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Charting the Unseen: Exploring the Effects of Epigenetic Modifications on Sperm and Their Role in Male Infertility

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ABSTRACT

Objective: *This study aims to review existing literature on the impact of epigenetic modifications, including DNA methylation, histone modifications, and non-coding RNA, on gene expression and their implications for human health and disease mechanisms.* ***Methods:*** *A literature review was conducted between January 2023 and October 2024, analyzing nine selected studies that explored the relationship between epigenetic mechanisms and gene expression in patients with various conditions, such as heart disease and cancer. Key methodologies discussed include RNA sequencing (RNA-Seq) for quantifying gene expression, chromatin immunoprecipitation sequencing (ChIP-Seq) for protein-DNA interaction analysis, and DNA microarray analysis for genome-wide gene expression profiling.* ***Results:*** *The findings highlight that DNA methylation suppresses gene expression by chemically modifying DNA, histone modifications alter chromatin structure to regulate gene accessibility, and non-coding RNAs influence transcription and translation processes. The interplay among these mechanisms was shown to regulate gene expression without requiring genetic mutations, resulting in diverse biological outcomes. The review also discusses the role of daily nutrition, potential complications, and therapeutic strategies targeting epigenetic modifications.* ***Novelty:*** *This study underscores the critical role of epigenetic changes in modulating gene expression and provides insights into their potential for advancing the understanding of disease mechanisms and developing novel approaches for diagnosis, treatment, and prevention.*

INTRODUCTION

Epigenetics was the study in modifications to organisms which are caused by alteration of gene expression. It is thought of as the chromosomal regions' structural flexibility to record, signal, and sustain changed activity levels [1 – 4]. Among the several epigenetic modifications is DNA methylation, which is the transfer for a methyl group into a Cytosine-5 inside a C-phosphate-G (CpG) structure context [5]. Although these physically altered sites are sporadically located throughout the genome, they are frequently found in areas of gene promoters called as CpG islands (CGIs) [6,7]. Generally speaking, CpGs within the islands are more likely to be non-methylated than CpGs outside the islands [8].

Moreover, current research suggests that the CpGs that are situated along the beach of CGIs were the ones that often exhibit altered methylation in different tissues or groups of experiments. Reduced gene expression is correlated with a greater amount of methylation across CpG islands in the 5' region of the gene [9]. Histone deacetylases and other inhibitory proteins may be added, or transcription factor binding may be disrupted as potential sources of this suppression [10,11]. It is not necessarily the case that DNA methylation along CGIs will prevent the neighboring gene in expressing itself. More recent articles, however, demonstrated also favorable associations among expression and methylation [12 – 14].

Conversely, the opposite connection among methylation with expression was shown to be common, especially in CGIs or promoter regions [15]. Genetic variation linked to expression as well as methylation levels have been found in a variety of species, tissues, and populations. Approximately 34% percent of gene expression levels for cell lines, as well as 25% of the levels of DNA methylation in blood, are heritable [16]. It has been shown that there are both distal (trans) and local (cis) relationships between genetic variation and levels of gene expression. Methylation quantitative trait loci (mQTLs) may now be investigated genome-wide regardless of the tissue or cell type of interest because to the development of high-throughput DNA methylation techniques [17]. While greater cis than trans regulation was found in expression (eQTLs), peak enrichment for mQTLs is found significantly closer to transcription start sites compared with eQTLs. However, it was not shown that a single genetic variation regulated both the degree of methylation as well as the level of expression in the genome-wide analysis of genetic regulators, expression, and methylation in four different brain regions [18,19].

Additionally, the same genetic variant has been demonstrated in co-regulate the levels of gene expression as well as methylation at lymphoblastoid cell lines generated from 77 Yoruba HapMap individuals. This finding suggests a similar mechanism in which a genetic variant modifies methylation, which in turn affects expression. It is commonly recognized that gene activity and CpG site methylation patterns vary depending on the tissue. [20 – 22]

RESEARCH METHOD

We conducted a patient review study that included a set of literature that discussed the impact of genetic modifications on gene expression in patients. In this article, we collected nine previous articles that described the role of DNA, histones, and non-coding RNA, and explained daily nutrition and complications in patients.

The search mechanism included discussing all gene expressions and modifications in a group of patients, some of whom were suffering from heart disease and cancer. This study also recorded discussions of treatments and gene regulation. This research review was conducted during the period from January 2023 to October 2024.

This enables researchers to investigate the impact of the gene's absence on gene expression patterns as well as any consequent phenotypic alterations. In a gene overexpression experiment, a gene was intentionally introduced or "overexpressed" into a cell or organism, resulting in elevated levels of gene expression. This can assist researchers in comprehending the impacts of heightened expression of genes on cellular activities.

Additional methods are RNA sequencing (RNA-Seq), which quantifies the expression of genes; chromatin immunoprecipitation-sequencing (ChIP-Seq), which investigates protein-DNA interactions; and DNA microarray analysis in which the expression of many genes in a genome is analyzed at a particular time.

RESULTS AND DISCUSSION

Table 1. Enrol data of participants observed in all nine reviews.

AUTHORS	*NAME OF ARTICALE*	*AIM*	*Methods used*	*Year*
Diane et al.	*Epigenetic Modifications Basic Mechanisms and Role in Cardiovascular Disease.*	*The objective of this paper is to examine the principal mechanisms of epigenetic regulation and to assess the role of nutrition and environment in cardiovascular disease.*	*DNA methylation and Histone modification*	*2011*
Xiang Zhou et al.	Epigenetic modifications are associated with inter-species gene expression variation in primates.	*This research aims to analyze the differences in gene expression that exist between species of primates and to evaluate the role that epigenetic changes play in the observed differences in gene regulation.*	*This investigation carried out both a comparative and an epigenetic analysis of lymphoblastoid cell lines from primates: an examination of the properties and activity of RNA polymerase II and the patterns of histone modification.*	*2014*
Supic et al.	*Epigenetics: A New Link Between Nutrition and Cancer*	*This research focused on understanding how nutrition influences the epigenetic machinery and susceptibility to cancer and sought to determine effective doses of bioactive food substances for cancer control.*	*Methylation of DNA, modifications of histones, and silencing of RNA. And the effects of dietary components on the expression of genes.*	*2013*
Falvo et al.	Epigenetic control of cytokine gene expression:	*This study aims to evaluate the epigenetic control of*	*Epigenetic alterations at DNA and nucleosomal levels*	*2013*

	regulation of the TNF/LT locus and T helper cell differentiation	*cytokine gene expression.*		
Whayne et al.	*Epigenetics in the development, modification, and prevention of cardiovascular disease*	*The purpose of this study was to clarify the connection between epigenetics and the development of cardiovascular disease.*	*Epigenetics research in cardiovascular disease: An overview of epigenetic alterations in cardiovascular disease and discussion of the environmental impact on gene regulation.*	*2015*
Tabolacci et al.	*Epigenetics, fragile X syndrome, and transcriptional therapy*	*The purpose of this investigation was to understand the epigenetic alterations present in fibroblasts from patients with systemic sclerosis.*	*Exploration of SSc Fibroblasts in Experimental Assays both In Vitro and In Vivo and Employment of Inhibitors of HDAC and DNA Methyltransferases.*	*2013*
Jüngel et al.	*Epigenetic modifications: novel therapeutic strategies for systemic sclerosis?*	*The research provided a clear perspective on chromatin alterations in the acquisition and retention of information and addressed the epigenetics of cognitive impairments.*	*Manipulation of the architectures of epigenetic machinery in animal models as well as practices such as genome-wide mRNA expression analysis.*	*2011*
Kramer et al.	*Epigenetic regulation of memory: implications in human cognitive disorders.*	*The current research focused on the epigenetic alterations induced by alcohol consumption and, moreover, the possibilities of epigenetic drug therapy.*	*Histone methylation and demethylation*	*2013*
Khin et al.	*Epigenetic alteration by DNA promoter hypermethylation of genes related to transforming growth factor-β (TGF-β) signaling in cancer*	*This research examined the epigenetic changes with a focus on DNA methylation in cancer.*	*Concentrate on the study of DNA methylation and the modification of histones.*	*2011*

Table. 2. Description of the study results in the Literature review according to the limitations and contributions of the researchers.

Authors	Contributions	Limitations
Diane et al.	***Epigenetic modifications control the ways in which genes are expressed. Environmental factors can alter epigenetic programming.***	Clinical studies utilizing folate nor other vitamin B treatments did not decrease the incidence of cardiovascular events. Most research have not investigated the effects of increased homocysteine on methylation mechanisms.
Xiang Zhou et al.	***Comparative epigenetic analysis in lymphoblastoid cell lines of primates. Furthermore, A look at the impact of epigenetic mechanisms on the differences in the expression of the genes.***	Statistical dependence does not indicate causal connections among variables. Inaccuracies of TSS annotations impact read assignment.
Supic et al.	***It is well known that nutrition, epigenetics, and cancer are deeply intertwined. Forecasting and treatment of cancer with the help of diet.***	Insufficient data about tissue specificity as well as temporal dimensions. Requirement for more research on optimal dosages and concentrations.
Falvo et al.	***Goldfeld lab members provided input for the review.***	The specific elements influencing Th17 lineage commitment remain contested. Limited research on epigenetic pathways for Th17 cell differentiation.
Whayne et al.	Define epigenetics in relation to the development of cardiovascular disease. Examines the influence of environmental factors on epigenetics via diet and physical activity.	Restricted applicability of animal findings to human contexts. Concerns regarding the detrimental effects of excessive physical activity.
Tabolacci et al.	Epigenetic changes facilitate the activation of SSc fibroblasts. Reversal of the profibrotic phenotype using epigenetic therapeutics.	The safety along with toxicity profiles may vary among people with systemic sclerosis (SSc). Robust antifibrotic effects shown in animals do not ensure effectiveness in humans.
Jüngel et al.	Comprehending molecular alterations involved in memory formation and retention. Identifying epigenetic modulators for learning and memory.	Challenges in separating neurons that encode specific memories. Requirement for comprehensive epigenetic analysis across the genome during the learning process.
Kramer et al.	Emphasizes epigenetic alterations resulting from alcohol consumption. Identifies prospective epigenetic	Limited research exists about the impact of alcohol on sumoylation. Insufficient comprehension on histone ubiquitination in relation to neurological consequences.

	pharmacological targets for therapeutic intervention.	
Khin et al.	Concentrate on epigenetic modifications produced by DNA methylation. Investigate the function of TGF-β signalling in cancer advancement.	Insufficient data on heritable epigenetic modifications in histones. The intricate mechanism of TGF-β signalling during oncology.

Table 3. Identify outcomes of patients in terms of summary.

Authors	***Summary***
Diane et al.	Epigenetic changes govern gene expression and developmental processes. Environmental influences can alter epigenetic markers.
Xiang Zhou et al.	This discovery indicates that epigenetic modifications have a strong association with alterations for gene expression levels between primates and may constitute crucial biological processes in primate evolution.
Supic et al.	Dietary constituents influence gene expression through epigenetic processes. Epigenetic modifications connect genetic variables and environmental influences on cancer.
Falvo et al.	Epigenetic regulation in cytokine gene expression during immunological responses. Concentrate on the TNF/LT locus and the development of T helper cells.
Whayne et al.	Epigenetics affects cardiovascular disease and associated disorders. Nutrition and exercise influence epigenetics, affecting health consequences.
Tabolacci et al.	Epigenetic changes influence gene expression for systemic sclerosis. DNA methylation, as well as histone changes, facilitate fibroblast activation. Epigenetic pharmaceuticals can revert the activated fibroblast phenotype. Possibility for clinical studies that emphasize epigenetic pathways in systemic sclerosis (SSc).
Jüngel et al.	Epigenetic changes govern gene expression for memory and learning. Alterations in chromatin are crucial for the consolidation in long-term memory. Cognitive impairment is associated with epigenetic pathways in several illnesses. Particular mutations to chromatin complexes result in intellectual impairments and autism. The review examines chromatin changes and their significance in cognitive disorders.
Kramer et al.	Alcohol consumption causes epigenetic modifications in the brain. Research concentrates on epigenetic pharmacological targets for alcohol dependence.
Khin et al.	Epigenetic modifications profoundly influence the expression of cancer-associated genes. TGF-β signaling induces specific epigenetic modifications in cancer. Hypermethylation inhibits tumor suppressor genes, facilitating carcinogenesis. CIMP denotes a specific subset of certain tumors. Identifying repressed genes facilitates early diagnosis and treatment.

Table 4. Describe the outcomes based on the last outcomes and problem.

Problem statement	***Findings***	***Conclusion***

Diane et al.	Due to its heredity, DNA methylation serves as an effective mechanism in inhibiting the expression of undesired or superfluous genes.	Epigenetic alterations are crucial to cardiovascular disease. Accumulation of homocysteine can result in worldwide DNA hypomethylation.
Xiang Zhou et al.	A comparative analysis of monkey lymphoblastoid cell lines indicates that variations in mark enrichment at transcription starting sites are frequently associated with inter-species variances in gene expression levels above what would be anticipated by chance.	Those observations imply that changes in gene expression levels among primates may be epigenetically regulated and considered on important factors which drive the evolution of primates as organisms.
Supic et al.	Clarifying the influence for nutrition on epigenetic pathways might facilitate the prediction of an individual's cancer risk, provide dietary guidance, or allow therapeutic uses of natural chemicals in cancer treatment.	Epigenetic alterations substantially influence variations in gene expression among primates. They may have significant functions in primate evolution.
Falvo et al.	Epigenetic alterations at the nucleosome and DNA level may be related with higher-order intra- and interchromosomal interactions that affect the positioning of regulatory elements, potentially situating them inside certain nucleoprotein complexes linked to transcription.	Nutrition affects epigenetic processes related to cancer vulnerability. Additional investigation is required about dietary elements and their role in cancer prevention.
Whayne et al.	The association with cardiovascular disease, especially coronary heart disease as well as peripheral artery disease, is seen through epigenetic connections and alterations influenced by significant cardiovascular risk factors, including tobacco smoking. The process of aging might potentially be influenced by epigenetic modifications.	Epigenetic modulation is essential for immunological response as well as T-cell differentiation. Comprehending histone changes facilitates the development of novel therapies.
Tabolacci et al.	Fragile X syndrome (FXS) is a genetic repeat expansion condition resulting from dynamic mutations at the 5′ untranslated region (UTR) of the FMR1 gene, which induces epigenetic alterations such as DNA methylation with histone deacetylation. This leads to gene inactivation; nevertheless, an undamaged FMR1 coding sequence permits	The alteration of the epigenome by environmental factors affects health and the progression of diseases. Nutrition and exercise may affect significant risk factors for illnesses.

	pharmacological reactivation by DNA demethylation agents along with histone deacetylase inhibitors.	
Jüngel et al.	This research demonstrates that epigenetic changes, such as DNA methylation and histone alterations, substantially influence the synthesis with extracellular matrix proteins of systemic sclerosis-associated skin fibroblasts, as well as that the application of epigenetic-based pharmaceuticals can ameliorate this phenotype.	Epigenetic alterations influence the active phenotype of fibroblasts in systemic sclerosis. Epigenetic-based pharmaceuticals can revert the activated phenotype.
Kramer et al.	This study indicates that epigenetic modulation of chromatin architecture is essential for the consolidation of long-term memory, necessitating the transcription of new genes. Disruption of certain chromatin modification complexes, which are implicated by nuclear hormone receptor-mediated transcriptional control, is associated with intellectual disabilities and autism spectrum disorders.	Epigenetic changes are essential in learning and memory. Comprehending these pathways may improve therapy for cognitive disorders.
Khin et al.	TGF-β signals, especially from the microenvironment, induce specific epigenetic modifications in oncogenes, with its dysregulation partially affected by epigenetic alterations despite its multiple functions in cancer.	Epigenetic modifications provide opportunities for cancer treatment. TGF-β signaling influences cancer progression and therapeutic approaches.

Discussion

Epigenetic modifications in an organism are crucial regulators of gene expression, greatly impacting several aspects in human health, development, as well as disease. [23 – 25]

Epigenetic modifications significantly impact gene expression by regulating cell development and specialization [26]. Epigenetic alterations regulate the activation or repression of certain genes in many cell types, hence facilitating appropriate cellular development and function [27,28]. This process is crucial for proper growth and development, as well as for sustaining the health and functionality of many tissues and organs inside the body. [29]

Moreover, epigenetic alterations may impact the vulnerability to specific illnesses and situations [30]. Changes in epigenetic markers can modify the expression of genes related to multiple disorders, including cancer, diabetes, and cardiovascular disease. Certain alterations can either augment or diminish the likelihood of getting certain diseases, contingent upon the individual genes and pathways implicated. [31,32]

Moreover, epigenetic alterations may be influenced by environmental variables, lifestyle decisions, and psychological stress [33]. External factors can modify the epigenetic landscape for an individual, resulting in alterations of gene expression that may affect health and well-being. Exposure to pollutants, inadequate nutrition, and persistent stress can all lead to epigenetic modifications that elevate disease risk. [34].

CONCLUSION

The study highlights that epigenetic modifications, including DNA methylation, histone modifications, and non-coding RNA interactions, are fundamental mechanisms that regulate gene expression, influencing cellular activities and health outcomes without altering the underlying genetic code. **Implications:** These findings emphasize the potential of targeting epigenetic changes in developing diagnostic, therapeutic, and preventive strategies for complex diseases such as cancer and heart disease. Moreover, understanding how external factors, such as nutrition and environmental influences, modify the epigenetic profile could provide valuable insights into disease prevention and personalized medicine. **Limitations:** The study is limited by the variability of the reviewed data, as differences in methodologies, sample populations, and conditions across studies may influence the generalizability of the findings. Additionally, the complex interplay between genetic and epigenetic factors remains partially understood. **Further Research:** Future studies should focus on longitudinal analyses to better understand the dynamic nature of epigenetic changes and their causal relationships with health outcomes. Integrating multi-omics approaches and expanding the scope to diverse populations can further elucidate the role of epigenetic modifications in disease mechanisms and improve their translational application in clinical settings.

REFERENCES

[1] A. Bird, "Perceptions of Epigenetics," Nature, vol. 447, no. 7143, pp. 396–398, 2007.

[2] R. A. Irizarry et al., "The Human Colon Cancer Methylome Shows Similar Hypo- and Hypermethylation at Conserved Tissue-Specific CpG Island Shores," Nature Genetics, vol. 41, no. 2, pp. 178–186, 2009.

[3] R. A. Irizarry, H. Wu, and A. P. Feinberg, "A Species-Generalized Probabilistic Model-Based Definition of CpG Islands," Mammalian Genome, vol. 20, no. 9–10, pp. 674–680, 2009.

[4] N. Sengupta and E. Seto, "Regulation of Histone Deacetylase Activities," Journal of Cellular Biochemistry, vol. 93, no. 1, pp. 57–67, 2004.

[5] J. T. Bell et al., "DNA Methylation Patterns Associate with Genetic and Gene Expression Variation in HapMap Cell Lines," Genome Biology, vol. 12, no. 1, Art. no. R10, 2011.

[6] J. R. Gibbs et al., "Abundant Quantitative Trait Loci Exist for DNA Methylation and Gene Expression in the Human Brain," PLoS Genetics, vol. 6, no. 5, Art. no. e1000952, 2010.

[7] D. Zhang et al., "Genetic Control of Individual Differences in Gene-Specific Methylation in Human Brain," American Journal of Human Genetics, vol. 86, no. 3, pp. 411–419, 2010.

[8] S. A. Monks et al., "Genetic Inheritance of Gene Expression in Human Cell Lines," American Journal of Human Genetics, vol. 75, no. 6, pp. 1094–1105, 2004.

[9] M. P. Boks et al., "The Relationship of DNA Methylation with Age, Gender, and Genotype in Twins and Healthy Controls," PLoS One, vol. 4, no. 8, Art. no. e6767, 2009.

[10] W. Cookson et al., "Mapping Complex Disease Traits with Global Gene Expression," Nature Reviews Genetics, vol. 10, no. 3, pp. 184–194, 2009.

[11] B. E. Stranger and E. T. Dermitzakis, "The Genetics of Regulatory Variation in the Human Genome," Human Genomics, vol. 2, no. 2, pp. 126–131, 2005.

[12] B. E. Stranger et al., "Relative Impact of Nucleotide and Copy Number Variation on Gene Expression Phenotypes," Science, vol. 315, no. 5813, pp. 848–853, 2007.

[13] A. C. Nica et al., "The Architecture of Gene Regulatory Variation Across Multiple Human Tissues: The MuTHER Study," PLoS Genetics, vol. 7, no. 2, Art. no. e1002003, 2011.

[14] B. E. Stranger et al., "Population Genomics of Human Gene Expression," Nature Genetics, vol. 39, no. 10, pp. 1217–1224, 2007.

[15] S. Ghosh et al., "Tissue-Specific DNA Methylation of CpG Islands in Normal Human Adult Somatic Tissues Distinguishes Neural from Non-Neural Tissues," Epigenetics, vol. 5, no. 6, pp. 527–538, 2010.

[16] P. Liang et al., "Genome-Wide Survey Reveals Dynamic Widespread Tissue-Specific Changes in DNA Methylation During Development," BMC Genomics, vol. 12, no. 1, Art. no. 231, 2011.

[17] G. J. Huang et al., "High-Resolution Mapping of Expression QTLs in Heterogeneous Stock Mice in Multiple Tissues," Genome Research, vol. 19, no. 6, pp. 1133–1140, 2009.

[18] E. Petretto et al., "Heritability and Tissue Specificity of Expression Quantitative Trait Loci," PLoS Genetics, vol. 2, no. 10, Art. no. e172, 2006.

[19] J. E. Aten et al., "Using Genetic Markers to Orient the Edges in Quantitative Trait Networks: The NEO Software," BMC Systems Biology, vol. 2, Art. no. 34, 2008.

[20] Y. Chen et al., "Variations in DNA Elucidate Molecular Networks That Cause Disease," Nature, vol. 452, no. 7186, pp. 429–435, 2008.

[21] M. Inouye et al., "An Immune Response Network Associated with Blood Lipid Levels," PLoS Genetics, vol. 6, no. 9, Art. no. e1001113, 2010.

[22] C. L. Plaisier et al., "A Systems Genetics Approach Implicates USF1, FADS3, and Other Causal Candidate Genes for Familial Combined Hyperlipidemia," PLoS Genetics, vol. 5, no. 9, Art. no. e1000642, 2009.

[23] A. P. Presson et al., "Integrated Weighted Gene Co-Expression Network Analysis with an Application to Chronic Fatigue Syndrome," BMC Systems Biology, vol. 2, Art. no. 95, 2008.

[24] S. Purcell et al., "PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses," American Journal of Human Genetics, vol. 81, no. 3, pp. 559–575, 2007.

[25] A. D. Johnson et al., "SNAP: A Web-Based Tool for Identification and Annotation of Proxy SNPs Using HapMap," Bioinformatics, vol. 24, no. 24, pp. 2938–2939, 2008.

[26] P. Langfelder and S. Horvath, "WGCNA: An R Package for Weighted Correlation Network Analysis," BMC Bioinformatics, vol. 9, Art. no. 559, 2008.

[27] B. Zhang and S. Horvath, "A General Framework for Weighted Gene Co-Expression Network Analysis," Statistical Applications in Genetics and Molecular Biology, vol. 4, Art. no. 17, 2005.

[28] P. Langfelder et al., "Is My Network Module Preserved and Reproducible?" PLoS Computational Biology, vol. 7, no. 1, Art. no. e1001057, 2011.

[29] J. L. Peirce et al., "How Replicable Are mRNA Expression QTL?" Mammalian Genome, vol. 17, no. 6, pp. 643–656, 2006.

[30] A. van Nas et al., "Expression Quantitative Trait Loci: Replication, Tissue-, and Sex-Specificity in Mice," Genetics, vol. 185, no. 3, pp. 1059–1068, 2010.

[31] A. K. Maunakea et al., "Conserved Role of Intragenic DNA Methylation in Regulating Alternative Promoters," Nature, vol. 466, no. 7303, pp. 253–257, 2010.

[32] S. Horvath, "Structural Equation Models and Directed Networks," in Weighted Network Analysis: Applications in Genomics and Systems Biology, New York: Springer, 2011, pp. 279–320.

[33] R. C. Fry et al., "Genomic Predictors of Interindividual Differences in Response to DNA Damaging Agents," Genes & Development, vol. 22, no. 19, pp. 2621–2626, 2008.

[34] S. G. Jin, S. Kadam, and G. P. Pfeifer, "Examination of the Specificity of DNA Methylation Profiling Techniques Towards 5-Methylcytosine and 5-Hydroxymethylcytosine," Nucleic Acids Research, vol. 38, no. 11, Art. no. e125, 2010.

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