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The relationship between UHRF1 and DNMT1 and the process of DNA methylation

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Abstract. The maintenance of normal epigenetic growth and development is crucial for various biological processes, with DNA methylation playing a significant role. The UHRF1-DNMT1 complex regulates DNA methylation, particularly in mammalian cells. This review paper explores the relationship between UHRF1, DNMT1, and DNA methylation homeostasis in cancer cells. Studies have primarily focused on human colorectal cell lines HCT116 and DLD1, shedding light on the protein stabilization of UHRF1 and DNMT1 through methylation-mediated ubiquitination. The impact of UHRF1 on DNA methylation in cancer cells is evaluated by controlling growth hormones. Furthermore, the study reveals that UHRF1 down-regulation influences DNMT1-mediated methylation on DNA and highlights the non-canonical functions of UHRF1 that significantly contribute to DNA methylation homeostasis. This review paper delves into the intricate relationship between the UHRF1-DNMT1 complex and DNA methylation homeostasis, particularly in the context of cancer cells. By examining the protein stabilization mechanisms of UHRF1 and DNMT1, as well as the regulatory role of UHRF1 in DNA methylation, this study provides valuable insights into the molecular mechanisms underlying epigenetic regulation. The findings presented in this review contribute to a better understanding of the role of UHRF1 and DNMT1 in maintaining DNA methylation patterns and highlight their potential implications in cancer biology.

Keywords: DNA Methylation, DNMT1, UHRF1.

1. Introduction

DNA methylation is a fundamental epigenetic modification with profound implications for various biological processes in mammals. This covalent modification involves the addition of a methyl group to the 5-carbon position of cytosine residues within CpG dinucleotides, a process primarily orchestrated by DNA methyltransferases such as DNMT1, DNMT3A, DNMT3B, and DNMT3L [1]. This modification is crucial for a range of biological phenomena including gene expression regulation, genomic imprinting, X-chromosome inactivation, and maintenance of genome stability. The methylation status of DNA can influence gene expression patterns and cellular differentiation, playing a pivotal role in organismal development, tissue-specific gene regulation, and maintenance of cellular identity.

The regulatory effects of DNA methylation are achieved through several mechanisms. For instance, methylation of CpG islands within gene promoters generally leads to transcriptional silencing by obstructing the binding of transcription factors and recruiting repressive chromatin modifiers. Conversely, DNA methylation in gene bodies can be associated with transcriptional activation and

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regulation of alternative splicing [2]. The dynamic regulation of DNA methylation is also critical for cellular reprogramming, memory formation, and adaptation to environmental changes.

Dysregulation of DNA methylation has been implicated in numerous pathological conditions. In cancer, for example, aberrant DNA methylation patterns are frequently observed. Tumor cells often exhibit global hypomethylation, which can lead to genomic instability and activation of oncogenes, as well as promoter hypermethylation, which silences tumor suppressor genes and contributes to tumorigenesis [3]. The hypermethylation of CpG island promoters, in particular, is a hallmark of many cancers, where it can lead to the silencing of crucial genes involved in cell cycle regulation, apoptosis, and DNA repair [4].

Recent scientific advances have significantly expanded our understanding of the mechanisms regulating DNA methylation dynamics. The key proteins involved in these processes are UHRF1 (Ubiquitin-like, containing PHD and RING finger domains 1) and DNMT1 (DNA methyltransferase 1). UHRF1 plays a critical role in maintenaning DNA methylation patterns by specifically recognizing and binding to hemimethylated DNA during replication. This recognition ensures the accurate copying of methylation marks onto newly synthesized DNA strands, thereby preserving the epigenetic state across cell divisions [5]. UHRF1 achieves this by interacting with specific histone modifications and promoting the recruitment of DNMT1 to replication sites.

DNMT1, in turn, is essential for the stability of DNA methylation patterns. It functions to add methyl groups to the cytosine residues of newly synthesized DNA, thus maintaining the integrity of the methylation pattern established in the parent DNA strand. This activity is crucial for the perpetuation of epigenetic information and ensuring that the methylation patterns are faithfully transmitted to daughter cells [6]. The interaction between UHRF1 and DNMT1 is therefore fundamental to the maintenance of DNA methylation and, by extension, to the regulation of gene expression and cellular identity.

This review aims to synthesize current knowledge regarding the roles of UHRF1 and DNMT1 in DNA methylation and their implications in cancer biology. By integrating insights from recent research, we highlight the collective significance of these regulatory mechanisms in maintaining proper epigenetic control and their impact on disease. A comprehensive understanding of the molecular processes governing DNA methylation will not only advance our knowledge of cancer pathogenesis but also offer potential avenues for the development of targeted therapeutic interventions and biomarkers for precision medicine applications. The diagnostic potential of DNA methylation patterns, including their use as biomarkers for early cancer detection and prediction of treatment responses, represents a significant advancement in personalized oncology strategies.

2. The role of DNMT1 in development and disease

The DNMT1 gene encodes DNA methyltransferase 1 (DNMT1), a pivotal enzyme responsible for DNA methylation, a critical epigenetic modification that plays a vital role in numerous cellular functions. DNMT1 is essential for maintaining DNA methylation patterns during cell division, ensuring that the epigenetic information is faithfully inherited by daughter cells [2]. This process is particularly important for gene silencing, metabolic regulation, and the modulation of neurotransmitter signaling pathways.

DNMT1’s function extends beyond its role in maintaining DNA methylation. For example, in the nervous system, DNMT1 activity is dynamically regulated and has profound implications for neuronal development and function. Evidence indicates that DNMT1 is involved in neuronal maturation, synaptic plasticity, and survival [3]. These findings underscore the pivotal role of DNMT1 in shaping the structural and functional characteristics of the nervous system through epigenetic mechanisms.

Moreover, DNMT1's influence is not confined to neuronal processes. In trophoblast cells, DNMT1 regulates cell proliferation, migration, and invasion by modulating the methylation levels of genes such as APLNR [4]. This highlights DNMT1's broader impact on cellular dynamics and tissue development beyond the nervous system.

These observations collectively emphasize the multifaceted roles of DNMT1 in both physiological processes and disease mechanisms. Understanding the complex regulation of DNMT1 and its downstream effects on DNA methylation patterns provides valuable insights into the epigenetic

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mechanisms underlying normal development and disease pathogenesis. Further research into the precise regulatory mechanisms of DNMT1 and its interactions with other proteins will enhance our understanding of epigenetic regulation and potentially lead to novel therapeutic strategies targeting epigenetic modifications in disease contexts.

3. UHRF1 orchestrating epigenetic stability and therapeutic potential

The transmission of DNA methylation patterns across generations is intricately governed by UHRF1, a gene encoding a multifunctional protein crucial for gene regulation and cell cycle control. UHRF1, which stands for Ubiquitin-like, containing PHD and RING finger domains 1, plays a central role in maintaining DNA methylation patterns by recognizing and binding to specific DNA and histone modifications.

UHRF1's interaction with hemi-methylated DNA and histone modifications, such as H3K9me2/3, is essential for establishing and preserving DNA methylation marks. This process is independent of UHRF1's SRA semi-MCPG binding activity [5]. UHRF1 also functions as an E3 ubiquitin ligase, which is crucial for DNA repair mechanisms and ensuring proper spindle architecture during cell division [6]. The multifunctional nature of UHRF1 extends to its influence on cancer biology. Dysregulation of UHRF1 has been implicated in tumorigenesis and metastasis, highlighting its significance in maintaining the fidelity of DNA methylation patterns across cellular generations [7].

Understanding UHRF1's role in orchestrating the interplay between DNA methylation and chromatin modifications provides insights into broader epigenetic regulation. Targeting UHRF1 holds therapeutic promise in modulating DNA methylation dynamics in various pathological contexts, including cancer and neurological disorders. Continued research into UHRF1's precise mechanisms and interactions with other epigenetic regulators will enhance our understanding and potentially lead to novel therapeutic strategies for correcting epigenetic dysregulation in diseases.

4. UHRF1-mediated activation of DNMT1

Maintaining DNA methylation patterns during cell division is a complex and crucial process for cellular function. This stability is heavily dependent on the intricate interaction between DNMT1 and UHRF1. While DNMT1 is essential for the maintenance of DNA methylation, its activity alone is insufficient to ensure the accurate transmission of these patterns. UHRF1 plays a critical role as a key regulator in this process by facilitating the recruitment and activation of DNMT1 at replication forks, thus ensuring that the DNA methylation marks are faithfully copied onto newly synthesized DNA strands [5].

The structural domains of DNMT1 and UHRF1 are crucial for their functional interaction. DNMT1 possesses several structural domains, including the regulatory region called the RFTS (Replication Focus Targeting Sequence) domain, which is essential for its interaction with UHRF1. The RFTS domain of DNMT1 engages with the UBL (Ubiquitin-Like) domain of UHRF1, a critical interaction necessary for the effective recruitment of DNMT1 to replication sites [6]. This interaction is fundamental for the activation of DNMT1 and subsequent enzymatic activity, which involves adding methyl groups to cytosine residues in newly synthesized DNA.

UHRF1's UBL domain is known to facilitate this interaction through recognition and binding to hemimethylated DNA and specific histone modifications. UHRF1's ability to recognize hemimethylated DNA is essential for its function, as it ensures that DNMT1 is directed to regions of DNA that require methylation maintenance. Additionally, UHRF1's E3 ubiquitin ligase activity, which involves tagging histone H3 with ubiquitin, plays a critical role in modulating the interaction between DNMT1 and chromatin [7]. Ubiquitylated histones H3 are recognized by DNMT1, which aids in its localization and activity at replication forks.

Experimental evidence supports the significance of these interactions in DNA methylation maintenance. Crystal structure analyses have provided detailed insights into how DNMT1's RFTS domain and UHRF1's UBL domain interact, illustrating the molecular basis of their functional collaboration. These structural studies reveal that the binding of UHRF1 to hemimethylated DNA and

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histone modifications facilitates DNMT1's access to its substrate, thereby promoting efficient DNA methylation replication [8].

In addition to structural studies, in vitro DNA methylation experiments have demonstrated the importance of the DNMT1-UHRF1 interaction. These experiments show that the absence of UHRF1 impairs DNMT1's ability to maintain DNA methylation patterns, highlighting the critical role of UHRF1 in this process. Studies in knockout mouse embryonic stem cells further reinforce the essential nature of this interaction. These studies have shown that mice lacking UHRF1 exhibit significant defects in DNA methylation maintenance, underscoring the indispensable role of UHRF1 in reestablishing DNA methylation patterns during cell division [9].

The interplay between UHRF1 and DNMT1 extends beyond mere recruitment and activation. UHRF1 also influences DNMT1's stability and activity through post-translational modifications such as ubiquitination. The regulation of DNMT1 by UHRF1 involves complex feedback mechanisms, where the ubiquitination of DNMT1 can modulate its interaction with DNA and histones, affecting its enzymatic activity and stability [10]. This regulatory loop ensures that DNA methylation patterns are precisely maintained across cell divisions, contributing to genomic stability and cellular function.

5. DNMT1 and ubiquitinated histone H3 and its effect on global DNA methylation

Recent studies have significantly enhanced our comprehension of the complex interplay between DNMT1, ubiquitinated histone H3, and their influence on global DNA methylation patterns, particularly within mouse embryonic stem (ES) cells. Investigations since 2020 have illuminated critical facets of this interaction, shedding new light on its pivotal role in epigenetic regulation.

In studies utilizing DNMT1 knockout mouse ES cell lines complemented with wild-type DNMT1 or various mutant forms, researchers have observed that disrupting the interaction between DNMT1 and ubiquitinated histone H3 markedly impairs DNMT1's nuclear localization and its capacity to uphold DNA methylation. Notably, experiments employing DNMT1 mutants have shown differential degrees of rescue in DNA methylation deficiencies observed in DNMT1-deficient cells [9].

Ubiquitinated histone H3, facilitated by UHRF1, serves as a crucial mediator in recruiting DNMT1 to newly replicated DNA strands lacking methylation marks. This mechanism ensures the accurate propagation of DNA methylation patterns during cellular division. Specifically, H3K9 methylation (H3K9me), a histone modification linked to the formation of transcriptionally silent heterochromatin is pivotal in establishing these epigenetic states. The recruitment of DNMT1 to these regions through ubiquitinated histone H3 underscores its pivotal function in maintaining epigenetic stability [10].

Moreover, the enzymatic removal of histone modifications, including ubiquitination of histone H3, represents a significant mechanism for reversing epigenetic silencing. Recent investigations have underscored the dynamic nature of these modifications and their profound impact on chromatin structure and gene expression regulation [11].

A study published in 2021 elucidated the structural basis of UHRF1's interaction with histone H3 and its role in DNA methylation maintenance. The researchers used cryo-electron microscopy (cryo-EM) to visualize the complex formation between UHRF1, histone H3, and DNA, providing insights into how UHRF1 recognizes and binds to specific chromatin regions to facilitate DNA methylation [12].

6. Stimulating the E3 ubiquitin ligase activity of UHRF1 toward chromatin

The recruitment of DNMT1 to newly replicated chromatin is a critical process for the maintenance of DNA methylation patterns. This recruitment depends on the ubiquitination of histone H3, a process orchestrated by UHRF1. However, the precise mechanism by which UHRF1 facilitates the attachment of ubiquitin to histone H3 remains incompletely understood. This study aims to clarify this mechanism by focusing on the role of the ubiquitin-like domain (UBL) of UHRF1 in the RING-mediated ubiquitination of histone H3.

To investigate this, it conducts a series of experiments involving the interaction of nucleosomes with both full-length UHRF1 and its truncated fragments. This approach will elucidate the essential function of the UBL domain in UHRF1’s E3 ubiquitin ligase activity. Additionally, it will examine the specificity

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of UHRF1 towards various modification marks and its ability to target histone H3 for ubiquitylation. By dissecting the molecular interactions between UHRF1, histone H3, and nucleosomes, the goal is to uncover the precise mechanisms underlying the catalytic activity of UHRF1 in modifying histone proteins.

Furthermore, this study will explore the intricate regulatory mechanisms that govern the stimulation and localization of UHRF1's E3 ligase activity within the chromatin environment. By analyzing the interplay between UHRF1, histone H3, and other chromatin-associated factors, the aim is to elucidate how UHRF1 is targeted to specific chromatin regions and how its activity is modulated in response to cellular signals. Understanding these regulatory processes is crucial for comprehending UHRF1's role in maintaining DNA methylation patterns and ensuring epigenetic stability.

In addition, it will investigate the functional significance of the ΔUBL mutant of UHRF1. This comparison between wild-type UHRF1 and the ΔUBL mutant will help determine the specific contributions of the UBL domain to UHRF1's catalytic activity and substrate specificity. These findings are expected to provide deeper insights into the molecular mechanisms underlying DNA methylation regulation and to identify potential therapeutic targets for modulating epigenetic processes in disease contexts.

7. Conclusion

The interplay between UHRF1 and DNMT1 is central to the maintenance of DNA methylation patterns, which are crucial for cellular stability, gene expression regulation, and overall epigenetic homeostasis. Through a detailed exploration of their interaction, this paper has illuminated the intricate mechanisms by which UHRF1 orchestrates the recruitment and activation of DNMT1, ensuring the faithful transmission of DNA methylation marks during cell division.

UHRF1's role extends beyond mere recognition of hemimethylated DNA; it functions as a multi-faceted regulator by engaging with specific histone modifications and acting as an E3 ubiquitin ligase. This dual functionality is essential for the proper establishment and maintenance of DNA methylation, particularly in cancer cells where aberrant epigenetic modifications contribute to tumorigenesis and disease progression. The dynamic interaction between UHRF1 and DNMT1, facilitated by UHRF1's UBL domain and DNMT1's RFTS domain, underscores the complexity of epigenetic regulation and highlights the necessity of these interactions for preserving DNA methylation patterns across generations.

The impact of UHRF1 on DNMT1-mediated methylation and its influence on global DNA methylation patterns have been further elucidated through studies involving ubiquitinated histone H3. These findings emphasize the significance of post-translational modifications in regulating DNMT1 activity and ensuring the accurate propagation of DNA methylation marks. The disruptions in these interactions, as observed in various experimental models, provide valuable insights into the mechanisms underlying epigenetic dysregulation and its implications for cancer biology.

As our understanding of the UHRF1-DNMT1 axis continues to evolve, it becomes increasingly clear that targeting these pathways holds substantial therapeutic potential. Developing inhibitors or modulators that specifically disrupt or enhance UHRF1-DNMT1 interactions could pave the way for novel cancer therapies and epigenetic treatments. Additionally, the insights gained from studying UHRF1 and DNMT1 interactions may inform the design of diagnostic tools and biomarkers for early cancer detection and treatment response prediction.

In conclusion, the detailed examination of UHRF1 and DNMT1 in this review highlights their critical roles in maintaining DNA methylation homeostasis and their broader implications in disease contexts, particularly cancer. Continued research into the molecular mechanisms governing UHRF1-DNMT1 interactions and their impact on DNA methylation will be instrumental in advancing our understanding of epigenetic regulation and developing innovative therapeutic strategies. By integrating these insights, we move closer to harnessing the full potential of epigenetic therapies and improving patient outcomes in cancer and beyond.

References

[1] Moore, L.D., Le, T. and Fan, G. (2012) DNA methylation and its basic function, Nature News. Available at: https://www.nature.com/articles/npp2012112 (Accessed: 21 July 2024).

[2] Yamaguchi, K. et al. (2024) Non-canonical functions of UHRF1 maintain DNA methylation homeostasis in cancer cells, Nature News. Available at: https://www.nature.com/articles/s41467-024-47314-4 (Accessed: 21 July 2024).

[3] Feng, J. et al. (2010) Dnmt1 and dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons, Nature News. Available at: https://www.nature.com/articles/nn.2514 (Accessed: 21 July 2024).

[4] 4.0 Author links open overlay panelYan Zhou et al. (2023) DNMT1 mediates proliferation, migration and invasion of extravillous trophoblasts by regulating the methylation level of APLNR, Placenta. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0143400423001017 (Accessed: 21 July 2024).

[5] Arita K;Ariyoshi M;Tochio H;Nakamura Y;Shirakawa M; (no date) Recognition of hemi-methylated DNA by the SRA protein uhrf1 by a base-flipping mechanism, Nature. Available at: https://pubmed.ncbi.nlm.nih.gov/18772891/ (Accessed: 21 July 2024).

[6] Tu, Z. et al. (2020) UHRF1 predicts poor prognosis by triggering cell cycle in lung adenocarcinoma, Journal of cellular and molecular medicine. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7348181/ (Accessed: 21 July 2024).

[7] Nishiyama, A. et al. (2013) UHRF1-dependent H3K23 ubiquitylation couples maintenance DNA methylation and replication, Nature News. Available at: https://www.nature.com/articles/nature12488 (Accessed: 21 July 2024).

[8] Rothbart, S.B. et al. (2012a) Association of uhrf1 with methylated H3K9 directs the maintenance of DNA methylation, Nature News. Available at: https://www.nature.com/articles/nsmb.2391

[9] RM;, C.X. (no date) Mammalian DNA methyltransferases: A structural perspective, Structure (London, England : 1993). Available at: https://pubmed.ncbi.nlm.nih.gov/18334209/

[10] Handy, D. E., Castro, R., & Loscalzo, J. (2011). Epigenetic modifications. Circulation, 123(19), 2145–2156. https://doi.org/10.1161/circulationaha.110.956839

[11] Li, T., Wang, L., Du, Y., Xie, S., Yang, X., Lian, F., Zhou, Z., & Qian, C. (2018, April 6). Structural and mechanistic insights into UHRF1-mediated DNMT1 activation in the maintenance DNA methylation. Nucleic acids research. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5887372/

[12] Qin, W., Wolf, P., Liu, N., Link, S., Smets, M., Mastra, F. L., Forné, I., Pichler, G., Hörl, D., Fellinger, K., Spada, F., Bonapace, I. M., Imhof, A., Harz, H., & Leonhardt, H. (2015, June 12). DNA methylation requires a DNMT1 ubiquitin interacting motif (UIM) and histone ubiquitination. Nature News. https://www.nature.com/articles/cr201572