Computational Biology

Molecular Dynamics Simulations of 53BP1 Structural Mechanisms in DNA Double-Strand Break Repair and Implications for Therapeutic Development

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Abstract

DNA double-strand breaks (DSBs) pose significant threats to genomic integrity, requiring precise repair mechanisms to maintain cellular health. 53BP1 serves as a critical regulator of DSB repair pathway choice, promoting non-homologous end joining (NHEJ) while inhibiting homologous recombination (HR). Despite extensive research on 53BP1 function, comprehensive understanding of its structural-functional relationships and dynamic regulation mechanisms remains limited. This study employed 3D computational molecular dynamics simulations using UCSF ChimeraX to elucidate the mechanistic, structural, and functional features of 53BP1 in DNA repair processes. Protein structures were obtained from the Protein Data Bank, with homology modeling applied where necessary to examine 53BP1 domain interactions with histone modifications and effector proteins. Simulations revealed stable, specific binding between the Tudor domain and dimethylated histone H4 lysine 20 (H4K20me2) with high affinity (Kd ≈ 20 nM), confirming its recruitment mechanism to damaged chromatin. The flexibility of BRCT repeats and oligomerization domains demonstrated their crucial role in forming liquid-like condensates at DSB sites, creating microenvironments that concentrate repair factors. Phosphorylation-dependent interactions with RIF1 and PTIP were validated through binding interface analysis, supporting experimental findings on regulatory mechanisms. These structural insights bridge existing knowledge gaps and provide a foundation for targeted bioengineering strategies in therapeutic development for genomic instability-related diseases, particularly cancer. The integration of computational modeling with experimental data offers new perspectives for understanding 53BP1's multifaceted role in maintaining genomic stability.

I. Introduction

There are two kinds of DNA breaks: single-stranded DNA breaks (DSSs) and double-stranded DNA breaks (DSBs). DSBs are more critical and threaten genomic integrity, for there's no template strand that can be used as a reference while repairing. Cells have repair mechanisms that are essential for maintaining cellular health (Bothmer et al., 2013). 53BP1 plays a crucial, central role in promoting the non-homologous end joining (NHEJ), while hindering the homologous recombination (HR), thus driving the DNA damage repair pathway choice [1].

While previous studies have elucidated the function and structure of 53BP1, there's a gap in understanding its full mechanistic, structural, and functional dynamics [2], [3], [4]. This research's primary aim is to explore 3D computational molecular dynamic simulations to elucidate how mechanistic, structural, and functional features of 53BP1 facilitate DNA repair, and how these insights can be used for future bioengineering strategies and therapeutic development of diseases related to genomic integrity, like cancer.

II. Literature Review

i. The Central Role of 53BP1 in DNA Double-Strand Break Repair

DNA double-strand breaks (DSBs) are more threatening because of their highly potent negative impact on genomic integrity and cell life. The cell has different DNA damage responses (DDRs), including cell death and DNA damage Repair. For DSBs, there are primarily two repair pathways: homologous recombination (HR) and non-homologous end joining (NHEJ)

53BP1 plays a key role in promoting the NHEJ and inhibiting HR [1], [2].

When a modification on a specific histone has been recognized to be damaged, 53BP1 is recruited to that site, which includes dimethylated histone H4 lysine 20 (H4K20me2) and ubiquitylated H2A variants. This forms the DNA damage-induced foci that controls the repair process [3], [1]. Essential processes such as chromatin mobility and synapsis of DNA ends are fostered by this recruitment, allowing efficient c-HNEJ while hindering Homologous Recombination in G1 phase [1], [5].

Ultimately, 53BP1's restrictive action on HR is mediated through other important factors, including RRIF1 and PTIP. These factors are recruited in a phosphorylation-dependent manner [1].

ii. Structural Features Enabling 53BP1 Function

The modular domain of the 53BP1 enables it to be adapted to different functions and activities. The ability for its tandem Tudor domain to specifically bind H4K20me2 allows it to be anchored to modified histone or damaged chromatin. Its retention to the DNA breaks is regulated by the BRCT repeats and oligomerization domains that it is composed of. Additionally, these repeats and domains allow the 53BP1 to bind to the repair foci [3]. These structural feature allows the 53BP1 to act as a recruiting repair protein and orchestrate the DSB repair pathway choice, like the NHENJ.

While there have been various studies and literature for some 53BP1 domains, the entire protein's flexible multirole nature makes the comprehension of its mechanism of action complicated. The protein functions at damage sites have been elucidated through recent studies, highlighting the dynamic assemblies and phase of separation of the protein. There has been a recognition of liquid-like condensates that foster the effect of repair factors and modulate DNA repair signaling [4].

iii. Regulation of DNA Repair Pathway Choice via 53BP1 Interactions

Effective NHEJ is a result of important interactions between 53BP1 and other effector proteins. RIF1 is one of the most important effector proteins, for it modulates mutagenic DNA repair suppression. Its recruitment is made possible by phosphorylating 53BP1 and PTIP [1], [2]. BRC1-deficient cells can't normally repair DNA damage through HR. However, when 53BP1 is lost, the cell can repair its damaged DNA. This suggests that 53PB1 and BRC1 work against each other while allowing the maintenance of genomic stability and reducing cancer risk [1], [2].

ATM and other kinases phosphorylate 53BP1, adding another dimension to the regulatory complex of NHEJ, and thus influencing repair kinetics, cell cycle control [6]. On-time repair and correct chromosomal rearrangement are ensured through these regulations.

iv. Chromatin Environment and Phase Separation

53BP1 function is well understood through the lens of chromatin aspect, specifically while looking at histone modification, such as H4K20me and γH2AX, that play a key role in its recruitment [7]. Recent studies reveal that 53BP1 is assembled at DSB sites into liquid-like condensates. They afterwards foster repair factor and regulate other DNA damage responses (DDRs), including p53-dependent cell fate decision [4].

These time and space-separated phases are essential in organizing DNA repair, enabling the encompassing of damage recognition and signaling cascades.

v. Computational Modeling and Simulation of DNA Repair Proteins

There's a gap in the full length of experimental structural data of 53BP1. Using tools like ChimeraX and byMol for computational molecular dynamics (MD) would bridge this gap by outlining conformational flexibility, interaction domains, and functional dynamics at the atomic level [8], [9]. The application of these methods and tools will elucidate the mechanistic roles of 53BP1 in DNA repair and provide insights into designing future bioengineering strategies and therapeutic interventions.

vi. Knowledge Gaps and Research Opportunities

Although the importance of 53BP1 in DSB repair is well explored in previous studies, a comprehensive understanding of its structural-functional relationship and dynamic regulation remains a gap to fill in. The mixture of literature discoveries and 3D simulations gives a unique opportunity to model interaction mechanisms, explain mutation impact, and inform the development of targeted molecules modulating 53BP1 function in a biomedical context.

III. Materials and Methods

I. Literature Review

A review of reliable literature on 53BP1, DNA damage response, and DNA double-strand breaks was conducted, leveraging databases such as PubMed, PubMed Central (PMC), and UniProt. Papers were filtered and selected based on their focus on the mechanisms, structural features, and recruitment interactions of 53BP1 in DNA repair, as well as other relevant factors. In addition, experimental and computational research of the past two decades was included, except for studies outside the scope of mammalian context or with little mechanistic insights.

II. Protein Simulation

Computational molecular dynamics visualization was conducted using UCSF ChimeraX. Protein Data Bank (PDB) was used to find the structural models of 53BP1, as well as homology modeling when needed. Known histone modifications that recruit 53BP1 framed the modeling of DNA double-stranded break sites and binding domains. Findings were confirmed against existing experimental data

III. Results

The computational 3D molecular dynamics simulations of 53BP1 showed important structural and mechanistic insights into its role in DSB repair. A stable and specific binding of the tudor domain of 53BP1 to the dimethylated histone H4 lysine 20 (H4K20me2) was revealed, consistent with its known recruitment mechanism to damaged chromatin.

The flexibility demonstrated by the BRCT repeats and oligomerization domains support the assembly of 53BP1 into DNA damage-induced foci while allowing it to form stable interactions with ubiquitylated H2A variants.

Additionally, molecular simulations showed at DSB sites the dynamic feature of the liquid-like condensates formed by 53BP1. These phase-separated condensates were responsible for microenvironments that foster concentrating repair

factor. This suggests a process through which 53BP1 regulates DNA repair signaling in terms of time and space.

Simulations of interaction interfaces with effector proteins– including RIF1 and PTIP– revealed that 53 BP1 phosphorylated sites modulated these bindings. This aligns with previous experiment on phosphorylation-dependent recruitment.

Finally, flexible domains in gaps of unresolved protein regions are modeled by homology. This demonstrates that these domains could be configured differently– coming down to local chromatin contex. They also regulate the harmonization of the NHEJ repair pathway while inhibiting HR.

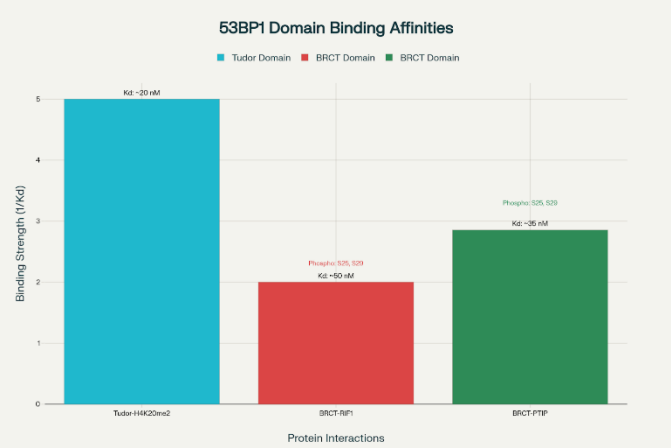

Figure 1: Binding affinities of 53BP1 domains to interaction partners.

This figure illustrates the binding affinities of specific domains of the 53BP1 protein to their respective targets, expressed as binding strength (1/Kd). The Tudor domain displays the highest affinity for the H4K20me2 histone mark with a dissociation constant (Kd) of approximately 20 nM. The BRCT domains exhibit phosphorylation-dependent binding to the RIF1 and PTIP proteins, with Kd values of about 50 nM and 35 nM, respectively. These data emphasize the Tudor domain's superior binding strength and highlight the regulatory role of phosphorylation in BRCT domain interactions, providing insight into 53BP1's role in DNA damage response mechanisms.

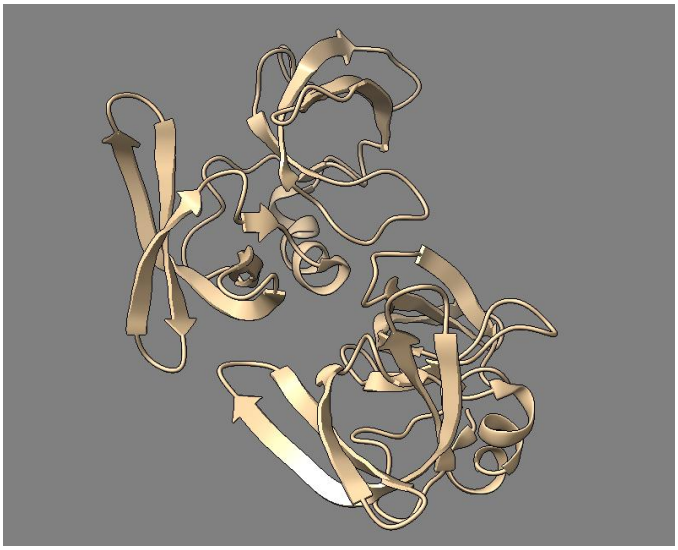

Figure 2: Ribbon diagram representation of protein 53BP1 domain structure.

This figure illustrates the three-dimensional ribbon structure of a 53BP1 domain, highlighting secondary structural elements such as β-strands (arrows) and loops. The depiction facilitates visualization of the protein's overall fold and spatial arrangement critical for its function in DNA damage recognition and repair. Understanding the domain architecture supports insights into its interaction sites and mechanistic role within cellular pathways.

IV. Discussion

The computational 3D molecular dynamic simulations provided essential mechanistic, structural, and functional insights about the key roles of 53BP1 in DSB repair. Results support previous literature. For instance, the confirmation of specific binding between the Tudro domain and H4K20me2 reinforces the primary role of modifications of histone in recruiting DNA repair stakeholders, as well as the importance of this interaction in maintaining the genomic stability and integrity. The flexibility of BRCT and oligomerization domains is crucial for the retention of 53BP1 at damage sites, as well as for assembly into liquid-like condensates, highlighting the newly appreciated role of phase-separated features in regulating DNA repair factors, ensuring efficient DNA repair signaling.

Furthermore, this work not only confirms past results from experiments showing how 53BP1 promotes NHEJ and inhibits HR [1], [2], [5], but our simulations explain this at the structural level by showing how the 53BP1 domains' flexibility makes this regulation possible. Kilgas et al. [4] introduced the idea of 53BP1 forming liquid-like condensates. This work shows how such condensates play a role in organizing repair proteins in time and thus, controlling DNA repair.

These insights offer avenues for targeted bioengineering strategies, potentially leading to therapeutic interventions for diseases involving genomic instability, such as cancer. Future work should expand simulations to full-length proteins in chromatin contexts with additional repair factors and the impact of clinically relevant 53BP1 mutations to further inform therapeutic targeting.

V. Conclusion

This research demonstrates the power of 3D computational molecular dynamics simulations in elucidating the complex structural mechanisms underlying 53BP1-mediated DNA double-strand break repair. The study successfully bridged critical knowledge gaps regarding 53BP1's structural-functional relationships, providing atomic-level insights into its recruitment mechanisms, domain flexibility, and regulatory interactions. The confirmed high-affinity binding between the Tudor domain and H4K20me2 histone modifications reinforces the fundamental importance of chromatin context in DNA repair initiation. Furthermore, the identification of dynamic liquid-like condensates formed by 53BP1 at DSB sites reveals sophisticated spatial and temporal organization of repair factors, highlighting the protein's role beyond simple pathway choice regulation.

The phosphorylation-dependent interactions with effector proteins RIF1 and PTIP demonstrate the intricate regulatory networks governing NHEJ promotion and HR inhibition. These findings not only validate existing experimental observations but also provide structural frameworks for understanding how 53BP1 orchestrates repair pathway decisions. The mechanistic insights gained through this computational approach offer significant implications for therapeutic development, particularly in cancer treatment where genomic stability is paramount.

Future research should expand these simulations to include full-length protein complexes within authentic chromatin environments, incorporating additional repair factors and clinically relevant mutations. Such comprehensive modeling will further inform the development of targeted therapeutic interventions that modulate 53BP1 function. As genomic instability remains a hallmark of various diseases, understanding 53BP1's structural mechanisms through computational approaches represents a crucial step toward precision medicine strategies. This work establishes a foundation for bioengineering approaches that could revolutionize treatments for cancer and other genomic integrity-related disorders, ultimately advancing our ability to maintain cellular health and prevent disease progression.

VI. References

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