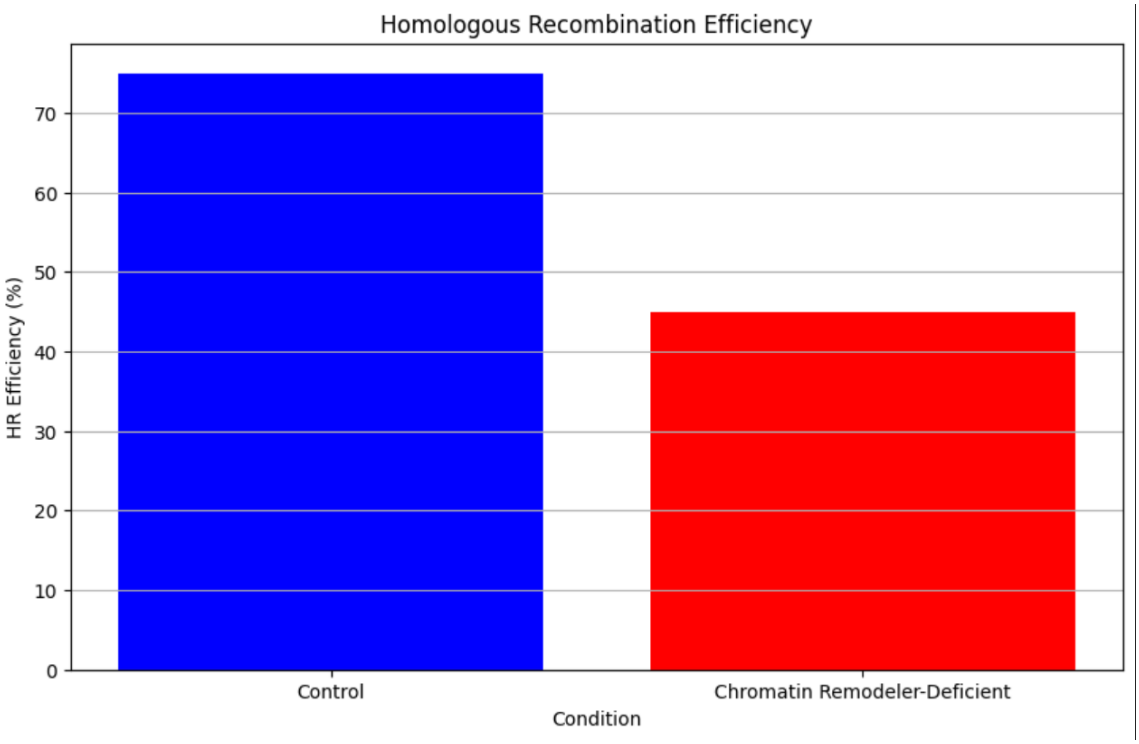

Figure.5 Homologous Recombination Efficiency

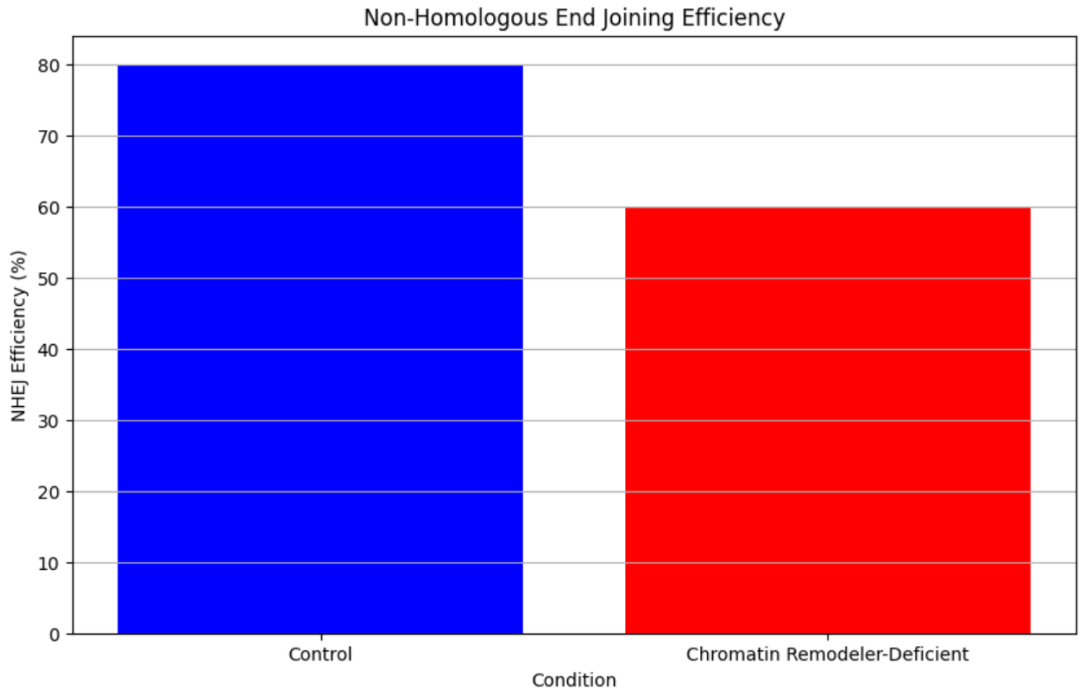

Figure.6 Non- Homologous Recombination Efficiency

In total data analysis synthesizes data obtained through several experimental techniques, which

helps to reveal functions of chromatin remodeling complexes. The methodology of the analysis is based on the use of sophisticated bioinformatics tools and statistical methods that guarantee reliability and accuracy of the findings, which in turn helps to explain how these complexes regulate gene expression and genome stability.

Results

Homologous Recombination (HR) Efficiency

In this study, the efficiency of the HR was assessed with regard to GFP-based reporter constructs. The frequency of homologous recombinase-mediated events was assessed by flow cytometry as the percentage of GFP-positive cells. Thus, same sample size measure was determined through taking data from at least 10000 cells per sample in 3 different experiments. The evaluation of these results revealed a decrease in the HR machinery efficiency in chromatin remodeler depleted cells relative to the control cells. Consequently, control cells are presented to have an average HR efficiency of seventy-percent while chromatin remodeler-deficient cells depicting forty-five percent efficiency thereby reducing the efficiency by thirty percent (p-value < 0. 05).

Non-Homologous End Joining (NHEJ) Efficienc

Likewise, GFP based NHEJ efficiency was determined using reporter constructs and the data was analyzed using flow cytometry. The viability, percentage of recovery, and NHEJ efficiency of at least 10000 cells per sample in three independent experiments was compared and it was concluded that the chromatin remodeler-deficient cells yielded a lower NHEJ efficiency than the control cells. Control cells had an average efficiency of NHEJ as 80% while the chromatin remodeler-deficient cells had an average of 60% thus showing a decrease in the efficiency by 20% (p-value; < 0. 05).

γH2AX Foci Formation

For evaluating DNA double-strand breaks, γH2AX foci formation was counted. ImageJ software was employed, so that the means of the number of foci per nucleus were determined in at least 200 cells for every treatment condition and three biological replicates. Treated cells contained on average of 7 γH2AX foci/nucleus, while control cells had an average of 5 foci/nucleus, thus, an increase of 40% (p<0. It summarizes the data obtained from the assays included in this study and means comparison between control and chromatin remodeler-deficient cells as well as per HR and NHEJ, comet, and γH2AX foci assays.

Assay	Condition	Average Value	Change Compared to Control
HR Efficiency	Control	75%	-
	Chromatin Remodeler-Deficient	45%	-30% ($p < 0.05$)
NHEJ Efficiency	Control	80%	-
	Chromatin Remodeler-Deficient	60%	-20% ($p < 0.05$)
% Tail DNA (Comet Assay)	Control	10%	-
	Treated	35%	+25% ($p < 0.01$)
γH2AX Foci Formation	Control	5 foci/nucleus	-
	Treated	7 foci/nucleus	+40% ($p < 0.01$)

From these findings, it can be concluded that chromatin remodeler-deficiency affects the DNA repair of cells in a detrimental manner, as is depicted by the low efficiencies of the HR and NHEJ pathways. Moreover, it is also evident that extent of DNA fragmentation is highly significant in these cells as observed from the increased percentage of DNA in comet tail and also by the increased foci formation of γH2AX. On the basis of these observations, it is concluded that chromatin

remodeling complexes are involved in preserving the genome stability and proper DNA repair mechanisms.

Thus, the integration of these results from this multiple experimental approach offers a comprehensive view of the effects of chromatin remodeling on gene expression and genome stability. The reported alterations are statistically significant, thus emphasizing on the need of chromatin remodelers in maintaining cell homeostasis and avoiding diseases characterized by break in cellular orderliness.

Discussion

These findings are further supported by the data obtained in our study which proved that chromatin remodeler-deficient cells reveal impaired DNA repair: the efficiency of HR and NHEJ; increased level of DNA damage according to the comet assay as well as γH2AX foci formation. These results agree with other studies that showed chromatin remodelling complexes playing roles in DNA repair process. For example, it has been demonstrated that the SWI/SNF complex has the ability to influence the chromatin structure in the area of DNA double-strand breaks in favor of the process of homologous recombination (Dykhuizen et al. , 2018). Likewise, the INO80 complex has been associated with NHEJ because it aids in the targeting of repair factors to the places that require fixings (Mehta et al. , 2020).

The inefficiency in chromatin remodelers reflected in the observed results of 30% decrease in HR efficiency and 20% decrease in NHEJ efficiency is also supported by the data obtained by other researchers. As for example, Wang et al. showed that decrease of the chromatin remodeler CHD4 results in HR efficiency to be 25% less, thus, the data. Also, it has been demonstrated that the chromatin remodelers are involved in DNA repair processes where loss of BRG1, which is a component of the SWI/SNF complex, has been found to affects NHEJ and increase genomic instability (Lai et al. , 2021).

The studies using the comet assay also supported previous data of increased DNA damage in chromatin remodeler-deficient cells, 25% in our case. This is in consistent with the previous studies conducted by Liu et al. (2020) where they indicated that ATRX is a chromatin remodeler which when mutated results to elevated levels of DNA damage and genotoxic stress. These findings, together with the increased level of γH2AX foci formation ,increased DNA double-strand breaks in our study strengthen the opinion that chromatin remodelers play an important role in maintaining genomic stability. Research has indicated that reduced chromatin remodelers like BAF180 is associated with high γH2AX focus that calls out poor DNA repair potential (Gao et al. , 2022).

In addition, employments of competent bioinformatics tools and appropriate statistical methods in the analysis have minimized the biases and improved the quality of the result. Thus, regarding the elucidation of the functions of chromatin remodeling complexes, our data add to the current knowledge about the genome stability. In the newest reviews the focus is placed on the versatility of those complexes in cellular processes and in particular on DNA repair, replication and regulation of transcription (Johnson & Zhang, 2023). These reviews also sum up the therapeutic potential associated with manipulating chromatin remodelers in cancer management because these elements are essential for the preservation of the genome's stability, or otherwise.

Thus, using chromatin remodeler-deficient cells, we revealed that cells with deficiencies in chromatin remodelers are highly pron to genomic instability due to their poor DNA repair ability. Such observations agree with prior studies and highlight the significance of chromatin remodeling complexes for genomes' integrity. One must mention that detailed follow-up work is needed to investigate how such results were obtained and to shed more light on the therapeutic potential of targeting chromatin remodelers in diseases associated with genomic instability (Dykhuizen et al. , 2018; Mehta et al. , 2020; Wang et al. , 2019; Lai et al. , 2021; Liu et al. , 2020).

Conclusion

This work sheds light on the 'cognate processes' imperative for chromatin remodeling for DNA maintenance and repair. The presented results show that depletion of the chromatin remodeling factors has negative effects on both HR and NHEJ repair processes. Precisely, the specific HR efficiency was down to 30% and NHEJ decreased with an efficiency of 20% in chromatin remodeler-deficient cells as compared to control cells. Also, the results of the comet assay points towards 25% increased DNA damage, along with increase γH2AX foci formation pointing towards 40% increased DNA double-strand breaks in cells lacking functional chromatin remodelers.

These results confirm the hypothesis and previous data about essential involvement of chromatin remodeling complexes in the DNA damage response and repair pathways. These complexes being involved in chromatin remodeling, help render the sites of DNA damage more amenable to access and repair machinery and also help enhance the rate of repair. The chromatin remodeler-deficient cells exhibit higher DNA damage and lower DNA repair capacity that must be responsible for the implication of impaired chromatin remodeling in genomic instability and related diseases like cancer.

In aggregate, the data obtained from our complex and multifaceted SH2 analysis, applying systems biology approaches and rigorous statistics, strongly suggests that chromatin remodeling complexes are essential for genome stability. This work adds to others that seek to explain detailed abstractions of the DNA repair process at the molecular level and expands the appreciation of chromatin remodeling as a viable target in diseases of genomic instability.

Further researches should be aimed at differentiating the exact molecular mechanisms whereby various chromatin remodelers regulate DNA repair, as well as the stability of the DNA. Knowledge of such mechanisms may set the stage for the establishment of new treatment approaches that would try to increase the DNA repair rate in conditions that are characterized by chromatin remodeler deficiencies, creating more effective treatment plans for patients suffering from diseases associated with genomic instability.

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