IN SILICO EXPLORATION OF MARCH2 PROTEIN: A SHARED E3 LIGASE IN NEURODEGENERATIVE DISEASES AND CANCER

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by:

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I, **Aastha Kaushik**, 2K22/BIO/01 student of M. Tech (Bioinformatics), hereby certify that the work which is being presented in the thesis entitled **"In Silico Exploration of MARCH2 Protein: A Shared E3 Ligase in Neurodegenerative Diseases and Cancer"** in partial fulfilment of the requirements for the award of the Degree of Master of Technology, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from January 2024 to May 2024 under the supervision of Prof. Pravir Kumar.

The matter presented in the thesis has not been submitted by me for the award of any other degree of this or any other Institute.

Aastha

Candidate's signature

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IN SILICO EXPLORATION OF MARCH2 PROTEIN: A SHARED E3 LIGASE IN NEURODEGENERATIVE DISEASES AND CANCER

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ABSTRACT

Aim: This study showcases an in-depth investigation into E3 ligases implicated in neurodegenerative diseases (NDDs) and cancer. It involved a comprehensive literature survey, structural studies, and molecular docking simulations (MDS) analysis with a particular focus on the MARCH2 protein.

Background: The literature review unravelled common E3 ligases in NDDs and cancer, highlighting their importance as common therapeutic targets. Among the common E3 ligases, the MARCH2 protein is documented to be overexpressed in colorectal cancer. It also showcases a regulatory function in CFTR-mediated autophagy shedding light on its relevance in NDDs. These instances highlight its significance in the mechanisms underlying cancer and NDDs.

Methodology: An in-depth structural analysis was conducted using the SWISS model of the RING domain to determine the structural features and therapeutic importance of the MARCH2 protein. Subsequently, MDS studies were performed using EasyDock Vina and CHARMM-GUI to predict the interaction of MARCH2 with drugs from DrugBank and Phytochemica, providing insights into potential therapeutic interventions.

Results: The MDS studies yielded promising results, highlighting the stability of the MARCH2-Tafluposide and MARCH2-Ergotamine complexes. The results of this project suggest that targeting MARCH2 could be a promising strategy for curing cancer and lays a strong foundation for understanding the common molecular mechanisms involving E3 ligases in both NDDs as well as cancer [1]. Additionally, it paves the way for precision medicine by rendering insights into the development of novel strategies that target the MARCH2 protein.

LIST OF PUBLICATIONS

1) **IEEE Conference Paper: A. Kaushik** and P. Kumar, "In-Silico Analysis for Differentially Expressed Genes in Multiple Sclerosis: Exploring Promising Biomarkers," 2023 3rd International Conference on Innovative Sustainable Computational Technologies (CISCT), Dehradun, India, 2023, pp. 1-5, doi: 10.1109/CISCT57197.2023.10351378. keywords: {Multiple sclerosis;Medical treatment;Biomarkers;Metabolism;Differentially Expressed Genes (DEGs);Multiple Sclerosis (MS)}

2) **IEEE Conference Paper: A. Kaushik** and P. Kumar, "In-Silico Structural Analysis of Membrane-Associated RING-CH Type 2 (MARCH2) Protein," 2023 International Conference on Integration of Computational Intelligent System (ICICIS), Pune, India, 2023, pp. 1-6, doi: 10.1109/ICICIS56802.2023.10430285. keywords: {Enzymes;Computational modeling;Control systems;Stability analysis;Intelligent systems;Cancer;Immune system;Membrane-Associated RING-CH type 2 (MARCH2); Really Interesting New Gene (RING);Molecular Dynamic Simulation (MDS)}

3) **Review Article: Aastha Kaushik**, Somya Parashar, Rashmi K Ambasta, Pravir Kumar, Ubiquitin E3 ligases assisted technologies in protein degradation: Sharing Pathways in Neurodegenerative Disorders and Cancer, Ageing Research Reviews, 2024, 102279, ISSN 1568-1637, https://doi.org/10.1016/j.arr.2024.102279. **(**https://www.sciencedirect.com/science/article/pii/S1568163724000977**) (IF:13.1)**

4) **Book Chapter:** Neetu Rani, **Aastha Kaushik**, Shefali Kardam, Sonika Kag, V. Samuel Raj, Rashmi K. Ambasta, Pravir Kumar, Reimagining old drugs with new tricks: Mechanisms, strategies and notable success stories in drug repurposing for neurological diseases, Progress in Molecular Biology and Translational Science, Academic Press, 2024,ISSN 1877-1173, https://doi.org/10.1016/bs.pmbts.2024.03.029.

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LIST OF ABBREVIATIONS

AR	Androgen Receptor
ARIH1	Ariadne-1 homolog
ABCB1	ATP-binding cassette sub-family B member 1
AUTAC	Autophagy Targeting Chimera
APP	The amyloid beta precursor protein
BMI1	B lymphoma Mo-MLV insertion region 1 homolog
BIRC7	Baculoviral IAP Repeat-Containing protein 7
BCL-XL	B-cell lymphoma-extra-large
BAG5	Bcl-2-associated athanogene 5
BECN1	Beclin-1
TrCP	Beta-Transducin repeats-Containing Proteins
BRI3	Brain Protein I3
BARD1	BRCA1-Associated RING Domain protein 1
BRD4	Bromodomain-containing protein 4
Cdh1	Cadherin-1
CREB	cAMP Response Element-Binding protein
CDC20	Cell Division Cycle 20
CPP	Cell Penetrating Peptide
cIAP	Cellular Inhibitor of Apoptosis
CRBN	Cereblon
CDT1	Chromatin licensing and DNA replication factor 1
CLL	Chronic lymphocytic leukemia
CMML	Chronic Myelomonocytic Leukaemia
CHIP	C-terminus of Hsc70 Interacting Protein
Cul1	Cullin 1
CRL	Cullin-RING Ubiquitin E3 Ligase
CNrasGEF	Cyclic Nucleotide ras Guanine-nucleotide-Exchange Factor
DUBs	Deubiquitinating enzymes
EMI1	Early Mitotic Inhibitor 1
MARCH	Membrane-Associated RING-CH type
NDD	Neurodegenerative Disease

CHAPTER 1

INTRODUCTION

In the current scenario, there is an urgent need to address the challenges posed by NDDs and cancer due to their severe impact on health across the globe. In 2020, 19.3 million cancer cases were diagnosed across the globe [2][3]. Furthermore, approximately 50 million cases of NDDs worldwide underscore the seriousness of diseases caused by neurodegeneration [4]. This concerning data casts light on the seriousness of the situation, underscoring the urgent requirement for novel therapeutic plans. However, in the anti-cancer regimen, there are two significant challenges. First, the diversity of targetable genomic changes restricts their practical importance in trials focused on biomarkers. The integration of Next Generation Sequencing (NGS) technologies for molecular screening in clinical studies faces difficulties related to the interpretation of extensive genomic data, which reduces their widespread clinical application. In addition, some primary obstacles to achieving success in precision cancer therapy such as tumour diversity and acquired resistance persist. Furthermore, well-validated predictive markers are absent, complicating optimization and selection processes for appropriate treatments [5]. In the context of NDDs, drug delivery to the brain remains a notable challenge due to factors such as the blood-brain barrier (BBB) and the drug's physio-chemical properties. However, the obstacles in curing NDDs go beyond these well-studied barriers. NDDs are marked by their complex and diverse nature and showcase variations in clinical manifestations and underlying pathologies, presenting a significant obstacle in developing effective treatments that are universally applicable. Also, the progressive NDDs are progressive, necessitating that the therapies must be capable of halting and significantly reversing damage caused to the neurons. The identification of targeted therapeutic approaches is further limited by the lack of knowledge about precise molecular mechanisms involved in NDDs. Additionally, NDDs are often diagnosed at late stages, therefore the effectiveness of interventions gets reduced. In a nutshell, a significant issue is highlighted due to the lack of effective treatments capable of reversing or slowing the mechanisms underlying NDDs and cancer. Hence, there is a crucial need to create innovative therapeutic approaches that are superior to the traditional methods in addressing the complex, diverse and progressive nature of NDDs and cancer [1]. Some recent studies have revealed a resemblance in the basic molecular mechanisms between NDDs and cancer, pointing out a common outlook of pathways [6][7][8]. E3 ligases, which are known for ubiquitinating and specifically degrading misfolded or toxic proteins, have emerged as promising targets for therapeutic interventions [9][10]. The urgent need to develop effective treatments for curing both NDDs and cancer has encouraged various studies into communal pathways. Intervening and targeting these pathways could provide twin benefits. A potential avenue for E3-ligases-based therapies is presented where E3 ligases can be targeted strategically and can simultaneously address the intricacies of both conditions. A promising framework

for creating comprehensive and innovative strategies to deal with these diverse yet intertwined health challenges can be done through a thorough evaluation of the intricate duties played by E3 ligases in common pathways between NDDs and cancer [10]. Amid ubiquitination, three proteins are involved- E1, E2 and E3 [11]. Their respective roles are as E1 is the enzyme that activates (the C- terminus of Ub is associated with the E1 enzyme through a thioester linkage). E2 is the enzyme for conjugation (a movement of activated ubiquitin occurs to the Cys residue of E2) [10]. E3: the enzyme ligase (it provides the target protein with the ubiquitin transferred from E2). These three enzymes, therefore, create a ubiquitin-protein complex. Among the three ubiquitination enzymes, the most specialised enzymes are E3 ligases. Both tumour promoters and suppressors are under their control. E3 ligases are an appealing field of research for the development of novel anti-tumour drugs because of their function in the activation or deactivation of tumour immunity. The mechanism of ubiquitination is - Ub gets added to the carboxyl end, and the E1 enzyme acts on it in the presence of ATP. In this reaction, ubiquitin gets attached to E1 through a thioester bond [12]. E2 then steps into the picture, transferring the active ubiquitin from the E1 to the cysteine residue of the E2 [13]. E2 conjugates ubiquitin. This ubiquitin protein needs to be attached to the target protein [14]. The last enzyme, an E3 ubiquitin ligase, then comes into play and an isopeptide bond with the target protein's Lys residue to transfer the Ub from E2 to it is formed [15]. The target protein can be monoubiquitinated, multi-ubiquitinated or polyubiquitinated [6]. For the destruction of the target protein, proteasomes are required. The huge protein complex, the proteasome, oversees the metabolically intensive process of degrading intracellular proteins. A key molecule known to work with the proteasome, polyubiquitin, sends a message for the destruction of many target proteins It has two alpha sheets and two beta sheets. The two beta sheets are sandwiched between two alpha-helices. This structure is called the 20S proteasome [16]. 19S cap is linked either to 1 alpha (α) chain or to both α chains. The 26-S proteasome comprises the 20S proteasome and the 19S cap [16]. It can completely break down the target protein into peptides. The 19-S cap can identify ubiquitinated proteins. The cap and ubiquitin chains interact to force the remaining protein into the proteasome. The Ub is reprocessed and the target protein is degraded into short peptides [17] [18][19]. The E3 ligase superfamily is divided into 4 categories: HECT (Homologous to the E6AP-Carboxy terminus), RING (Really Interesting New Genes), RBR (RING Between RING), and U-box types. HECT ligases create a covalent interaction with the Ub molecule before attaching it to the target protein (substrate). RING-type ligases serve as a bridge between the E2 and the target protein, enabling a direct transfer of the Ub molecule to the protein [20]. RBR ligases have combined traits of both HECT and RING types. They can facilitate both direct as well as indirect transfer of Ub to the target protein. U-box shares a resemblance with RING types in structure but lacks a Zn-finger domain. However, they can facilitate a direct transfer of Ub to the protein target. Each group has distinct traits and mechanisms that contribute to the diversity of the UPS, and regulate several cellular processes [21]. When it comes to determining the substrates which are to be marked for degradation, the specificity of E3 ligases becomes crucial. Different E3 ligases recognize their specific substrates, rendering precise control over proteins inside a cell. If the UPS functioning gets dysregulated, specifically if E3-ligase activity gets disturbed, then it can foster the development of deadly conditions like NDDs such as AD, PD, HD, and ALS, and several types of cancer [12][22][23] (Fig.I).

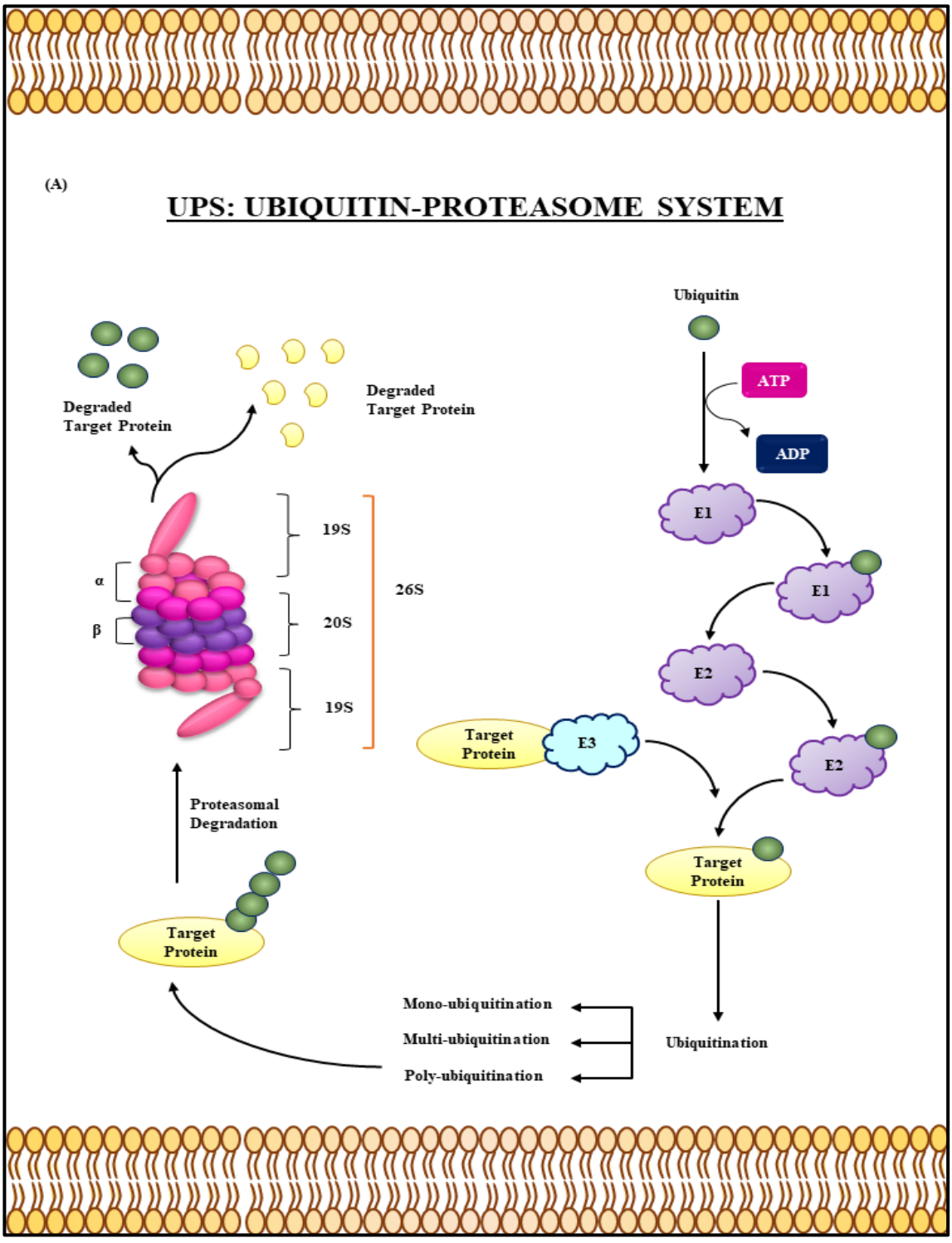

Fig.I. The Ubiquitin-Proteasome System(UPS) involves E1, E2, and E3 enzymes for targeted protein degradation.

CHAPTER 2

LITERATURE REVIEW

2.1. Therapeutic Potential of E3 ligases

The E3 ligases hold immense significance in playing their crucial roles in cellular processes, particularly in the degradation and governance of target proteins by an approach called ubiquitination [25]. Different E3 ligases have distinct functions and have a huge impact on the mechanisms underlying NDDs and cancer. One can gain crucial insights that can guide potential therapeutic interventions by studying the analogous pathways and common targets of E3 ligases in these diseased conditions. E3 ligases are indulged in new innovative approaches such as PROTAC and other advanced methods. These approaches highlight the significant potential of designing ligands and compounds to precisely target E3 ligases, leading to highly effective, efficient, and tailored personalized therapeutic results. Furthermore, various E3 ligase inhibitors are undergoing clinical trials, indicating advancements in therapeutic strategies for NDDs and cancer. Although, there is a need for a balanced approach in medical research to ensure both safety and efficacy for which ethical considerations associated with emerging treatments must be addressed. Looking forward, targeting E3 ligases emerges as a promising and dynamic way poised to revolutionize the current treatments for NDDs and cancer. To significantly contribute to the ongoing development of E3-based therapeutics, a focused understanding and exploration of targeting E3 ligases are expected which will ultimately offer novel and precise strategies for combating such challenging and complex diseases. In short, studying E3 ligases will expand the knowledge of how cellular regulation occurs and accelerate the evolution of personalized and effective therapeutic interventions.

2.2. E3 ligases in neurodegenerative diseases (NDDs)

In the zone of neurodegenerative diseases (NDDs) such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and Amyotrophic Lateral Sclerosis (ALS), E3 ligases hold immense importance [26] [27]. These enzymes regulate the pathways for the disintegration of proteins, including the elimination of proteins that are toxic, aggregated, or misfolded which are the key characteristics of these diseases [28]. For instance, in AD, E3 ligases such as CHIP are involved in breaking down amyloid-

beta (Aβ) plaques and tau proteins, which are the hallmarks of AD. Similarly, E3 ligase such as Parkin is essential for maintaining the health of mitochondria and managing the processes linked with protein aggregation in PD. In HD and ALS conditions, disrupted E3 ligase functioning can contribute to the development of mutant huntingtin (mHtt) and misfolded proteins, respectively, intensifying the progression of the disease [29]. Therefore, gaining an in-depth knowledge of E3 ligases and their roles holds immense potential for creating targeted therapies to cure these life-threatening NDDs (Fig. II).

Alzheimer's Disease (AD) is a progressively deteriorating NDD and has a range of symptoms initiating from the impairment of memory and decline and cognitive skills. It extends to motor, behavioural and visuospatial motor impairments [30]. In pathological samples, AD is identified by the Aβ plaques accumulation and neurofibrillary tangles (NFTs) which contain the tau protein [31][32]. CHIP, an E3 ligase play a crucial role in various aspects of AD [33]. It mediates Aβ and tau pathology, mitochondrial function modulation, calcium homeostasis regulation, and supervision of the cell cycle in different parts of the brain [15]. CHIP eliminates Aβ and tau proteins through phagocytosis. Aβ precursor protein (APP) causes autophagy dysfunction and CHIP modulates it by regulating APP processing through BACE1 (β-site APP-cleaving enzyme 1) [34]. In addition, CHIP restricts p-tau seeding by targeting tau proteins for degradation [35]. In AD, several other E3 ligases such as NEDD4-1, NRBP1, Mdm2, Peli1, and STAT1 also play roles in tau and Aβ regulation. This affects processes such as Aβ degradation, APP processing, and proper functioning of mitochondria [36]. Furthermore, Parkin (PK), another E3 ligase is found to play a function in mitochondrial control and tauopathy in AD condition[37][38] [39][40]. In addition, the APC/C-Cdh1 complex is regulated by Fzr1 and it is necessary for the process of neurogenesis and survival of neural cells. This affects the cell cycle in AD [41]. If this complex gets dysregulated then this may contribute to the apoptosis of neuronal cells and excitotoxicity [42] [43].

Parkinson's Disease (PD), is the nearly second most prevalent NDD and it is identified by the Lewy bodies and degenerative loss of dopaminergic neurons in the substantia nigra of the brain [44][1][45]. This process occurs by the α-Synuclein accumulation and involves mitochondrial impairment, oxidative burden, endoplasmic reticulum (ER) burden and other cellular stressors [46][47]. PD is also marked through some non-motor symptoms such as sleep issues, psychiatric manifestations, olfactory disturbances, and autonomic dysfunction [27]. Some cases of PD are associated with mutations in SYN, Parkin (PARK2), PTEN-induced putative kinase-1 (PINK-1), Leu-rich repeat kinase 2 (LRRK2), and DJ-1 gene, shedding light on the genetic intricacies of the PD [12][48]. PINK1 and Parkin are E3 ligases and crucial players in the pathology of PD. They are involved in operations such as protein turnover, mitophagy, and regulation of neuroinflammation [49][50]. In addition, other E3 ligases such as SIAH1, SIAH2, HDR1, Pellino1, and E6-AP are found in the decline of α-Synuclein, apoptosis induced by ER-stress, activation of microglia, and Ub-mediated protein degradation in PD. All of them reflect the diverse and significant roles of E3 ligases in this type of NDD[51] [52] [53] [54].

Huntington's Disease (HD) arises from an expansion of trinucleotide (CAG) repeats in the HD gene's exon 1, which leads to the accumulation of mutant huntingtin (mHtt) containing expanded polyQ repeats [55][56]. This genetic anomaly disrupts the UPS, playing an important role in the onset of HD [12]. Clinically, HD is characterized by movement disorders, neuropsychiatric symptoms, and progressive cognitive decline and is an inherited disease [12][57]. CAG expansion mutation in exon 1 of the HTT gene on human chromosome 4 is the primary hallmark of HD [12]. mHtt undergoes abnormal post-translational modifications (PTMs), impacting transcription, mitochondrial functions, and immune responses. Importantly, mHtt is detectable in the serum as an early biomarker in HD patients [58]. E3 ligases are closely associated with mHtt and antioxidant mechanisms in HD. TRAF6, a RING-type E3 ligase, promotes the formation of mHtt aggregates, while WWP1, a NEDD4-like E3, increases cellular mHtt levels through K63-linked ubiquitination. UBE3A/E6AP, a HECT-type E3 ligase, exhibits reduced levels in aged mouse models, affecting differential ubiquitination and deterioration of Htt fragments, thus supporting age-related NDD [12]. CHIP/STUB1, a U-Box E3 ligase, inhibits mHtt oligomerization, while HRD1/SYVN1, a RING-type E3 ligase, aids in HttN clearance. Parkin also plays a role in mHtt clearance. The SCF complex is crucial for maintaining postmitotic neuron integrity and is involved in mHtt clearance. Overexpression of Cul1, a component of the SCF complex, negatively impacts mHtt aggregation. Increased CHIP activity facilitates the degradation of mHtt, but this activity is hindered in neurons by elevated HSPA (Hsp70)-Binding Protein 1 (HspBP1) expression, potentially contributing to neuronal sensitivity in HD [59]. UBR5, UBR7, UBE3A, and RNF181 are other E3 ligases with implications in HD pathogenesis. Heat shock transcription factor 1 (HSF1) expression is crucial for mHtt aggregate clearance and is reduced in HD brains [12], with mHtt upregulating FBXW7, leading to HSF1 degradation via ubiquitin-dependent pathways [60][61]. PIAS1 deficiency improves behavioural phenotypes and microglial activation in HD mouse models, influencing the PIAS1-DDR pathway crucial for HD progression [62]. HACE1, associated with antioxidant stress, activates NRF2 protein synthesis and nuclear localization, although its expression is decreased in HD tissue. Furthermore, Htt aggregates in HD are enriched with linear ubiquitin chains formed by the LUBAC-catalyzed linkage of the donor Ub's C'- Gly to the Gly residue, which contributes to reducing protein toxicity in HD [63][64].

Amyotrophic Lateral Sclerosis (ALS) is characterized by progressive muscle weakness in the bulbar and limb regions, primarily impacting motor neurons in the brainstem, cerebral cortex, and spinal cord [65][66][67]. ALS pathology also involves frontotemporal lobes, showing the presence of ubiquitinated inclusions that are immunoreactive to TAR DNA Protein-43 (TDP43). Familial ALS often presents mutations in the Cu-Zn Superoxide Dismutase-1 (SOD1) gene [28], TARDP mutation, and repeated amplification of C9orf72, with proposed neuroprotection through SOD1 degradation [68]. Multiple E3 ligases such as CHIP, Dorfin (Rnf19a), GP78, NEDL1, PK, ZNF179, HRD1, MuRF1/TRIM63, and SCFCyclin F play roles in facilitating the degradation of mutant SOD1, influencing disease progression. For instance, CHIP is involved in the proteasomal elimination of defective SOD1, aided by chaperones like Hsp70 or heat shock cognate Hsc70 [69]. Dorfin specifically targets mutant SOD1 for ubiquitination and degradation, reducing its levels and mitigating spinal motor neuron degradation. GP78 promotes ER-related degradation of mutant SOD1, offering protection

against mutant SOD1 mutation-induced stress and neurotoxicity [70] [71]. TDP43 aggregates in ALS are targeted for polyubiquitination by PK and ZNF179, with autophagy playing a crucial role in clearance. Mutations in CCNF disrupt ubiquitination, contributing to autophagic defects and TDP43 accumulation. OPTN gene mutations, such as the E478G variant, impact UPS-mediated degradation and aggregation, highlighting the complex interplay between E3 ligases, misfolded proteins, and neurodegeneration in ALS [72][27]. Other ligases like NEDL1 [73], MITOL [74], Mitofusin2 [75], and MuRF1/TRIM63 also play significant roles in protein clearance and muscle protein degradation in ALS-related muscular atrophy [76] [45] [36].

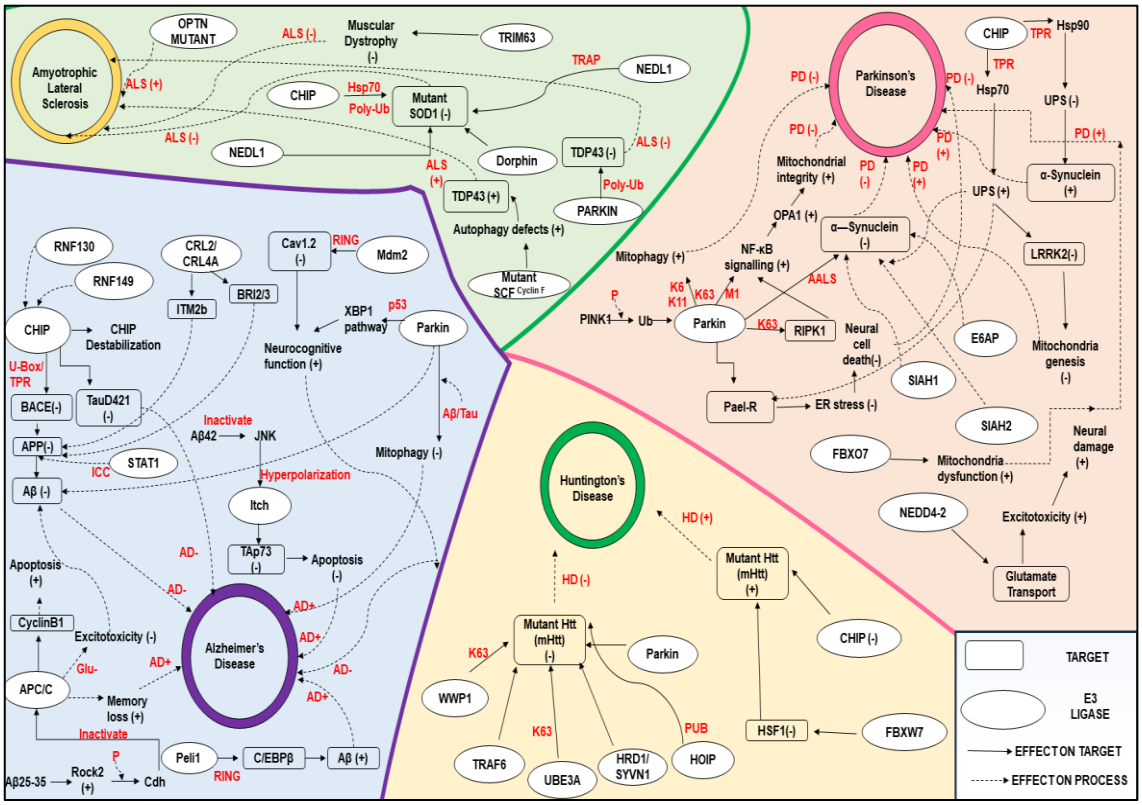

Fig. II illustrates the importance of E3 ligases in the development of Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and Amyotrophic Lateral Sclerosis (ALS) [77]**.** In AD, CHIP plays a role in reducing the levels of BACE and tauD421, inhibiting the aggregation of APP and Aβ [46]. RNF130 and RNF149 influence CHIP's stability, while CRL2/CRL4A ubiquitinate ITM2b and BRI2/3 to mitigate Aβ aggregation. Mdm2 inhibits Vav1.2 to enhance neurocognitive function, and Parkin, along with p53, affects the XBP1 transcription factor, improving neurological functions and promoting mitophagy in the presence of Aβ and tau protein. Peli1 elevates the AD condition, and APC/C has a role in promoting apoptosis, improving memory, and reducing excitotoxicity. In PD, CHIP targets α-Synuclein, and Hsp70 promotes the UPS machinery to reduce its accumulation, while Hsp90 opposes this process. E6AP, SIAH1, and SIAH2 also improve PD symptoms. FBXO7 contributes to mitochondrial dysfunction, and NEDD4-2 influences glutamate transport, increasing excitotoxicity and neuronal damage. Parkin enhances mitophagy and NF-κB signalling, ubiquitinating α-Synuclein through the aggresome-autophagy lysosome system (AALS) to reduce PD symptoms.

2.3. E3 ligases involved in Cancer

The HECT E3 ligases play a vital function in cancer biology through their distinct catalytic HECT domains, which comprise an N' end E2 binding domain and a C' end catalytic Cys residue [78]. They are categorized into 3 subfamilies: NEDD, HERC, and "other" HECT [46], each with specific domains and target proteins [79][80]. Among these, NEDD4 has been extensively studied for its regulatory effects on PTEN expression. NEDD4 can negatively regulate PTEN by binding through its C2 or HECT domain, leading to PTEN ubiquitination and subsequent deterioration [81][82][83]. Conversely, NEDD4-1 can bind with pAkt-Ser473, resulting in increased PTEN levels and suppression of liver cancer cell growth, impacting various signalling pathways like PI3K-AKT, cell adhesion, and kinase activities [84] [85] [86]. Moreover, NEDD4-1 interacts with other proteins such as CNrasGEF, active FGFR1, N-MYC, HER3, and SAG, influencing cell migration, downstream signalling, neuroblastoma cell proliferation, and apoptosis in cancer cells [87][88]; [89]; [90]; [91]. Additionally, RING-type E3 ligases, characterized by a meshed structure with zinc-coordinating residues, regulate cell cycle progression and apoptosis. For instance, APC/CCDH1 degrades proteins promoting mitotic exit, while SCFSKP2 and SCFβ-TrCP regulate proteins involved in G1/S transition and cell cycle advancement [92][93][94][95] [96][97]. The RBR-type E3 ligases, including Parkin, HHARI, TRIAD1, HOIP, and HOIL1, also contribute significantly to cancer biology by influencing several cytoplasmic processes (mitophagy, apoptosis, and cell proliferation) [98] [99]; [100]; [101]; [102][103]. These E3 ligases' dysregulation is observed in different cancers, highlighting their potential as therapeutic targets or biomarkers for cancer diagnosis and prognosis [104][105]; [106]; [107]; [108]. Understanding the structural features and functional roles of E3 ligases in cancer is essential for creating target-specific therapies and improving cancer management strategies (Fig. III). The diverse roles of various types of E3 ligases are consolidated in Table-I management strategies (Fig. III). The diverse roles of various types of E3 ligases are consolidated in Table-I.

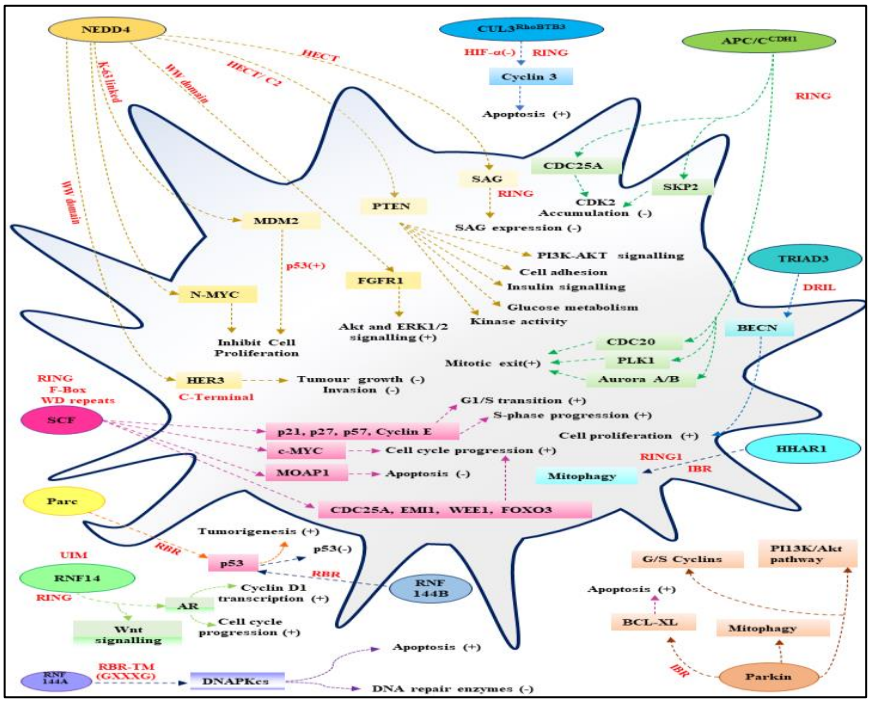

Fig. III: Involvement of E3 ligases in the development of cancer.

TABLE-I: A consolidated list of different types of E3 ligases and their significance in NDDs and Cancer and their interplay with Deubiquitinases (DUBs)

E3-LIGASES IN CANCER AND NDDs										
Type	**Name**	**UniProt ID**	**Size (kDa)**	**Chromosomal Location**	**Ubiquitination/ Interacting Domain/ Motif/Residues**	**Cancer**	**AD**	**PD**	**HD**	**ALS**
HECT	NEDD4-1	P46934	149.11	15q21.3	K63-mediated	PTEN, MDM2, CNrasGEF, FGFR1, Myc, HER3, SAG, pAkt-Ser473, Notch (PPSY motif), RNAPII, N4BP, AKT	ABCB1, Autophagy-related proteins (Beclin1, p62, LC3, SQSTM1)	α-Synuclein	-	Mutant SOD1
	NEDD4-2	Q96PU5	111.93	18q21.31	K29, K48, and K63-mediated, WW domain, PY motif	ACK1, AMPAR, c-Myc, all SMAD proteins	Glutamate	α-Synuclein, Glutamate	-	-
	ITCH	Q96J02	102.80	20q11.22	K63 and K48-mediated	FLIP, p63, p73, RASSF5/NORE1, LATS1, LAPTM5, ErbB4, NOTCH1, SMAD2	Tap73	-	-	-
	WWP1	Q9H0M0	105.20	8q21.3	WW domain	Smad2, Smad4, ErbB4/HER4, JunB, p53	-	-	mHtt	-
	WWP2	O00308	98.91	16q22.1	K63-mediated	ENac, RNAPII, PTEN, SMAD2, SMAD3, SMAD7, TRIF, RBP, p73, NDP52, OPTN, SQSTM1	-	-	-	-
	SMURF2	Q9HAU4	86.19	17q23.3-q24.1	PY motif, WW domain, C2 domain	SMAD-specific, Lamin A, Progerin, SNON in TGF-β signalling	SNON	SNON	SNON	SNON

	SMURF1	Q9HCE7	86.11	7q22.1	PPxY motif at K517 and K559, HECT domain, LRR repeats	UVRAG	-	-	-	SMAD 2/3
	HECW1	Q76N89	179.55	7p14.1-p13	Linker between C2 and WW domain	Smad4, DVL1	-	-	-	Mutant SOD1
	HECW2	Q9P2P5	193	2q32.3	WW domain	TP73	TP73	-	-	TP73
	HERC3	Q15034	117.18	4q21.1	HECT domain	RPL23A, EIF5A2, RelA in NF-κB signalling	-	-	-	-
	HERC5	Q9UII4	116.85	4q22.1	Cys994 within the HECT domain	CtBP1	-	-	-	-
	E6AP	Q05086	100.68	15q11.2	HECT domain	p53, PML, CDKN1B	Arc	α-Synuclein	Htt	-
RBR	ARIH1	Q9Y4X5	64.11	15q24.1	RING1 and IBR domains	Cullins, PD-L1, hnRNP-E1	-	-	-	-
	Triad1	O95376	57.819	3p21.31	K6, K48, and K63-mediated	ECS complex, IκBβ in the nucleus, PABN1, NLRP3, MLL-ELL	-	-	-	-
	ANKIB1	Q9P2G1	122	7q21.2	RBR domain	-	-	-	-	-
	PARC	Q81WT3	281.22	6p21.1	RBR domain	p53/TP53, CUL7, Cyt c,	-	-	-	-
	Parkin	O60260	51.64	6q26	Mono, K6, K11, K48, and K63-mediated, Ubl domain, IBR domain	PHGDH, HKI, GAPDH, TKT, Cyclin D/E, Cdc20/Cdh1, Tubulin, BC-XL	Mitochondrial proteins, tau, Aβ, XBP1	OPA1, RIPK1, Pael-R, NEMO, Multiple targets inducing mitophagy	mHtt	TDP43
	HOIL1	Q9BYM8	57.57	20p13	IBR domain	p53	-	-	-	-
	RNF14	Q9UBS8	53.83	5q31.3	Ub Interaction Motif (UIM), RING Zinc finger motif	Androgen Receptor, Wnt signalling	-	-	-	-

	RNF19A	Q9NV58	90.69	8q22.2	C-terminal region	TRAF6	-	SNCAIP	-	SOD1 variants
	RNF19B	Q6ZMZ0	77.92	1p35.1	RING domain	-	-	-	-	-
	HOIP	Q96EP0	119.65	14q12	RBR domain	ERα, p53/Mdm2, FOXP3, TNFR1 signalling, BCL10	-	-	mHtt	-
	RNF144A	P50876	32.89	2p25.1	RING1 and Transmembrane (TM) domain	DNA PKcs, DNA-repair proteins, HSPA2	-	-	-	-
	RNF144B	Q7Z419	33.69	6p22.3	RBR domain	p53	-	-	-	-
	Triad3	Q9NWF9	99.40	7p22.1	At K48 through the DRIL domain	BECN1	-	-	-	-
	RNF217	Q8TC41	59.37	6q22.31	C-terminal RING finger motif	HAX	-	-	-	-
RING	BRCA1-BARD1 complex	Q99728	86.64	BC: 17q21.31, BD: 2q35	K6, K27, K29, K63-linked poly-ubiquitination, K60/96, K123/125/127/129-linked mono-ubiquitination, RING domain, BD: BRCTs, BC: BRCTs	Aurora Kinase B, Cdc25C, Claspin, Erα, H2A, LARP7, NF2, P50, RPB1, RPB8, TFIIE, Topoisomerase IIa, γ-tubulin	Accumulation within NFTs and co-localization with tau proteins suggest a potential role of the complex	-	-	-
	MDM2-MDMX complex	O15151	54.86	MDM2: 12q15, MDMX: 1q32.1	C-terminal residues of the RING domain	p53	p53, Cav1.2	-	p53, mHtt	-
	CRL4	Q9NRM6	55.88	3p21.1	C/N-terminal domain	CDT1, XPC, p21, p27, Cyclin D	BRI2/3	-	-	-
	SCF^{FBXW7}	Q969H0	79.66	22q12.3	F-box, WD repeats	Cyclin E, c-MYC, NOTCH1, NOTCH2, JUN	PSEN1	-	-	-
	FBXO7	Q9Y3I1	58.50	22q12.3	FP domain	BIRC2, IAP	-	Mitophagy-associated proteins	-	-

	SIAH1	Q8IUQ4	31.12	16q12.1	N-terminal RING domain	p53, TRAF, β-catenin, c-Myb	-	Synphilin-1, α-Synuclein	-	-
	SIAH2	O43255	34.61	3q25.1	RING domain	SPRY2, p53	-	α-Synuclein	-	-
DEUBIQUITINASES (DUBs) IN CANCER AND NDDs										
USPs	USP7	Q93009	128.30	16p13.2	C-terminus for oligomerization, USP-domain	HDM2, p53, H2B, FOXO4, MDM2, ERCC6,	mTOR signalling, p53, TP53, PTEN,	mTOR signalling, PTEN	-	NEDD 4L
	USP8	P40818	127.52	15q21.2	Target proteins with K48/63 ubiquitination, USP-domain	NRDP1	-	α-Synuclein	-	-
	USP9	Q93008	290.46	Xp11.4	Target proteins with K29/33/48/63 ubiquitination, USP-domain	β-catenin, epsin, AF-6	-	-	-	-
	USP10	Q14694	87.13	16q24.1	Cys in USP-domain	p53, c-MYC	-	P62	-	-
	USP15	Q9Y4E8	112.49	12q14.1	DUSP domain	RBX1	-	-	-	-
	USP24	Q9UPU5	294.36	1p32.3	UBA domain	-	-	ULK1	Increase mHtt formation	-
	USP30	Q70CQ3	58.50	12q24.11	Cleaves K6 Ub linked proteins, USP domain	-	-	PARK2	-	-
	USP33	P07550	46.45	1p31.1	ZF UBP domain	HIF1-α	-	Parkin	-	-
	USP46	P62068	42.44	4q12	No deubiquitinating activity by itself;	-	AMPARs	-	-	-

2.4. Shared E3 ligases in NDDs and Cancer

E3 ligases are vital for cellular regulation, particularly in the controlled deterioration of harmful proteins by utilizing the UPS system [12]. This understanding has led to exploring their involvement in diseases like cancer and NDDs for targeted therapies. Focusing on common E3 ligases involved in both cancer and NDDs presents a promising therapeutic avenue due to shared molecular pathways. Notably, HECT domain-containing ligases like NEDD4-1 target proteins linked to cancer (e.g., PTEN, MDM2) and NDDs (e.g., α-Synuclein, mutant SOD1). Similarly, Itch, WWP1/2, and SMURF1/2 exhibit ubiquitination activities affecting substrates in both cancer and NDDs, showcasing their potential as therapeutic targets. RBR ligases like Parkin, BRCA1-BARD1, and MDM2-MDMX complex also play crucial roles in ubiquitinating proteins linked to cancer and neurodegenerative pathways. Additionally, U-box domain-containing enzymes such as CHIP have dual roles in cancer and NDDs, suggesting their importance as therapeutic targets. This dual-targeting strategy recognizes the complex interplay between cancer and NDDs, providing opportunities for more effective interventions (Fig. IV).

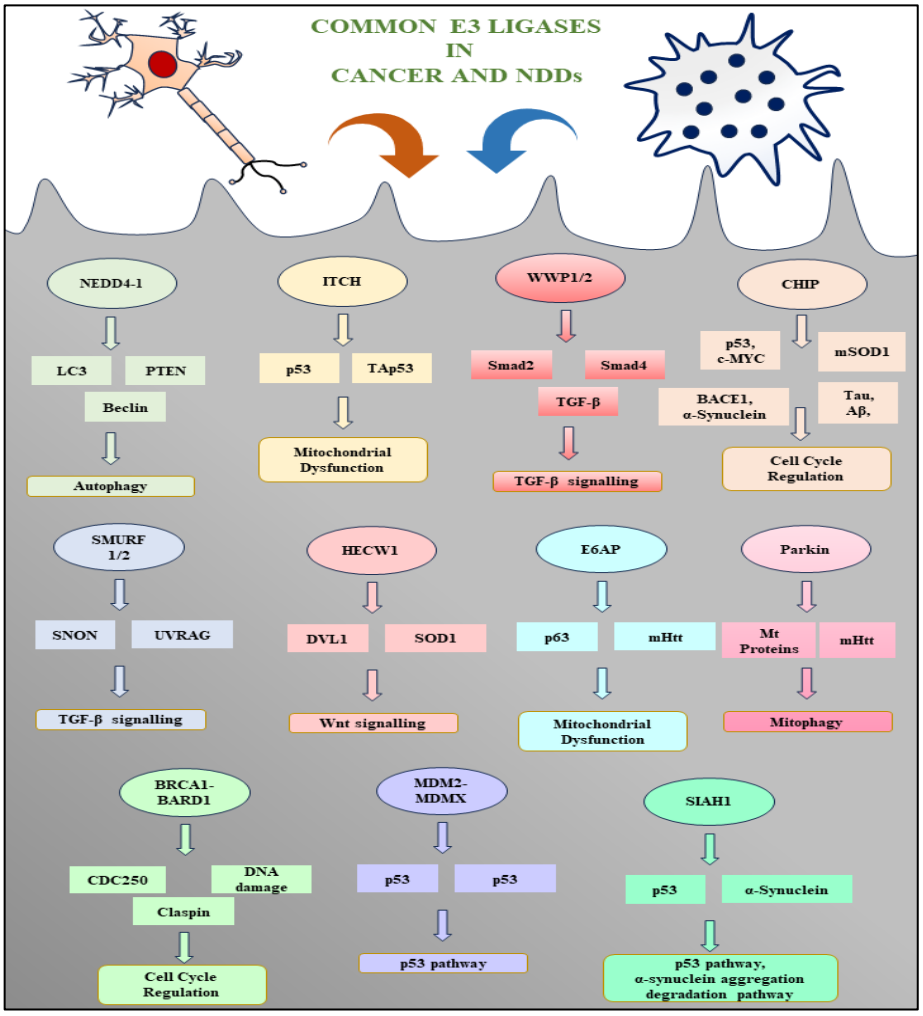

Fig. IV outlines key E3 ligases influencing different pathways underlying Cancer and Neurodegenerative Diseases (NDDs).

2.5. MARCH Proteins

A sub-division of RING-type E3 Ub ligases is known as MARCH (Membrane-associated RING -CH type) proteins [109]. Viral immunomodulatory ligases- K3 and K5 of the Kaposi's sarcoma-associated Herpes virus are structural homologs of MARCH proteins [110][111]. At the N-terminus, they have the RING-CH domain, which is followed by the transmembrane (TM) domain. They suppress MHC-I molecule surface expression. The first and most MARCH protein to be identified was named c-MIR (cellular modulator of immune recognition)[112][113]. The MARCH family consists of eleven members. All eleven members have a RING-CH type domain with E3 ubiquitin ligase activity [114]. These MARCH proteins catalyze the ubiquitination of several immunological receptors, membrane-associated organelles, and other components involved in the innate immune response [115].

2.6. Structure and Localization of MARCH proteins

Members of the MARCH family have a transmembrane domain and a RING-CH domain on the N-terminus. MARCH 7 and MARCH 10 are the exceptions (Fig. V). They do not have the transmembrane domain but have the RING-CH domain at the C' end [10]. MARCH 7 and MARCH 10 are therefore also called the non-canonical members of the MARCH family [116].

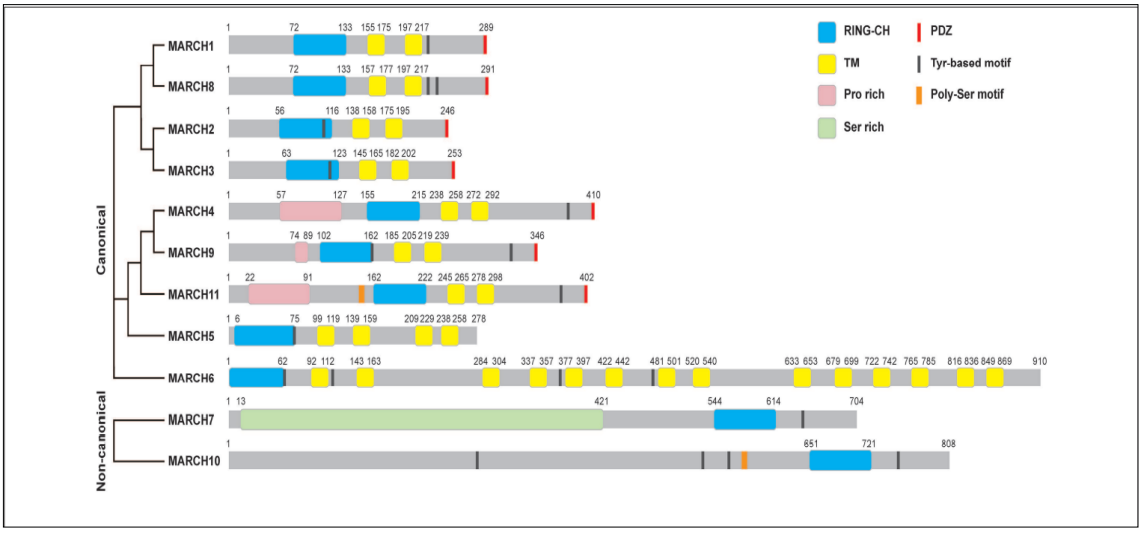

Fig. V. Structural Domains of MARCH family members [117]

The MARCH proteins are mostly present in the plasma membrane, endosome and lysosome. PDZ-binding domain decides the sub-cellular location of MARCH proteins. The respective locations of MARCH family members are as follows in Table-II and Fig. VI [110][118].

Table-II: Sub-cellular location of the members of the MARCH family.

MARCH FAMILY MEMBER	LOCATION
MARCH 1	LAMP-1 + late endosome/lysosome
MARCH2	Endosome/lysosome/PM
MARCH 3	ER
MARCH 4	Golgi
MARCH 5	The outer membrane of Mt
MARCH 6	ER
MARCH 7	Nuclear speckles
MARCH 8	PM
MARCH 9	Endosome/lysosome/PM
MARCH 10	Cytosol
MARCH 11	MVBs, TGN

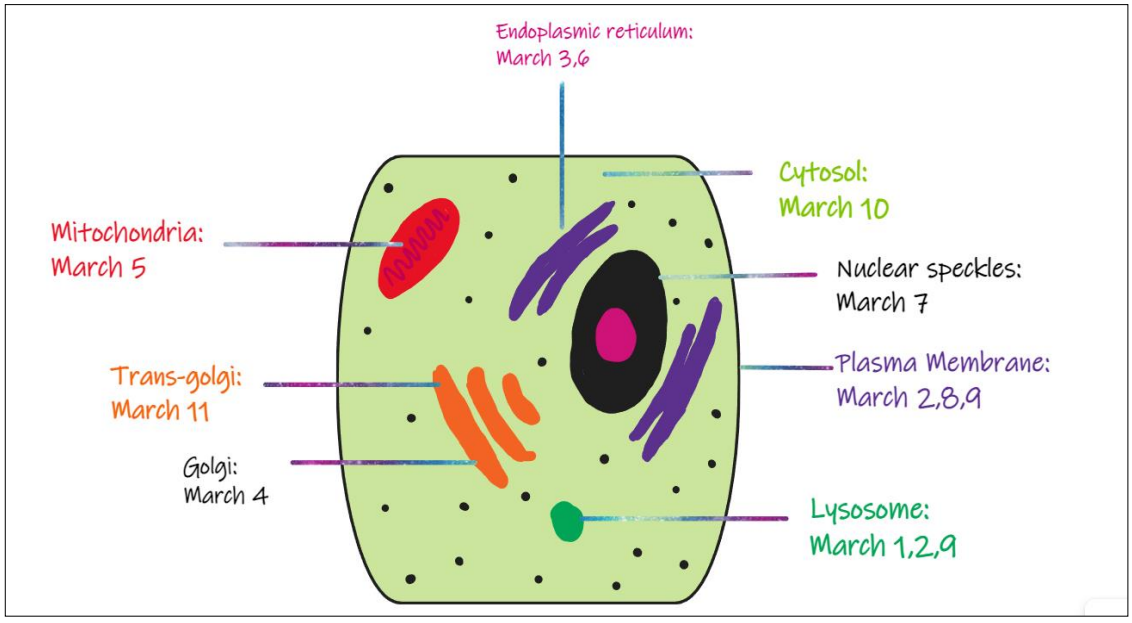

Fig. VI illustrates the sub-cellular location of various MARCH proteins

2.7. PTMs of MARCH proteins

MARCH proteins are under the strict control of PTMs. Ubiquitination is one of the important PTMs and is vital for regulating the stability of the MARCH proteins [119]. For instance- MARCH 5,6,7,8 and 10 are tightly regulated by the autoubiquitination mediated by their RING domain [120]. Phosphorylation also plays a function in controlling MARCH ligases [28]. For instance, when TYRO3-mediated phosphorylation takes place in the unstimulated cells, MARCH 3 is inactive. IL-1beta stimulates MARCH 3 CDC 25A dephosphorylates MARCH 3 and MARCH 3 becomes activated. Activated March 3 degrades the IL-1 type 1 receptor through K48-linked polyubiquitination. This inhibits IL-1beta signalling.

2.8. Role of MARCH proteins

MARCH proteins play a crucial role in immune regulation. MHC-1 molecules are present on CD8+ T cells and help in their activation which allows T cells to fight against foreign antigens. MARCH proteins are known for downregulating the MHC expression on T cells. For example- if MARCH 9 gets overexpressed then there is an increase in the endocytosis of MHC-1 molecules. Additionally, MARCH 9 gene knockout impairs MHC-1 translocation from TGN to endosomes. The access of MHC-1 to endosomes gets hindered and antigen presentation efficiency gets affected. MHC-2 molecules are present on the CD4+ T cells' surface and are involved in their activation. MARCH 8 protein causes polyubiquitination of the beta chain of the MHC-2 molecule and causes its degradation [110][121]. APCs express CD86, which is crucial for immunological control. MARCH 8 causes polyubiquitination of CD86 at the C' end which leads to its degradation. MARCH 1 also similarly degrades CD86 by polyubiquitinating the at K267 [122]. TRAIL binds to the TRAIL-R1 receptor and causes the death of breast cancer cells [123]. However, MARCH 8 polyubiquitinates TRAIL-R1 which prevents apoptosis of cancer cells [124]. MARCH 2 is induced upon HIV infection and it prevents viral replication by destroying the proteins of the viral envelope. An $Fc\gamma R$ inhibitor called FcRIIb blocks the $Fc\gamma R$-mediated response to tumour cells that have been coated with an antibody.

2.9. MARCH2 Protein

MARCH2 is a part of the MARCH family of proteins, which are E3 Ub ligases involved in the regulation of protein trafficking and degradation within cells [125].

Structure and Function: MARCH2 contains a RING-CH domain, which is accountable for its E3 Ub ligase functioning [110]. This activity allows MARCH2 to link Ub molecules to target protein molecules, marking them for deterioration by the proteasome or altering their cellular localization and function.

Role in Ubiquitination: MARCH2 protein ubiquitinates various target proteins, such as MHC-II (Major Histocompatibility Class II), CD44, CD86 as well as CD98. MARCH2 protein becomes crucial in controlling cell adhesion, immune responses, and nutrient transportation by ubiquitinating them [46].

Role in Cellular Processes: MARCH2 protein targets surface proteins for degradation and is thus involved in the downregulation of surface proteins. For instance, on the surface of dendritic cells, it regulates MHC-II molecules' expression [126]. This is necessary for the modulation of antigen presentation process to T cells.

Role in diseases: In various diseases, the dysregulation of MARCH2 protein has been implicated. For example, the MARCH2 protein gets overexpressed in colorectal cancers and affects autophagy-mediated pathways, underscoring its indirect potential role in neurodegenerative disorders (NDDs). The abnormal expression of MARCH2 has been linked with autoimmune diseases (AIDs) because it influences the presentation of self-antigens by MHC-II molecules.

Role in Therapeutics: Understanding the molecular mechanisms of MARCH2 and its role in cellular processes has potential therapeutic implications. Targeting MARCH2 activity could be explored as a strategy for modulating immune responses in autoimmune disorders, and NDDs or as a therapeutic approach in cancer treatment by influencing protein degradation pathways.

CHAPTER-3

METHODOLOGY

3.1. Predicting the 3D structure of MARCH2

To model the structure of the MARCH2 protein, a 246-amino acid long sequence from UniProt was retrieved. Using the Swiss Model, a trusted homology modelling technique, a model based on this sequence was created. The model's stability and quality through a Ramachandran Plot (obtained from SAVES v6.0) was assessed. This process provided insights into the potential structure and features of the MARCH2 protein.

3.2. Virtual Screening of Drugs

Ligands, comprising drugs and phytochemicals, were obtained from the Drug Bank and Phytochemica databases for virtual screening. Utilizing the EasyDock Vina tool, PDBQT files of both the ligands and the modelled structure of MARCH2 (the receptor) were prepared. Blind docking was conducted with a 60X60X60 grid centred at coordinates (29.971, 39.371, 35.279). Following the docking process, a table presenting the binding energies of the protein-drug complexes was generated. Table-1 offered valuable insights into the associations and adhesion affinities between the ligands and the MARCH2 receptor [127].

3.3. Molecular Dynamic Simulation

For Molecular Dynamics Simulations (MDS) to be conducted, specific system prerequisites are necessary, including a computer with either a GPU or a capable CPU for tasks that don't require GPU acceleration. Adequate RAM is also essential to manage simulation data, alongside sufficient storage space for storing input and output files generated during the simulation process. The MDS process, as per the recommended procedure outlined on the website, involved several steps. Initially, preparations such as protein structure preparation, protein topology file preparation, solvation, and system ionization were carried out using CHARMM-GUI to ensure a well-prepared system. Subsequently, energy minimization, system equilibration, and MDS production were conducted using the CHARMM-GUI force field, with equilibration performed at a temperature of 300K. The actual MDS simulation was executed using GROMACS, with

a simulation duration of 35ns. After acquiring the MDS files, analysis was conducted in a Linux environment to extract crucial insights. This analysis included obtaining the RMSD curve, RMSF curve, Radius of gyration curve, and the number of hydrogen bonds curve, which offered valuable information regarding the stability of the drug-protein complexes under physiological conditions. The entire methodology for performing in-silico structural analysis of the MARCH2 protein is depicted in Fig. VII.

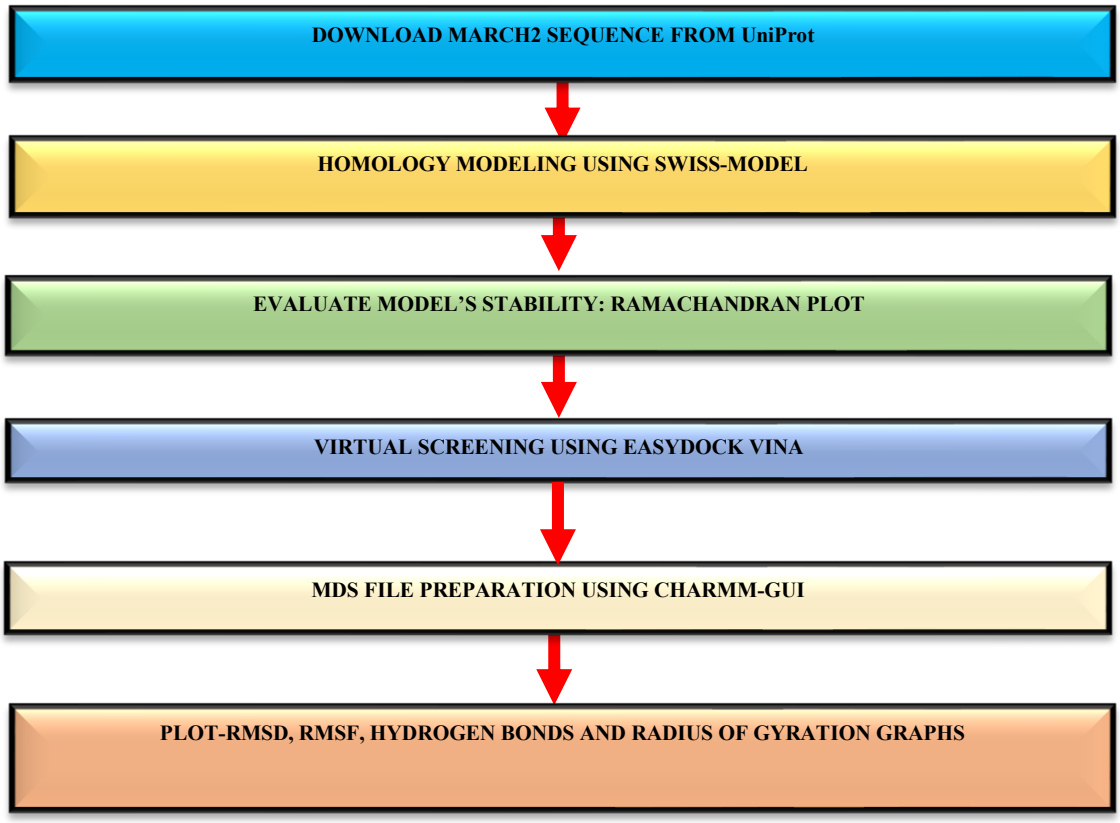

Fig.VII outlines the methodology to analyse the structural behaviour of the predicted model of MARCH2 protein.

CHAPTER 4

RESULTS

4.1. Predicted structure of MARCH2

The RING domain of the protein was successfully modelled using SWISS-MODEL, with 82.4% of its residues falling within the favourable region, indicating a reasonably good quality model. For a visual representation of the model and its structural assessment, Ramachandran Plot was generated. Fig.1 illustrates the predicted model of the RING domain of MARCH2 protein and its corresponding Ramachandran Plot.

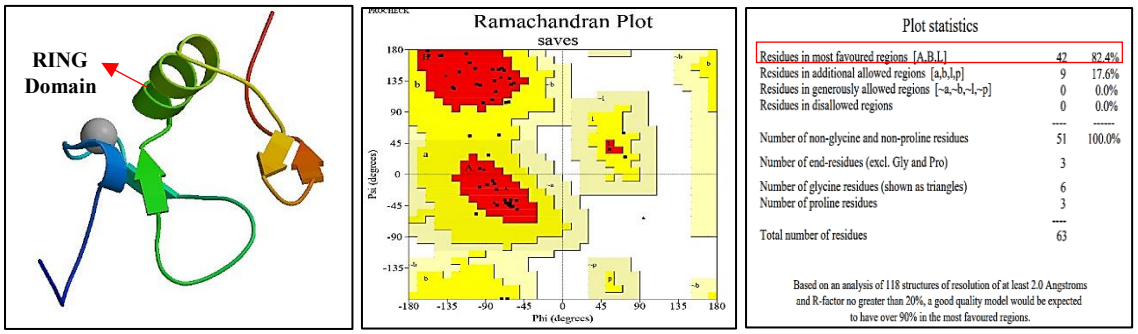

Fig. 1. Predicted model of MARCH2 RING domain and its Plot Statistics. Ramachandran Plot and plot statistics of the RING domain of MARCH 2 with 82.4% of residues in the favourable region. These visual representations provide a comprehensive assessment of the model's structural quality, offering insights into the favourable and unfavourable backbone conformations, ultimately ensuring the reliability of the predicted protein structure.

4.2. Virtually Screened Drugs

A total of 1919 drugs from the Drug Bank database and 295 drugs from the Phytochemica database were subjected to docking to analyze their binding affinities. After completing the virtual screening, the best complex conformations were selected based on their minimal binding affinity values from both databases. The top 10 complexes from each database were compiled in Table-III. Among the Drug Bank database compounds,

Ergotamine (DB00696) exhibited the highest effectiveness against the RING domain of the MARCH 2 protein, displaying a significant binding affinity of -9.2 kcal/mol. Meanwhile, in the Phytochemica database, Tafluposide (POHX0073) derived from Podophyllum hexandrum emerged as the most promising ligand, with a remarkable binding affinity of -8.4 kcal/mol against the RING domain of the MARCH 2 protein. For a visual representation, the binding interactions of Ergotamine (DB00696)-RING and Tafluposide (POHX0073)-RING complexes are illustrated in Fig.3. and Fig.4., respectively. These findings shed light on potential candidates for further investigation as promising drug leads targeting the RING domain of the MARCH 2 protein.

Table-III: Top 10 virtually screened drug-MARCH2 RING complexes

DATABASES	COMPOUND-ID	COMPOUND NAME	BINDING ENERGY (kcal/mol)
Drug Bank	DB00696	Ergotamine	-9.3
Drug Bank	DB09074	Olaprib	-9.1
Drug Bank	DB11986	Entrectinib	-9
Drug Bank	DB00320	Dhe-45	-9
Drug Bank	DB12457	Rimegepant	-8.9
Drug Bank	DB13292	Pimethixene	-8.8
Drug Bank	DB00673	Aprepitant	-8.7
Drug Bank	DB13246	Quinupramine	-8.6
Drug Bank	DB09048	Netupitant	-8.6
Drug Bank	DB00246	Zeldox	-8.6
PhytoChemica	POHX0073	Tafluposide	-8.4
PhytoChemica	POHX0071	NPF	-8.2
PhytoChemica	HEIN0055	Estradiol	-8.1
PhytoChemica	HEIN0056	Lupeol	-7.9
PhytoChemica	ATBE0004	Belladonnine	-7.9
PhytoChemica	ATBE0054	Atroposide G	-7.8
PhytoChemica	PIKU0068	Curcubitacin R	-7.6
PhytoChemica	HEIN0069	Chalinasterol	-7.6
PhytoChemica	HEIN0057	Pestalamide B	-7.6
PhytoChemica	ATBE0053	Atroposide F	-7.6

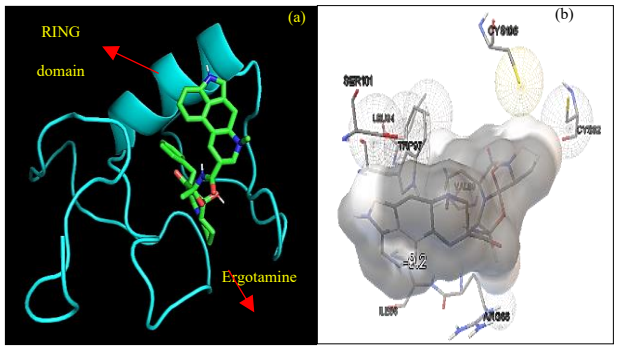

Fig.2 Ergotamine-MARCH2 RING complex. Docked structure of Ergotamine and RING of MARCH 2 with a binding affinity of -9.2kcal/mol; Fig.3b. Interacting residues in the docked complex.

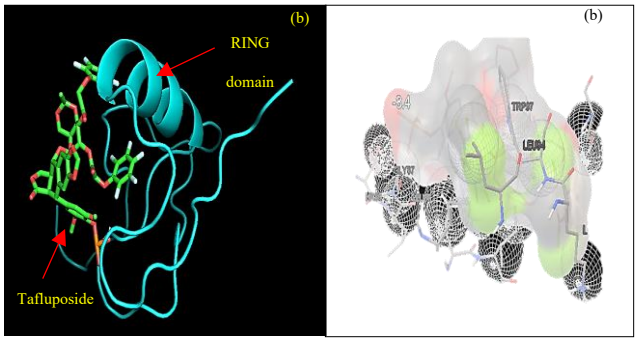

Fig.3. Tafluposide-MARCH2 RING complex. Docked structure of Tafluposide and RING of MARCH 2 with a binding affinity of -8.4kcal/mol; Fig.4b. Interacting residues in the docked complex.

4.3. Molecular Dynamic Simulation of MARCH2

After conducting the Molecular Dynamics Simulations (MDS) for 35 ns, several graphs were plotted to gain insights into the behaviour and durability of the protein-ligand complexes. The **RMSD (Root Mean Square Deviation) curve**, depicted in Fig.4a and Fig. 4b, illustrates how the protein's structure changes throughout the simulation. Interestingly, the RMSD graphs do not exhibit significant fluctuations over time for both the Ergotamine-RING and Tafluposide-RING complexes [127]. This observation indicates that these complexes remain stable throughout the simulation, suggesting promising interactions between the ligands and the RING domain of the MARCH 2 protein.

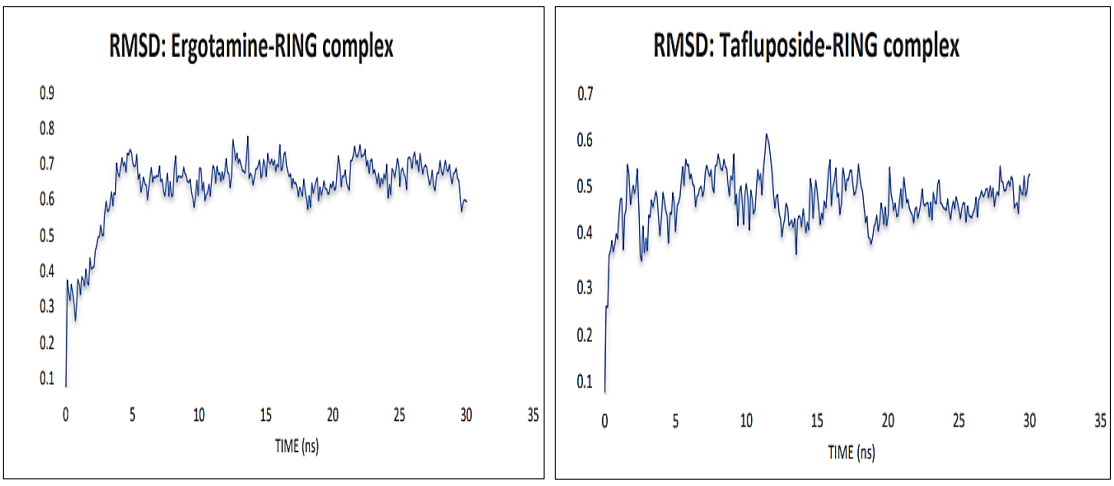

Fig. 4a RMSD curve for the Ergotamine-RING complex, The graph shows low RMSD and insignificant changes throughout the simulation indicating that the Ergotamine-RING complex is maintaining its overall structure. **Fig. 4b RMSD curve of Tafluposide-RING complex.** The graph shows that the Tafluposide-RING complex is maintaining its overall structure and not undergoing overall significant changes.

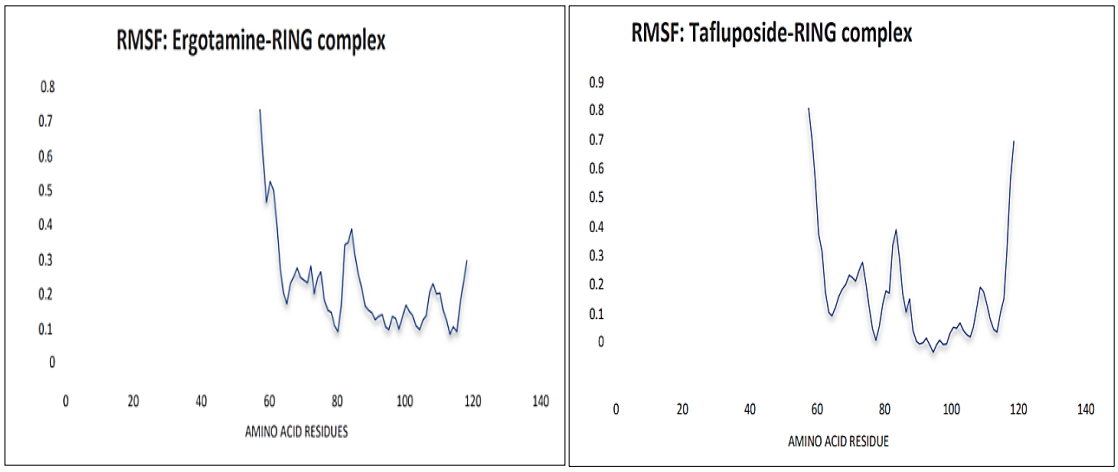

Fig. 5a RMSF curve of Ergotamine-RING complex. Y-axis: RMSF in nm. X-axis: Amino acid residues. The graph shows that the residues from 55-60 are highly flexible throughout the simulation indicating the crucial role of these residues in ligand binding. **Fig. 5b RMSF curve of Tafluposide-RING complex.** Y-axis: RMSF in nm. X-axis: Amino acid residues. The graph shows that the residues from 110-120 are highly flexible throughout the simulation indicating the crucial role of these residues in ligand binding.

The **RMSF (Root Mean Square Fluctuation)** curve, shown in Fig. 5a and Fig. 5b, highlights regions with high flexibility within the protein-ligand complexes. Notably, in both the Ergotamine-RING and Tafluposide-RING complexes, peaks of flexibility are observed in residues 55-60 and 110-120. These regions might play crucial roles in ligand binding or induce conformational changes in the protein during the simulation.

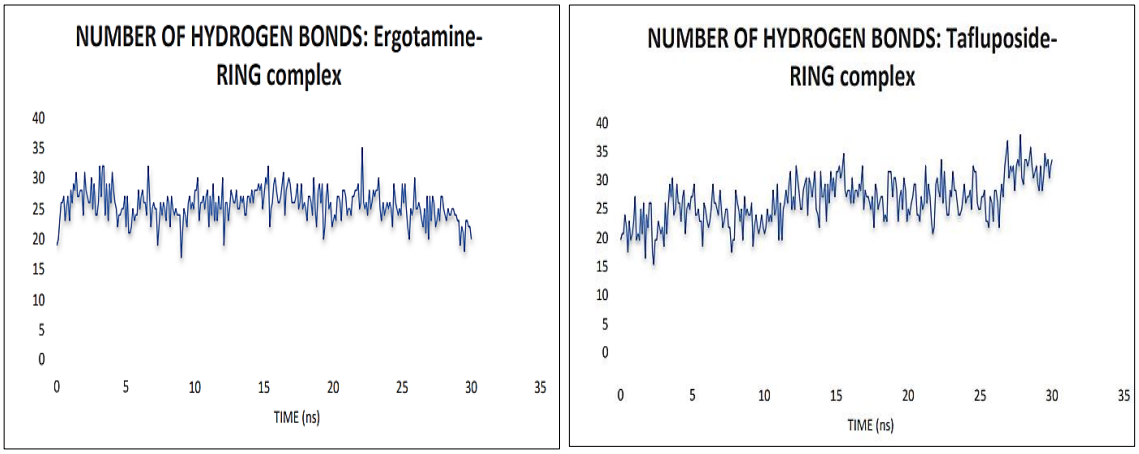

Fig. 6a Hydrogen bonds curve of Ergotamine-RING complex. The number of hydrogen bonds formed in this complex remains relatively constant indicating stable interactions throughout the simulation period. **Fig. 6b Hydrogen bonds curve of Tafluposide-RING complex**. The number of hydrogen bonds formed in this complex remains relatively constant indicating stable interactions throughout the simulation period.

Analyzing the **number of hydrogen bonds** during the MDS, as depicted in Fig. 6a and Fig. 6b, provides insights into the stability of the complexes. Notably, the number of hydrogen bonds does not decrease with time, indicating that both the Tafluposide-RING and Ergotamine-RING complexes maintain stable interactions throughout the simulation.

The radius of gyration (Rg) graph, presented in Fig. 7a and Fig. 7b, sheds light on the compactness of the protein-ligand complexes. In the Ergotamine-RING complex, the Rg value consistently decreases during the simulation, suggesting tight packing and stabilization of the complex. However, in the case of the Tafluposide-RING complex, the Rg value displays both increases and decreases during the MDS, indicating a less compact and potentially dynamically fluctuating complex.

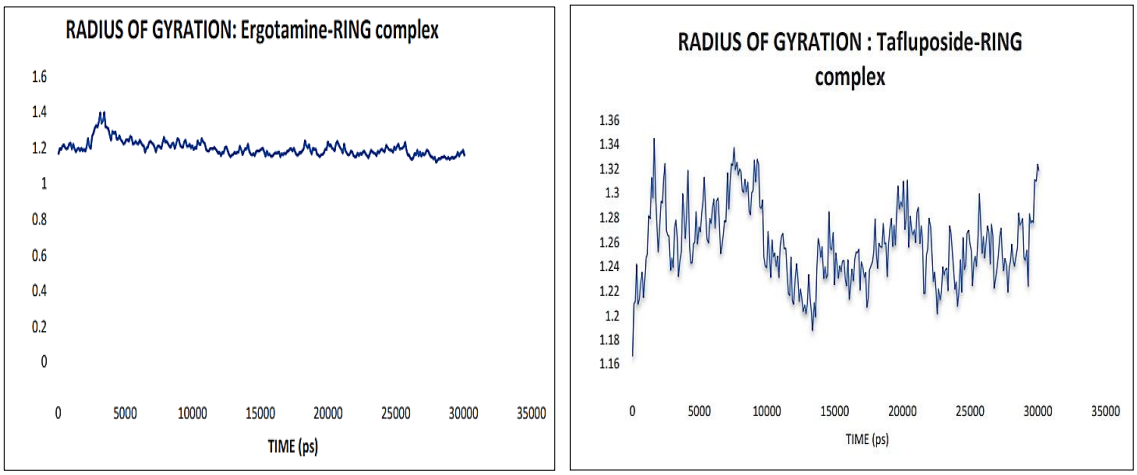

Fig. 7a Rg curve of Ergotamine-RING complex. The complex gets stabilized with time as the value of Rg decreases with time. **Fig. 7b Rg curve of Tafluposide-RING complex.** The value of Rg is fluctuating with time. This indicates the need for further optimization

These fluctuating observations suggest the need for further optimization to obtain more precise results for the Tafluposide-RING complex. In a nutshell, useful insights into the stability and dynamics of the Ergotamine-RING and Tafluposide-RING complexes are provided by the MDS analysis. These findings behave like a stepping stone for future studies related to drug discovery and repurposing

CHAPTER 5

CONCLUSIONS AND DISCUSSION

E3 ligases which share an analogy in both NDDs as well as cancer are vital for discovering the intricate molecular mechanisms underlying these pathological conditions. A detailed summary of E3 ligases converging on specific pathways has unravelled intertwined signalling cascades, contributing to the pathogenesis of NDDs and cancer. The multifaceted contribution of different types of E3 ligases such as CHIP, SIAH1, MDM2-MDMX, BRCA1-BARD1, E6AP, PKN, ITCH, WWP1, WWP2, NEDD4-1, and ITCH in different cellular processes highlights the shared molecular mechanisms of NDDs and cancer. These common pathways and substrates can be targeted, presenting potential therapeutic strategies to address the intricate nature of these seemingly uncommon diseases. Furthermore, VHL Cullin E3 ligase and CRBN are underscored as promising candidates for E3 ligase-assisted therapeutic interventions in both NDDs and cancer. Cutting-edge technologies such as PROTAC, molecular glues, Hydrophobic tagging, SNIPER, and TRIM-away are utilizing the UPS to enable the degradation of pathogenic proteins with higher precision and efficiency. Although AUTAC, PROTAC, and molecular glues have kept their feet in clinical trials for treating various types of malignancies, however, their application remains unexplored in NDDs. This has raised several questions about their ability to serve the intricate molecular interactions underlying NDDs like AD, HD, PD, and ALS across a diverse range of populations and emphasized the need to tailor these technologies specifically for synucleinopathies and tauopathies. New therapeutic avenues in molecular medicine are anticipated if these modern technologies are applied in NDDs. However, some ethical considerations like equitable access to medication, informed consent, and addressing disparities in healthcare must be given utmost priority in research and clinical studies. MARCH2 protein has been reported to get overexpressed in certain cancers such as colorectal cancer and has a potential role in autophagy. This implies that either directly or indirectly, MARCH2 can mediate certain mechanisms underlying NDDs. However, its 3D structure remains unexplored. This study aimed to develop the 3D structure of MARCH2 protein and explore potential compounds from DrugBank and Phytochemica databases that can target the RING domain of MARCH2 protein and block its activity. A successful model of MARCH2 was formed using SWISS-MODEL, and 82.4% of residues lied in the fabourable region, indicating its stability. However, further loop refinement can be done. Docking analysis of many compounds revealed Ergotamine and Tafluposide as the most promising drugs with high binding energies of -9.2kcal/mol and -8.4kcal/mol respectively. To assess the stability and behaviour of the complexes over time, an MDS analysis was performed. The results unravelled that both the Ergotamine-RING and Tafluposide-RING complexes remained consistent and stable throughout the simulation, with fewer fluctuations in their structures. There was a formation of stable interactions as

the number of hydrogen bonds in the complexes did not decrease. The Root Mean Square Fluctuation (RMSF) analysis highlighted specific residues with high flexibility in both complexes, indicating that they potentially play crucial roles in ligand binding or conformational changes. However, the Ergotamine-RING complex showcased consistent decreases in radius of gyration (Rg), while the Tafluposide-RING complex displayed more fluctuations. This indicated that the former complex is comparatively a more compact and stable structure. In brief, the in-silico analysis suggests that Ergotamine and Tafluposide are promising candidates for targeting the RING domain of the MARCH 2 protein but further investigation as potential drug leads is required. Important insights into the ligand-protein complexes' behaviour are provided by the stable interactions observed in the MDS. These findings contribute valuable information to the field of drug discovery, guiding future experimental studies and potential drug optimization efforts for targeting the RING domain of the MARCH 2 protein or similar targets. One limitation of this study is its reliance on computational methods without experimental validation, necessitating future in vitro or in vivo experiments to validate the anticipated ligand-protein associations. Additionally, the repurposing potential of drugs like Ergotamine and Tafluposide, which are currently used for migraines and cancer treatment, respectively, presents an interesting opportunity.

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LIST OF PUBLICATIONS

1) IEEE Conference Paper: A. Kaushik and P. Kumar, "In-Silico Analysis for Differentially Expressed Genes in Multiple Sclerosis: Exploring Promising Biomarkers," 2023 3rd International Conference on Innovative Sustainable Computational Technologies (CISCT), Dehradun, India, 2023, pp. 1-5, doi: 10.1109/CISCT57197.2023.10351378. keywords: {Multiple sclerosis;Medical treatment;Biomarkers;Metabolism;Differentially Expressed Genes (DEGs);Multiple Sclerosis (MS)}

2) IEEE Conference Paper: A. Kaushik and P. Kumar, "In-Silico Structural Analysis of Membrane-Associated RING-CH Type 2 (MARCH2) Protein," 2023 International Conference on Integration of Computational Intelligent System (ICICIS), Pune, India, 2023, pp. 1-6, doi: 10.1109/ICICIS56802.2023.10430285. keywords: {Enzymes;Computational modeling;Control systems;Stability analysis;Intelligent systems;Cancer;Immune system;Membrane-Associated RING-CH type 2 (MARCH2); Really Interesting New Gene (RING);Molecular Dynamic Simulation (MDS)}

3) Review Article: Aastha Kaushik, Somya Parashar, Rashmi K Ambasta, Pravir Kumar, Ubiquitin E3 ligases assisted technologies in protein degradation: Sharing Pathways in Neurodegenerative Disorders and Cancer, Ageing Research Reviews, 2024, 102279, ISSN 1568-1637, https://doi.org/10.1016/j.arr.2024.102279**.** **(**https://www.sciencedirect.com/science/article/pii/S1568163724000977**) (IF:13.1)**

4) Book Chapter: Neetu Rani, **Aastha Kaushik**, Shefali Kardam, Sonika Kag, V. Samuel Raj, Rashmi K. Ambasta, Pravir Kumar, Reimagining old drugs with new tricks: Mechanisms, strategies and notable success stories in drug repurposing for neurological diseases, Progress in Molecular Biology and Translational Science, Academic Press, 2024,ISSN 1877-1173, https://doi.org/10.1016/bs.pmbts.2024.03.029

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Review article

Ubiquitin E3 ligases assisted technologies in protein degradation: Sharing pathways in neurodegenerative disorders and cancer

Aastha Kaushik [a,1], Somya Parashar [a,1], Rashmi K. Ambasta [b], Pravir Kumar [a,*,2,3]

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Abbreviations: AR, Androgen Receptor; ARIH1, Ariadne-1 homolog; ABCB1, ATP-binding cassette sub-family B member 1; AUTAC, Autophagy Targeting Chimera; APP, Aβ precursor protein; BMI1, B lymphoma Mo-MLV insertion region 1 homolog; BIRC7, Baculoviral IAP Repeat-Containing protein 7; BCL-XL, B-cell lymphoma-extra-large; BAG5, Bcl-2-associated athanogene 5; BECN1, Beclin-1; β-TrCP, Beta-Transducin repeats-Containing Proteins; BRI3, Brain Protein I3; BARD1, BRCA1-Associated RING Domain protein 1; BRD4, Bromodomain-containing protein 4; Cdh1, Cadherin-1; CREB, cAMP Response Element-Binding protein; CDC20, Cell Division Cycle 20; CPP, Cell Penetrating Peptide; CIAP, cellular Inhibitor of Apoptosis; CRBN, Cereblon; CDT1, Chromatin licensing and DNA replication factor 1; CLL, Chronic lymphocytic leukemia; CMML, Chronic Myelomonocytic Leukaemia; CHIP, C-terminus of Hsc70 Interacting Protein; Cul1, Cullin 1; CRL, Cullin-RING Ubiquitin E3 Ligase; CNrasGEF, Cyclic Nucleotide ras Guanine-nucleotide-Exchange Factor; CCNF, Cyclin F; CDK, Cyclin-dependent Kinase; DAXX, Death domain-associated protein; DUBs, Deubiquitinating enzymes; DLBCL, Diffuse large B cell lymphoma; DDR, DNA-damage response; E6-AP, E6-associated protein; EMI1, Early Mitotic Inhibitor 1; 4EHP, eIF4E-Homologous Protein; EBPβ, Enhancer-Binding Protein β; ErbB4, Erb-B2 Receptor Tyrosine Kinase 4; EBV, Epstein Barr Virus; ER, Estrogen Receptor; ERα,

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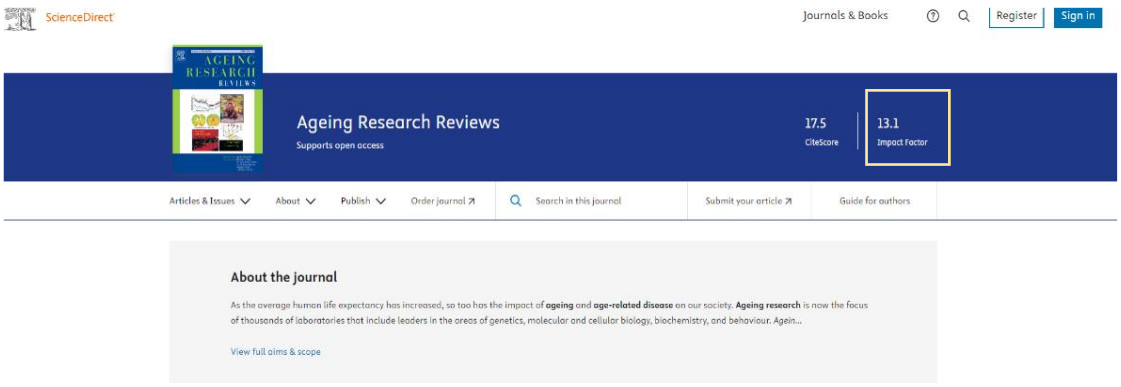

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Reimagining old drugs with new tricks: Mechanisms, strategies and notable success stories in drug repurposing for neurological diseases

Neetu Rani[a], Aastha Kaushik[a], Shefali Kardam[a], Sonika Kag[a], V. Samuel Raj[b], Rashmi K. Ambasta[b], and Pravir Kumar[a,*]

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of Delhi Technological University has Participate as *Delegate* in the Two Days National Symposium on *"Recent Advances in Neurochemistry and Neurosciences"* held from 25th April 2024 to 26th April 2024 at Convention Centre, Jamia Hamdard, New Delhi. He/She has also presented in Young Investigator/Poster Session.

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ANNEXURE-IV

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In silico exploration of MARCH2 protein: A shared E3 ligase in neurodegenerative diseases and cancer.

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CGPA: 9.06

Delhi Technological University, Shahbad Daulatpur, Delhi
— *M.Tech (Bioinformatics)*
Session: 2022-2024

Achievements

- Lifetime Member of SNCI
- GATE (BT-2024): 98.3 percentile
- GATE(XL-2024): 97.3 percentile
- GATE(BT-2022): 97.63 percentile
- GATE(XL-2022):96.84 percentile
- IIT-JAM (Biotechnology-2022)

Project Details

- *Carried out an experiment on "**Preparation of Culture Medium and Streaking of Bacterial Culture**" during Senior Secondary School Project under the supervision of Dr. Rekha Kansal, Principal Scientist, NRCPB, Delhi*
- *Training at CSIR-IGIB on "**Techniques Used In Preparation And Characterization of Nanostructures**" under the supervision of Dr. Pradeep Kumar, Senior Principal Scientist, IGIB, Delhi.*
- *Observational training at PATH LAB- microbiology, hematology and serology were learnt.*
- B.Tech project on ***Structure and expression analysis of Membrane-Associated RING-CH type 8 (MARCH8) protein'*** *under the supervision of Dr. Rinu Sharma, Assistant Professor, GGSIPU, New Delhi*
- Online Training on ***Genomic Variance Analysis and Clinical Interpretation (GVACI)*** offered by Dr. Vinod Scaria at IGIB, South Campus

Publications

- Review Article: Aastha Kaushik, Somya Parashar, Rashmi K Ambasta, Pravir Kumar, Ubiquitin E3 ligases assisted technologies in protein degradation: Sharing Pathways in Neurodegenerative Disorders and Cancer, Ageing Research Reviews, 2024, 102279, ISSN 1568-1637, https://doi.org/10.1016/j.arr.2024.102279. (https://www.sciencedirect.com/science/article/pii/S1568163724000977) (IF:13.1)
- IEEE Conference Paper: A. Kaushik and P. Kumar, "In-Silico Structural Analysis of Membrane-Associated RING-CH Type 2 (MARCH 2) Protein," 2023 International Conference on Integration of Computational Intelligent System (ICICIS), Pune, India, 2023, pp. 1-6, doi: 10.1109/ICICIS56802.2023.10430285.
- IEEE Conference Paper: A. Kaushik and P. Kumar, "In-Silico Analysis for Differentially Expressed Genes in Multiple Sclerosis: Exploring Promising Biomarkers," 2023 3rd International Conference on Innovative Sustainable Computational Technologies (CISCT), Dehradun, India, 2023, pp. 1-5, doi: 10.1109/CISCT57197.2023.10351378.
- 4) Book Chapter (Releasing Date: 1 June, 2024): "Reimagining Old Drugs with New Tricks: Mechanisms, Strategies and Notable Success Stories in Drug repurposing for Neurological diseases" in Progress in Molecular Biology and Translational Science (IF: 3.6)

Conferences and Poster Presentations

- International Conference on Integration of Computational Intelligent System (ICICIS), Pune, India, 2023
- 3rd International Conference on Innovative Sustainable Computational Technologies (CISCT), Dehradun, India, 2023
- Poster Presentation at INCD organized by the University of Delhi, 2022.
- Poster Presentation at IMMUNOCON organized at AIIMS, Delhi, 2023.
- Poster Presentation at 3rd International Conference on "Antimicrobial Resistance, Novel Drug Discovery and Vaccine Development: Challenges and Opportunities", 2024
- Poster Presentation in Symposium on "Recent Advances in Neurochemistry an Neurosciences" organized at Jamia Hamdard, 2024

Online courses

- Introduction to breast cancer course, Coursera
- Next generation sequencing, Udemy
- Learn Bioinformatics from Scratch (Theory and Practical), Udemy
- BSL-3 Laboratory Working practices and *Mycobacterium tuberculosis* handling protocols, University of Delhi, South Campus
- Industrial Biotechnology, BioTecNika
- Bioinformatics: Learn Docking & Molecular Dynamic Simulation, Udemy

Technical Knowledge

- Dynamic Light Scattering, Spectrophotometry, Lyophilization, Sonication, Centrifugation, Pipetting skills, Light microscope, Rotary Evaporator, Agarose Gel Electrophoresis, SDS-PAGE, Western blotting, Protein expression at the pilot scale, In-silico protein modelling, Molecular docking, Molecular Dynamic Simulation, Bacterial transformation and cloning