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Original Article

Computational Exploration of Functional and Structural Impact of Single Nucleotide Changes in DNMT3A Gene among Acute Myeloid Leukemia Patients

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ABSTRACT

Acute myeloid leukemia (AML) is a blood cell malignancy of the myeloid line, characterized by fast proliferation of aberrant cells that build up in the bone marrow and blood, interfering with normal blood cell synthesis. DNMT3A is a DNA methyltransferase that plays a role in DNA methylation, an epigenetic modification associated with gene expression regulation. DNMT3A mutations are frequently found in AML and are associated with poor prognosis. **Objective:** To evaluate the impact of DNMT3A mutations on protein structure and function, specifically in the context of AML. **Methods:** SNPs of DNMT3A gene reported in AML (R882P, R882L, R882S, R882G, and R882C) were retrieved from National Centre for Biotechnology Information (NCBI) database and different *in silico* approaches were used to investigate how these mutations affect protein structure and function. **Results:** Prediction tools indicated that mutations are pathogenic affecting DNMT3A function and were found in evolutionarily conserved regions. Protein stability analysis showed that mutations reduce DNMT3A's structural stability, alter secondary structure of the protein, particularly helices, interacts with other proteins and reduce protein-protein affinity. RNA folding analysis revealed abnormal folding patterns caused by mutant, affecting protein translation. DNMT3A expression was reported to be considerably greater in AML compared to normal tissues, and mutations were associated with poor overall survival in AML patients. Methylation levels and post-translational modification sites of DNMT3A were also investigated. **Conclusions:** Overall, this research highlighted the negative impact of DNMT3A mutations on protein structure and function, emphasizing their importance in the development and prognosis of AML.

INTRODUCTION

Leukemia is a kind of blood and bone marrow malignancy characterized by the fast development of abnormal white blood cells [1]. Based on the kind of stem cell involved and whether the leukemia is acute or chronic, there are four primary subtypes of leukemia [2]. Acute myeloid leukemia (AML) is the most frequent kind of leukemia in adults and is associated with a high number of annual deaths in the United States [3]. It is distinguished by an overabundance of undifferentiated myeloid cells in the bone marrow, resulting in a lack in normal blood cell synthesis [4]. The symptoms may include fever, weight loss, fatigue, breathlessness, frequent infections, and abnormal bleeding [5]. The causes of AML are diverse and can include exposure to therapeutic or environmental agents that damage DNA, although the exact etiology is often unclear. AML is caused by a combination of hereditary and ecological factors, such as genetic mutations, age, radiation exposure, chemical exposure, and previous treatments [6]. Genomic profiling advances have thrown some insight on the function of genetics in AML, but further research is needed to fully understand the mechanisms involved. Diagnosing AML involves analyzing peripheral blood or bone marrow samples for the presence of abnormal myeloid cells. It should be diagnosed when marrow or blood has > 20% blasts of myeloid lineage [7]. The prognosis of AML depends on factors such as the patient's age and subtype of the disease. It is mostly diagnosed at the median age of 68 years. Treatment

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typically involves chemotherapy, sometimes combined with targeted therapy drugs, and may be followed by a stem cell transplant. Recent studies have focused on the molecular pathogenesis of AML, identifying genetic variations that influence the prognosis and altering the classification of the disease. One of the commonly altered genes in AML is DNMT3A (~20%), which plays a role in DNA methylation [8]. It consists of 35 exons and encodes a protein of 912 amino acid. It was mapped to chromosome 2p23.3. Mutations in DNMT3A are linked to cytogenetically normal acute myeloid leukemia (CN-AML). These mutations impair the enzyme's ability to methylate DNA fully, leading to changes in gene activity and the production of abnormal white blood cells [9]. In the field of research, bioinformatics methods and in silico techniques have revolutionized the study of life sciences. These computational approaches help categorize proteins based on structure and function and assist in the development of servers for molecular sorting using machine learning methods [10]. In silico methods also facilitate screening potential therapeutics against molecular targets, reducing the need for extensive laboratory work and conserving resources [11].

METHODS

To perform in silico analysis, 18 computational tools were employed (Table 1).

Table 1: Tools applied for analysis

In silico tools	Function
NCBI	For retrieval of SNPs
SIFT, Align GVGD, FATHMM, PANTHER	For the identification of deleterious SNPs
MUpro, I-Mutant Suite, mCSM	For protein stability analysis
ConSurf	For estimation of the conservation profile
HOPE Project	For analysis of structural effects of DNMT3A gene mutation
SWISS-MODEL	For protein modeling
SOPMA	For secondary structure analysis
STRING	In order to anticipate protein-protein interactions
Vienna package	For the prediction of effect of mutations on RNA secondary structure
GEPIA, UALCAN, cBioPortal, Cytoscape	For analysis of post-translational modifications

RESULTS

From NCBI, five SNPs were reported to be found in AML. To identify the damaging and deleterious effects of SNPs that could interfere with the structure and function of DNMT3A gene, four in silico tools (SIFT, Align GVGD, FATHMM, and PANTHER) were used. All the SNPs were predicted as damaging and deleterious by these computational algorithms (Table 2).

Table 2: Analysis of damaging effects of DNMT3A mutations on structure and function of gene using in silico tools

DBSNP RS#	SNPs	SIFT	Align GVGD	FATHMM	PANTHER
rs147001633	R882P	Affect protein	C65	Damaging	Probably damaging
rs147001633	R882L	Affect protein	C65	Damaging	Probably damaging
rs377577594	R882S	Affect protein	C65	Damaging	Probably damaging
rs377577594	R882G	Affect protein	C65	Damaging	Probably damaging
rs377577594	R882C	Affect protein	C65	Damaging	Probably damaging

To analyze the effects of point mutations on the stability of protein structure, three software (MUpro, mCSM, and I-Mutant) were used. The SNPs were shown to reduce the protein structural stability (Table 3).

Table 3: Effects of SNPs on the structural stability of DNMT3A protein

	Mupro		mCSM		I-Mutant	
SNPs	Delta Delta G value	Prediction	ΔΔG Kcal/mol	Prediction	DDG value (kcal/mol)	Prediction
R882P	-0.94342015	Decrease stability	-0.335	Destabilizing	-0.78	Decrease
R882L	0.073176169	Increase stability	-0.301	Destabilizing	-0.62	Decrease
R882S	-0.64353902	Decrease stability	-0.228	Destabilizing	-1.46	Decrease
R882G	-1.2625806	Decrease stability	-0.302	Destabilizing	-1.65	Decrease
R882C	-0.47408111	Decrease stability	0.096	Stabilizing	-1.14	Decrease

To analyze the evolutionary conservation, ConSurf interpret the results, according to which all the SNPs were predicted as highly conserved (Figure 1).

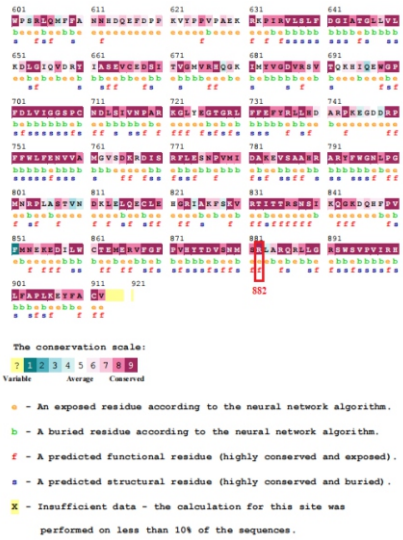

Figure 1: ConSurf results of DNMT3A gene amino acid sequence on a multi-colored bar sheet with conservation scale below

To assess the consequences of amino acid changes on physical and chemical characteristics, spatial organization, hydrophobicity, size, charge and function of the protein, HOPE was used. It predicted that all the mutant

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residues were smaller than the wild-type residue (Table 4 and Figure 2). External interactions will be lost by the smaller size.

Table 4: HOPE's interpretation of the effect of amino acid changes on DNMT3A protein structure and stability

SNPs	Size	Change of Charge
R882P	W>M	+ve to neutral
R882L	W>M	+ve to neutral
R882S	W>M	+ve to neutral
R882G	W>M	+ve to neutral
R882C	W>M	+ve to neutral

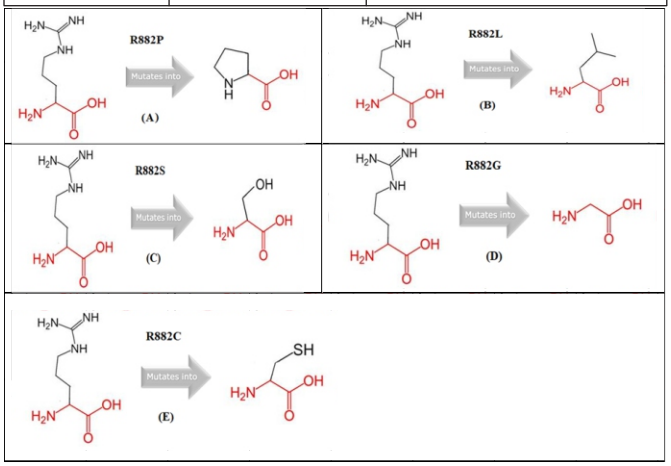
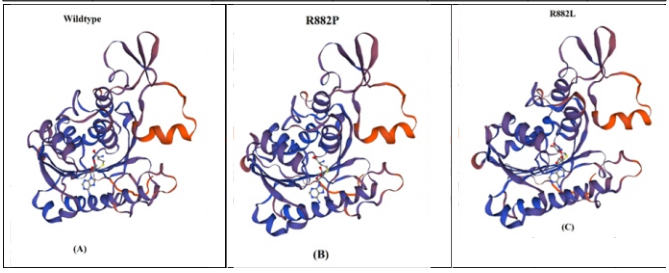


Figure 2: The native (left) and mutant (right) amino acid residues of DNMT3A gene mutations are shown in schematic form

For the homology modeling of the wild and mutant variants, SWISS MODEL was used. It is designated to construct 3D structure of protein (Figure 3). Different parameters (GMQE, QMEAN) were obtained using this tool (Table 5).

Table 5: Representing the different QMEAN Z score, GMQE, Identity, and template query number of wild type and mutant proteins via SWISS-Model

SNPs	Total number of amino acids	Number of amino acids in model	Template query number	Sequence identity	GMQE	QMEAN
Wildtype	912	613-912	6pa7.1. K	100.00%	0.39	-1.14
R882P	912	613-912	6pa7.1. K	99.85%	0.39	-1.07
R882L	912	613-912	6pa7.1. K	99.85%	0.39	-1.19
R882S	912	613-912	6pa7.1. K	99.85%	0.39	-1.19
R882G	912	613-912	6pa7.1. K	99.85%	0.39	-1.26
R882C	912	613-912	6pa7.1. K	99.85%	0.39	-1.23



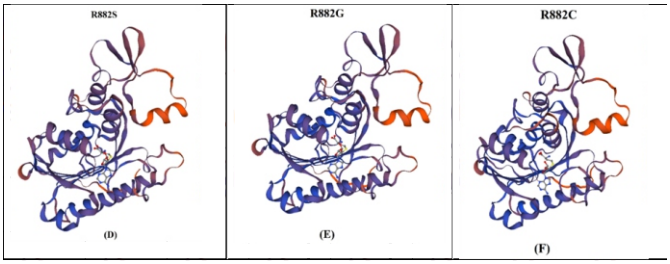


Figure 3: Protein structures of wildtype and mutants using SWISS-MODEL

Self-Optimized Prediction Method from Alignment abbreviated as SOPMA, was used to interpret the secondary structure of protein. It predicted different characteristics of secondary structure i.e., alpha helix, 310 helix, Pi helix, beta bridge, ambiguous states, and other states (Figure 4).

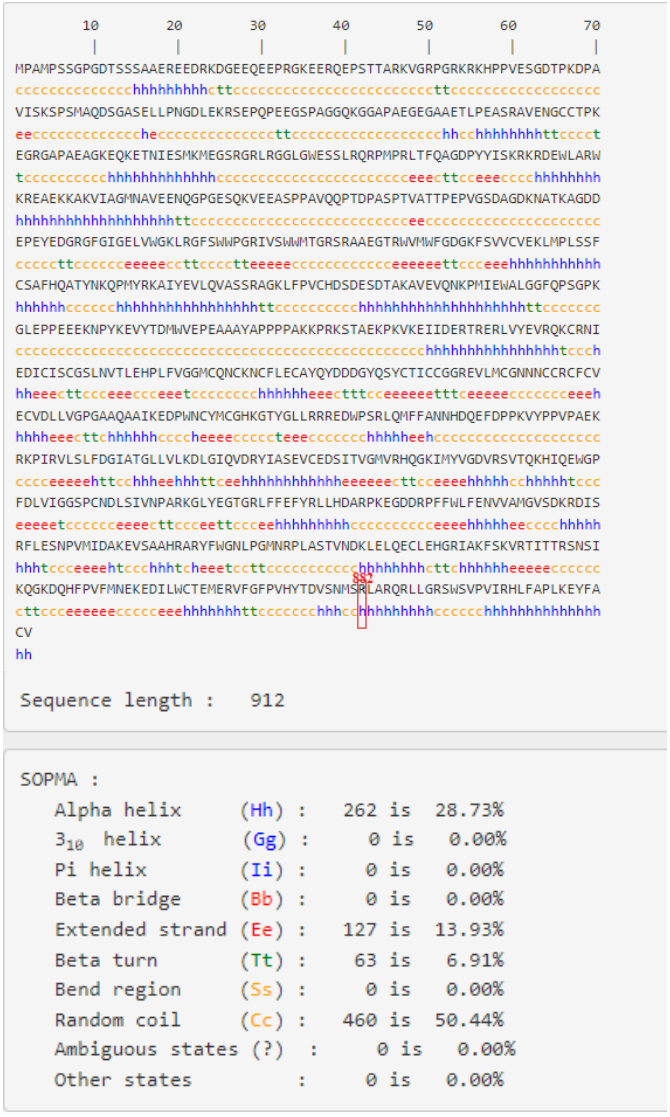


Figure 4: Presenting secondary structure of DNMT3A protein using SOPMA

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To create protein interaction network of DNMT3A protein, STRING Database was used. It predicted that DNMT3A is functionally associated with five other proteins (EZH2, DNMT3L, MYC, DNMT1 and HIST2H3PS2). Any change in the structure of DNMT3A protein can affect the functions of these proteins related to it (Figure 5).

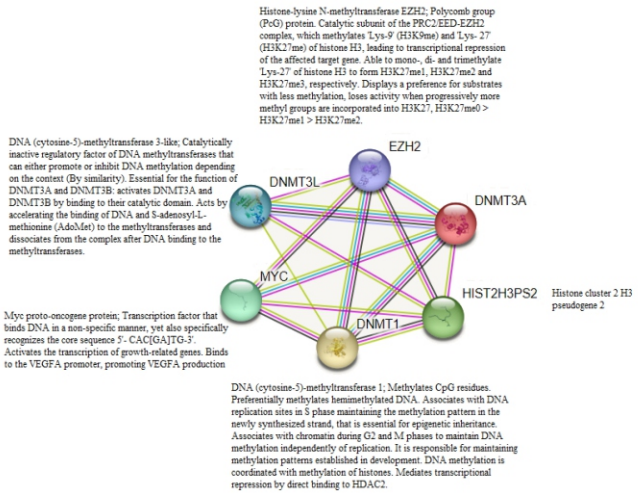

Figure 5: Network of protein-protein interactions of DNMT3A using STRING Database

For the assessment of secondary structure of mRNA, RNA fold webserver on the Vienna package was used. All the mutations resulted in anomalous RNA folding, thus affecting mRNA localization and influencing the translation of protein (Figure 6).

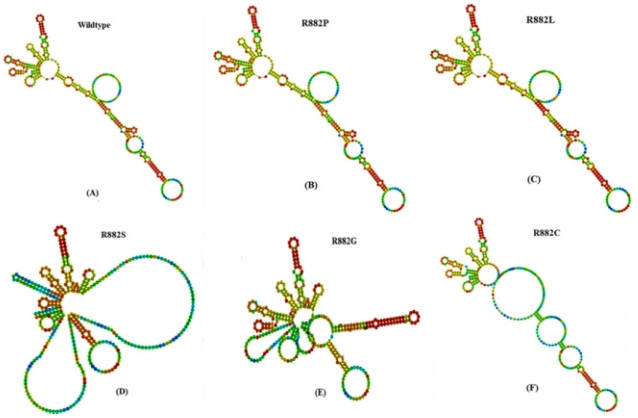

Figure 6: Impacts of SNPs on RNA secondary structure by Vienna package

To check the expression of DNMT3A in AML and overall survival analysis, GEPIA2 was used. In figure 7 (A), Transcripts per million (TPM) graph showed that mRNA expression of DNMT3A gene was significantly higher T (n=173) in LAML as compared to normal tissues N (n=70). In figure (B), the relationship between DNMT3A mRNA expression and the prognosis of patient, including overall survival (OS), was analyzed by the Log-rank test. As log-rank p value is < 0.05 (0.32), it was considered statistically significant as it was explaining that both high and low DNMT3A groups have different distribution curve.

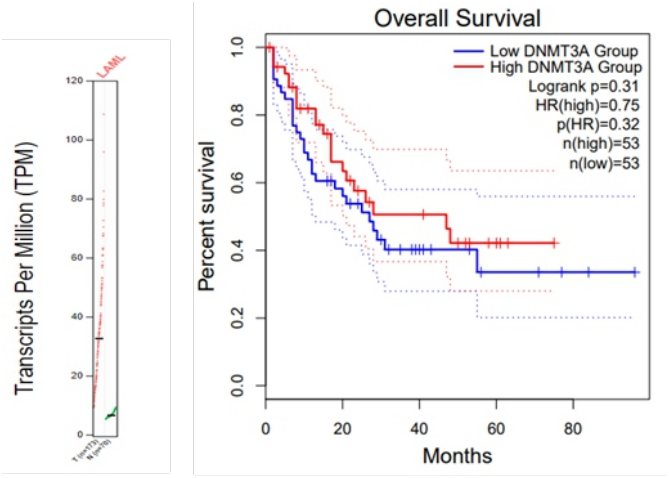

Figure 7: (A) Overview of DNMT3A mRNA expression in AML from TCGA obtained from GEPIA2 (B) Kaplan-Meier plots showing overall survival between high and low DNMT3A groups in AML

For comprehensive and interactive analysis of expression and methylation levels of DNMT3A gene in AML, UALCAN was used. In Figure 8 (A), a significant variation of DNMT3A methylation was seen between male and female. In Figure 8 (B), methylation levels in different age groups of AML patients were shown. Individuals with age of 21 to 80 years were found to have significant methylation levels.

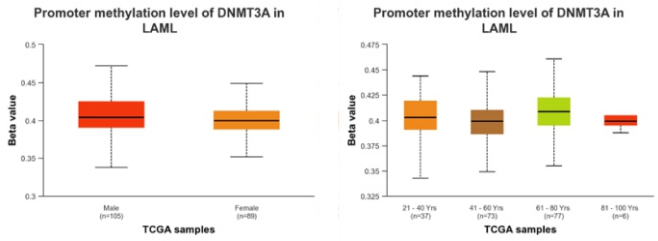

Figure 8: (A) DNMT3A promoter methylation profile based on patient's gender in LAML (B) DNMT3A promoter methylation profile based on patient's age in LAML

To check the PTM sites, cBioPortal was used. It predicted that 23% (altered / profiled = 131 / 560) of genetic alterations were reported in DNMT3A including in-frame, missense, splice and other mutations etc. It displayed all Post Translational Modifications (PTMs) available for the transcript. Different PTM types were shown with varying color codes such as green for phosphorylation, red for ubiquitination and purple for sumoylation (Figure 9).

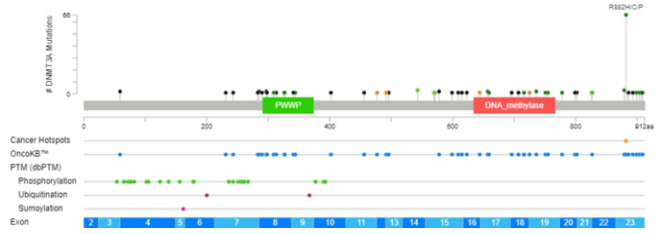

Figure 9: Representing genetic alterations of DNMT3A and PTM

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sites by using cBioPortal

Cytoscape was used for the visualization and analysis of network graphs of DNMT3A gene involving nodes and edges. A gene hub was identified by Cytoscape network analysis (Figure 10). Each gene represented by a node was predicting the high correlation of DNMT3A with them. The thickness of the line connecting the two nodes reflected the strength of the link favorably.

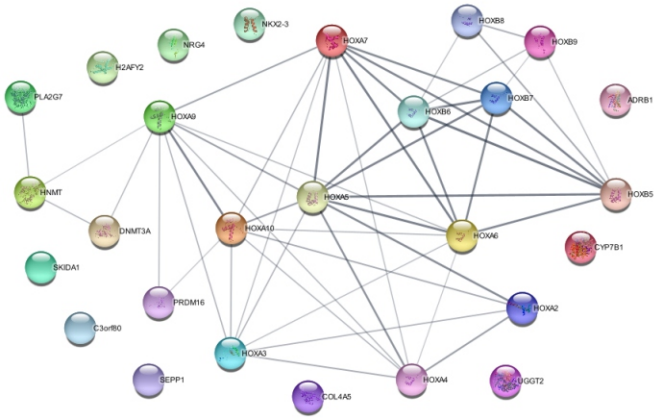

Figure 10: Gene hub presenting correlation of DNMT3A with other genes

DISCUSSION

In silico methods are preferred in many scientific disciplines because they are cost-effective, time-efficient, flexible, reduce ethical concerns, and can make predictions about complex systems so they are used to better understand how mutations might disrupt protein structure and function [12]. DNMT3A is a de novo DNA methyltransferase that has lately gained attention as a result of its common mutation in a variety of immature and mature hematologic neoplasms. DNMT3A mutations occur early in cancer formation and tend to be associated with a poor prognosis in persons with acute myeloid leukemia (AML), making this gene an intriguing target for innovative therapies [13]. Disease-causing SNPs are frequently detected in evolutionarily conserved areas. Five SNPs of DNMT3A were found to be involved in AML. All the five SNPs (R882P, R882L, R882S, R882G, R882C) were retrieved from dbSNP of NCBI database. For the assessment of function, different prediction tools (SIFT, Align GVGD, FATHMM, and PANTHER) were used. These tools predicted that all the mutations are pathogenic and affecting the function of DNMT3A. In recent studies, it was revealed that DNMT3A is required for methylation of unaltered DNA in CpG islands by converting cytosine to 5-methylcytosine, which is linked with gene silencing[14]. In vitro, enzymatic assays revealed that mutated DNMT3A reduced DNA methylation and overexpression of the two most common DNMT3A mutants (R882H and R882C) advanced proliferation in cell culture trials [15]. It strongly demonstrated a substantial pathogenetic involvement of DNMT3A mutations in AML [16]. To investigate the impact of point mutations on DNMT3A structural stability, MUpro, mCSM and I-Mutant were used. The outcome revealed that almost all the SNPs were found to diminish the structural stability of the protein. The previous studies have found that mutations in the DNMT3A protein's catalytic region were anticipated to result in function loss [17]. Protein stability regulates protein conformational structure and consequently dictates function. Any change in protein stability can result in misfolding, disintegration, or abnormal protein aggregation [18]. To evaluate if a mutation has a deleterious effect on the host, evolutionary conservation in the protein sequence is critical. The degree to which an amino acid position has been evolutionarily preserved reveals its structural and functional significance [19]. ConSurf was used for the evolutionary conservation analysis, according to which all the five substitutions were found to be present in exposed region and are highly conserved, having high conservation scores. Therefore, increases the risk of tumorigenesis[20]. HOPE was used to assess the consequences of amino acid replacements on the protein's physical and chemical characteristics, spatial structure, hydrophobicity, size, charge, and function. All of the mutant residues were anticipated to be smaller than the wild-type residue. The lower size will eliminate external interactions. The variations in hydrophobicity and size between mutant and wildtype residues may cause protein framework disruption by disrupting H-bonding connections with adjacent residues [21]. The mapping of amino acid substitutions can be accomplished using 3D protein structure analysis. To create 3D models of the mutated residues, SWISS-MODEL was used. Chakravarty explained the changes in secondary structure during the transition suggest that helices and strands are likely to be extended at the expense of turns and coils. The decrease in bounding factors of the interface residues as they transition from the unbound to the bound form reflects a loss in flexibility during complex formation [22]. For the analysis of secondary structure, SOPMA was used. This software predicted that all the mutations were present in the exposed region in the form of helix. Helices can tolerate more mutations than strands without change, because they have more inter-residue interactions [23]. Mutations that alter secondary structure inside the protein core are more likely to produce proteins that do not fold correctly, making their structures more difficult to crystallize. Hence, anomalous proteins are formed[24]. STRING database was used to analyze how DNMT3A is associated with other proteins. It showed the association of DNMT3A gene with five other genes (EZH2, DNMT3L, MYC, DNMT1 and

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HIST2H3PS2). DNMT1 maintains methylation during DNA replication. Trowbridge *et al.*, demonstrated that DNMT1 haploinsufficiency impaired leukemia stem cell (LSC) activity by depressing bivalent chromatin domains [25]. DNMT3A can reside in the nucleus as dimers, tetramers, and larger oligomeric complexes. The oligomers are made of either homo-dimeric DNMT3A molecules or heterodimeric DNMT3A–DNMT3L molecules [26]. Apart from programmed changes in oligomerization, such as those caused by developmental changes in DNMT3L expression and differential DNMT3A/3B isoform usage, a number of pathologic changes, such as mutations at DNMT3A binding interfaces have been shown to influence oligomerization and alter cell behavior [27]. For the prediction of changes in RNA secondary structure, RNA fold webserver in Vienna Package was used, according to which all the mutants of DNMT3A led to the abnormal folding of RNA, thus influencing RNA localization and affecting the protein translation. In the current study, it was elaborated that in addition to its known involvement in HSC differentiation, DNMT3A has been linked to the preservation of RNA splicing and genomic integrity, both of which are dramatically altered when DNMT3A is mutated [28]. The loss of DNMT3A resulted in the downregulation of spliceosome genes and aberrant RNA splicing. Mutations induce abnormalities of DNMT3A splicing, likely through changing exonic splicing silencers [29]. GEPIA2 was utilized to examine the expression of DNMT3A in AML and overall survival analysis. It was illustrated by GEPIA2 that mRNA expression of DNMT3A gene was significantly higher in AML as compared to normal tissues. DNMT3A expression was shown to be higher in AML in a prior study [30] and its mutations were independently linked with poor outcome in AML patients with an intermediate-risk cytogenetic profile or CN-AML [31]. UALCAN was employed for a thorough and interactive investigation of the expression and methylation levels of the DNMT3A gene in AML. A statistically significant overrepresentation of DNMT3A methylation status was reported in patients ≥ 50 years old in recent research. There was no evident relationship between DNMT3A methylation status and gender. While the prevalence of AML is increasing, no difference in frequency has been seen between males and females [32]. For prediction of post translational modification sites, cBioPortal was used. Large-scale studies have recently revealed that overlap between PTMs and SNPs results in damaged PTMs, which severely influence both gene and protein function and are linked to human cancer [33]. Radivojac *et al.*, also discovered a link between phosphorylation site disruptive variations and somatic cancer mutations [34]. Cytoscape was used to visualize molecular interaction networks of human DNMT3A protein with other associated proteins [35]. Maintaining protein interactions is critical for maintaining system homeostasis [36]. Any change in the gene leads to disruption of functioning of correlated genes. Thus, various studies have found a high relationship between DNMT3A genetic variants and prognosis in AML patients, with mutations predicting a markedly bad prognosis in AML patients [37].

CONCLUSIONS

In this study, *in silico* tools were used to analyze the impact of DNMT3A mutations, which are associated with hematologic neoplasms, particularly AML. The findings shed light on the potential mechanisms underpinning DNMT3A's function in cancer formation and emphasize its therapeutic potential. All the analyzed SNPs were found to be deleterious and damaging, destroying the DNMT3A structure and function. These SNPs may reduce the DNMT3A capacity to fully methylate DNA which abrupt its activity preventing the normal differentiation, ultimately leading to AML. Overall, this study demonstrates the importance of *in silico* methods in elucidating the complex molecular processes involved in cancer development and progression, particularly AML.

Authors Contribution

Conceptualization: AMA
Methodology: SA, KJ, AT
Formal analysis: SA, KJ, AT
Writing-review and editing: SA, AMA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] Heidari A and Gobato R. Evaluating the effect of anti-cancer nano drugs dosage and reduced leukemia and polycythemia vera levels on trend of the human blood and bone marrow cancers under synchrotron radiation. Trends in Research. 2019 Jan; 2(1): 1-8. doi: 10.15761/TR.1000126.

[2] Bibi N, Sikandar M, Ud Din I, Almogren A, Ali S. IoMT-based automated detection and classification of leukemia using deep learning. Journal of Healthcare Engineering. 2020 Dec; 2020: 1-2. doi: 10.1155/2020/6648574.

[3] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA: A Cancer Journal for Clinicians. 2014 Jan; 64(1): 9-29. doi: 10.3322/caac.21208.

DOI: https://doi.org/10.54393/pbmj.v6i04.882

[4] Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. Blood Reviews. 2019 Jul; 36: 70-87. doi: 10.1016/j.blre.2019.04.005.

[5] Williams LA, Garcia-Gonzalez A, Ahaneku HO, Cortes JE, Garcia-Manero G, Kantarjian HM, *et al.* A patient-reported outcome measure for symptoms and symptom burden of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). Blood. 2015 Dec; 126(23): 2094. doi: 10.1182/blood.V126.23.2094.2094.

[6] Tebbi CK. Etiology of acute leukemia: A review. Cancers. 2021 May; 13(9): 2256. doi: 10.3390/cancers13092256.

[7] Estey EH. Acute myeloid leukemia: 2013 update on risk-stratification and management. American Journal of Hematology. 2013 Apr; 88(4): 317-27. doi: 10.1002/ajh.23404.

[8] Sergeev AV, Kirsanova OV, Loiko AG, Nomerotskaya EI, Gromova ES. Detection of DNA methylation by Dnmt3a methyltransferase using methyl-dependent restriction endonucleases. Molecular Biology. 2018 Mar; 52: 272-8. doi: 10.1134/S0026893318020139.

[9] Khrabrova DA, Yakubovskaya MG, Gromova ES. AML-associated mutations in DNA methyltransferase DNMT3A. Biochemistry (Moscow). 2021 Mar; 86: 307-18. doi: 10.1134/S000629792103007X.

[10] Behbahani M, Nosrati M, Moradi M, Mohabatkar H. Using Chou's general pseudo amino acid composition to classify laccases from bacterial and fungal sources via Chou's five-step rule. Applied Biochemistry and Biotechnology. 2020 Mar; 190: 1035-48. doi: 10.1007/s12010-019-03141-8.

[11] Sadeghi M, Miroliaei M, Fateminasab F, Moradi M. Screening cyclooxygenase-2 inhibitors from Allium sativum L. compounds: in silico approach. Journal of Molecular Modeling. 2022 Jan; 28: 1-2. doi: 10.1007/s00894-021-05016-4.

[12] Namadyan N, Seyedalipour B, Hosseinkhani S, Baziyar P. Biochemical and biophysical properties of the novel ALS-linked hSOD1 mutants: an experimental study accompanied by in silico analysis. Journal of the Iranian Chemical Society. 2023 Jan; 20(1): 125-38. doi: 10.1007/s13738-022-02660-2.

[13] Brunetti L, Gundry MC, Goodell MA. DNMT3A in leukemia. Cold Spring Harbors Perspectives in Medicine. 2017 feb; 7(2): a030320. doi: 10.1101/cshperspect.a030320.

[14] Baylin SB and Ohm JE. Epigenetic gene silencing in cancer–a mechanism for early oncogenic pathway addiction? Nature Reviews Cancer. 2006 Feb; 6(2): 107-16. doi: 10.1038/nrc1799.

[15] Yan XJ, Xu J, Gu ZH, Pan CM, Lu G, Shen Y, *et al.* Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nature Genetics. 2011 Apr; 43(4): 309-15. doi: 10.1038/ng.788.

[16] Fried I, Bodner C, Pichler MM, Lind K, Beham-Schmid C, Quehenberger F, *et al.* Frequency, onset and clinical impact of somatic DNMT3A mutations in therapy-related and secondary acute myeloid leukemia. Haematologica. 2012 Feb; 97(2): 246. doi: 10.3324/haematol.2011.051581.

[17] Bruno S, Bochicchio MT, Franchini E, Padella A, Marconi G, Ghelli Luserna di Rorà A, *et al.* Identification of Two DNMT3A Mutations Compromising Protein Stability and Methylation Capacity in Acute Myeloid Leukemia. Journal of Oncology. 2019 Oct; 2019: 5985923. doi: 10.1155/2019/5985923.

[18] Witham S, Takano K, Schwartz C, Alexov E. A missense mutation in CLIC2 associated with intellectual disability is predicted by in silico modeling to affect protein stability and dynamics. Proteins: Structure, Function, and Bioinformatics. 2011 Aug; 79(8): 2444-54. doi: 10.1002/prot.23065.

[19] Yasmin T. In silico comprehensive analysis of coding and non-coding SNPs in human mTOR protein. Plos One. 2022 Jul; 17(7): e0270919. doi: 10.1371/journal.pone.0270919.

[20] Aggarwal V, Tuli HS, Varol M, Tuorkey M, Sak K, Parashar NC, *et al.* NOTCH signaling: Journey of an evolutionarily conserved pathway in driving tumor progression and its modulation as a therapeutic target. Critical Reviews in Oncology/Hematology. 2021 Aug; 164: 103403. doi: 10.1016/j.critrevonc.2021.103403.

[21] Zhou J, Park CY, Theesfeld CL, Wong AK, Yuan Y, Scheckel C, *et al.* Whole-genome deep-learning analysis identifies contribution of noncoding mutations to autism risk. Nature Genetics. 2019 Jun; 51(6): 973-80. doi: 10.1038/s41588-019-0420-0.

[22] Chakravarty D, Janin J, Robert CH, Chakrabarti P. Changes in protein structure at the interface accompanying complex formation. IUCrJ. 2015 Nov; 2(6): 643-52. doi: 10.1107/S2052252515015250.

[23] Ferrada E and Wagner A. Protein robustness promotes evolutionary innovations on large evolutionary time-scales. Proceedings of the Royal Society B: Biological Sciences. 2008 Jul; 275(1643): 1595-602. doi: 10.1098/rspb.2007.1617.

[24] Fujiwara K, Toda H, Ikeguchi M. Dependence of α-helical and β-sheet amino acid propensities on the overall protein fold type. BMC Structural Biology. 2012

DOI: https://doi.org/10.54393/pbmj.v6i04.882

Dec; 12(1): 1-5. doi: 10.1186/1472-6807-12-18.
[25] Trowbridge JJ, Sinha AU, Zhu N, Li M, Armstrong SA, Orkin SH. Haploinsufficiency of Dnmt1 impairs leukemia stem cell function through derepression of bivalent chromatin domains. Genes & Development. 2012 Feb; 26(4): 344-9. doi: 10.1101/gad.184341.111.
[26] Yoo NJ, Kim MS, Lee SH. Mutational analysis of DNMT3A gene in acute leukemias and common solid cancers. Cancer Research. 2012 Apr; 72(8_Supplement): 2191. doi: 10.1158/1538-7445.AM2012-2191.
[27] Holz-Schietinger C, Matje DM, Reich NO. Mutations in DNA methyltransferase (DNMT3A) observed in acute myeloid leukemia patients disrupt processive methylation. Journal of Biological Chemistry. 2012 Sep; 287(37): 30941-51. doi: 10.1074/jbc.M112.366625.
[28] Banaszak LG, Giudice V, Zhao X, Wu Z, Gao S, Hosokawa K, *et al*. Abnormal RNA splicing and genomic instability after induction of DNMT3A mutations by CRISPR/Cas9 gene editing. Blood Cells, Molecules, and Diseases. 2018 Mar; 69: 10-22. doi: 10.1016/j.bcmd.2017.12.002.
[29] Kapahnke M, Banning A, Tikkanen R. Random splicing of several exons caused by a single base change in the target exon of CRISPR/Cas9 mediated gene knockout. Cells. 2016 Dec; 5(4): 45. doi: 10.3390/cells5040045.
[30] Zhang TJ, Zhang LC, Xu ZJ, Zhou JD. Expression and prognosis analysis of DNMT family in acute myeloid leukemia. Aging (Albany NY). 2020 Jul; 12(14): 14677. doi: 10.18632/aging.103520.
[31] Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, *et al*. DNMT3A mutations in acute myeloid leukemia. New England Journal of Medicine. 2010 Dec; 363(25): 2424-33.
[32] Asfour IA, Hegab HM, El-Salakawy WA, Hamza MT, Mansour DA, Saeed AM. Prognostic significance of DNMT3a gene expression and reactive nitrogen species in newly diagnosed Egyptian de novo adult acute myeloid leukemia patients. Egyptian Journal of Medical Human Genetics. 2020 Dec; 21: 1-6. doi: 10.1186/s43042-020-00066-4.
[33] Awan FM, Obaid A, Ikram A, Janjua HA. Mutation-structure-function relationship based integrated strategy reveals the potential impact of deleterious missense mutations in autophagy related proteins on hepatocellular carcinoma (HCC): A comprehensive informatics approach. International Journal of Molecular Sciences. 2017 Jan; 18(1): 139. doi: 10.3390/ijms18010139.
[34] Radivojac P, Baenziger PH, Kann MG, Mort ME, Hahn MW, Mooney SD. Gain and loss of phosphorylation sites in human cancer. Bioinformatics. 2008 Aug; 24(16): i241-7. doi: 10.1093/bioinformatics/btn267.
[35] Franz M, Lopes CT, Fong D, Kucera M, Cheung M, Siper MC, *et al*. Cytoscape. js 2023 update: a graph theory library for visualization and analysis. Bioinformatics. 2023 Jan; 39(1): btad031. doi: 10.1093/bioinformatics/btad031.
[36] Hossain MS, Roy AS, Islam MS. In silico analysis predicting effects of deleterious SNPs of human RASSF5 gene on its structure and functions. Scientific Reports. 2020 Sep; 10(1): 14542. doi: 10.1038/s41598-020-71457-1.
[37] Yuan XQ, Zhang DY, Yan H, Yang YL, Zhu KW, Chen YH, *et al*. Evaluation of DNMT3A genetic polymorphisms as outcome predictors in AML patients. Oncotarget. 2016 Sep; 7(37): 60555. doi: 10.18632/oncotarget.11143.