THE P53/SOX2 AXIS IN MODULATING THE CELL FATE IN NEURONS: A PARADIGM OF NEURODEGENERATION AND BRAIN TUMORS

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HARSH VARDHAN

23/MSCBIO/21

Under the supervision of

PROF. PRAVIR KUMAR

Professor and Dean of International Affairs

Department of Biotechnology

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, New Delhi, 110042

May, 2025

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, New Delhi, 110042

DECLARATION

I, Harsh Vardhan 23/MSCBIO/21 hereby certify that the work which is being presented in the thesis entitled **"The p53/SOX2 axis in modulating the cell fate in neurons: A paradigm of neurodegeneration and brain tumors"** in partial fulfillment of the requirements for the award of the Degree of Master of Science, submitted in the Department of Biotechnology, Delhi Technological University is an authentic record of my own work carried out during the period from 2023 to 2025 under the supervision of Prof. Pravir Kumar.

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DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, New Delhi, 110042

SUPERVISOR'S CERTIFICATE

Certified that Harsh Vardhan (23/MSCBIO/21) has carried out their search work presented in this thesis entitled "**The p53/SOX2 axis in modulating the cell fate in neurons: A paradigm of neurodegeneration and brain tumors"** for the award of Master of Science from Department of Biotechnology, Delhi Technological University, Delhi, under my supervision. The thesis embodies results of original work, and studies are carried out by the student himself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

Date:

Signature

Prof. Pravir Kumar

Professor and Dean IA

Department of Biotechnology

Delhi Technological University

Signature

Prof. Yasha Hasija

Head of the Department

Department of Biotechnology

Delhi Technological University

"THE P53/SOX2 AXIS IN MODULATING THE CELL FATE IN NEURONS: A PARADIGM OF NEURODEGENERATION AND BRAIN TUMORS"

HARSH VARDHAN

(23/MSCBIO/21)

ABSTRACT

Tumor suppressor p53 is a transcription factor associated with apoptosis or programmed cell death as it activates several caspases and downstream apoptotic signaling cascades, leading to cell death. Apoptosis is crucial for maintaining different cellular and biological processes within the organism, like genome stability and integrity, and cell cycle regulation. Apoptosis plays a major role in the progression of neurodegenerative diseases (NDDs) like Parkinson's disease (PD), where substantial loss of neuronal cells, which secrete Dopamine, takes place, mediated by upregulated expression of p53 protein. p53 also interacts with the gene promoter of Bax, a Bcl2 family pro-apoptotic protein, directly upregulating its expression in the cell. Upregulation of Bax has many significant consequences within a cell, such as mitochondrial membrane disruption and excessive release of cytochrome-c from the mitochondria, which triggers a caspase-dependent apoptotic pathway. Interestingly, the Bax/Bak axis can also function in a p53-independent manner in response to TNF-α to activate apoptosis. Thus, exploring the relationship between p53 and Bax is crucial for marking the progression of apoptosis in neurodegenerative diseases like Parkinson's disease. In this study, we have focused on the effect of such mutations on the structural configuration of p53 and its relationship with MDM2 and p53-Bax-mediated mitochondrial dysfunction which contribute to apoptosis and neuron death. We have targeted the missense single-nucleotide polymorphic (msSNPs) variants of p53, obtained from NCBI and UniProtKB, which have not been extensively studied. Structural stability and evolutionary studies, using tools like I-Mutant and ConSurf, identified eight SNPs as the most conserved, which were further utilized in this study. In MutPred2, PANTHER. and SNP&GO, all eight msSNPs were found to be deleterious according to their structural implications on the protein, suggesting that apoptosis in cells with these p53 mutations might be altered. To confirm these hypotheses, we conducted docking studies of these p53 variants with the MDM2 (inhibitor of p53) and Bax gene promoter, which, interestingly, showed low binding affinities with the former and high binding affinities with the latter as compared to the WT-p53, suggesting an increase in mitochondrial stress and dysfunction, which activates a series of apoptotic events leading to death of the cell. On the other hand, SOX2 is reported to be involved in several signaling pathways such as *EGFR/MAPK/P13K-mTOR-AKT signaling pathway, SHH pathway, HIPPO signaling pathway, Wnt/β-catenin signaling pathway* which plays a crucial role in the maintenance of cancer stem cell-like properties, tumor aggression, poor prognosis, drug resistance, invasion and migration in several brain tumors including Glioblastoma. Furthermore, recent studies suggest that p53 directly upregulate the gene expression of SOX2 in certain conditions. Additionally, there function is involved and overlap in the AKT signaling which suggest that these interplay between these proteins is crucial and can play an important role in determining the fate of the neuronal cells in diseased conditions, whether they take the path of programmed cell death and contribute to neurodegeneration or proliferation indefinitely to form brain tumors. Further in vivo and in vitro studies are required to validate these hypotheses and

provide new insights into drug targeting that disrupts this p53/SOX2 axis and potential therapeutic strategies for treating neurodegenerative diseases and brain tumors

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Harsh Vardhan

23/MSCBIO/21

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ABBREVIATIONS

Bax	Bcl-2-associated X protein
Bak	Bcl-2-homologous antagonist/killer
NDD	Neurodegenerative diseases
PD	Parkinson's disease
AD	Alzheimer's disease
MDM2	Murine double minute 2
MOMP	Mitochondrial outer membrane permeabilization
PRD	Proline rich domain
LRRK2	Leucine rich repeat kinase 2
PRKN	Parkin
SNCA	Synuclein alpha
GBA	Glucocerebrosidase
SOX2	Sex determining region Y box 2
GBM	Glioblastoma
HMG	High mobility group
DIM	DIM: Dimerization domain
TAD	Transactivation domain
GSC	Glioma stem cells
ESA	Erythropoiesis stimulating agents
CD	Cluster of differentiation
ALDH1	Aldehyde dehydrogenase 1
GFP	Green fluorescent protein
BMP4	Bone morphogenetic protein 4
CKD	Conditional knockdown
TUSC3	Tumor suppressor candidate 3
miRNA/miR	Micro-RNA
EMT	Epithelial-Mesenchymal transition
TIF1γ	Transcriptional intermediary factor 1- γ
ZO-1	Zonula occludens-1
ESCC	Esophageal squamous cell carcinoma
STAT3/HIF-1α	Signal transducer and activator of transcription 3/Hypoxia-inducible factor-1α
MGMT	O6-Methylguanine-DNA Methyltransferase
Bcl	B-cell lymphoma
Bcl-XL	B-cell lymphoma extra-large
IAP	Inhibitor of apoptosis
TNF-α	Tumor necrosis factor- α
Nf-kB	Nuclear factor kappa B
TMZ	Temozolomide
IDH-4	Isocitrate dehydrogenase 4
FOXM1	Forkhead box M1
WT	Wild type
Ki67/MIB	Antigen Kiel 67/ Mycoplasma immunoglobulin binding protein
NESTIN	Neuroepithelial stem cell protein
POU3F2	POU class 3 homeobox 2
OLIG2	Oligodendrocyte transcription factor 2
SALL2	Spalt-like transcription factor 2
FACT	Facilitates chromatin transcription
HMGA1	High mobility group A1 protein
TRIM	Tripartite motif

WWP2	WW domain containing E3 ubiquitin protein ligase 2
TIF	Transcription initiation factor
TET2	Tet-methylcytosine dioxygenase 2
5mC	5-methylcytosine
5hmC	5-hydroxymethylcytosine
SRR	SOX2 regulatory regions
CRISPR	Clustered regularly interspaced short palindromic repeats
RNAi	RNA interference
FOXG1	Forkhead box G1
PDGF	Platelet derived growth factor
IGF-1	Insulin-like growth factor 1
HLA	Human leukocyte antigen
CTL	Cytotoxic T-lymphocyte
ABCC	ATP-binding cassette superfamily C
MMPs	Matrix metalloproteinases
VEFG	Vascular endothelial growth factor
MAPK	Mitogen activated protein kinase
MEK	Mitogen activated protein kinase kinase
GSK3β	Glycogen synthase kinase 3 beta
WWC1	WW and C2 domain containing 1
NF2	Neurofibromatosis type 2 protein
NLS	Nuclear localization signal
E2F3	E2F transcription factor 3
MALAT1	Metastasis-associated lung adenocarcinoma transcript-1
UTR	Untranslated region
BCSC	Breast cancer stem cell
CDK1/2	Cyclin-dependent kinase 1/2
AKT1 or PKB	AK mouse plus thymoma or Protein kinase B
EGFR	Epidermal growth factor receptor
ERK1/2	Extracellular signal-regulated kinase 1/2
HKMT	Histone methyltransferase
SET7	Su(var)-3-9, Enhancer of Zeste, Trithorax containing domain protein 7
OGT	O-GlcNAc transferase
UBE2S	Ubiquitin conjugating enzyme E2S
p300/CBP	p300/CREB binding protein
PARP1	Poly(ADP-ribose) polymerase 1
ESC	Embryonic stem cells
OCT4	Octamer binding transcription factor 4
SOX1	Sex determining region Y box 1
BEX-1	Brain expressed X-linked 1
SHH	Sonic hedgehog
IGFBP3	Insulin-like growth factor binding protein 3
ETS1	E26 transformation-specific or Erythroblast transformation specific protein 1
SOX18	Sex determining region Y box 18
BMPR1B	Bone morphogenetic protein receptor type 1B
RUNX1	Runt-related transcription factor 1
CDC20	Cell division cycle 20
FGF13	Fibroblast growth factor 13
UTF1	Undifferentiated embryonic cell transcription factor 1
TGF-β	Transforming growth factor-β
mTOR	Mechanistic target of Rapamycin

1. INTRODUCTION

Apoptosis or programmed cell death is a process in which a cell undergoes a series of events ultimately leading to its death. It is an essential phenomenon to regulate several cellular and biological processes like disease control, growth, cell turnover [1] Apoptosis is a complex process which operates with intertwined mechanisms involving cohesive interactions between a variety of signals and protein molecules like p53 and is vastly studied in cancer [2]. Human tumor suppressor p53 is a DNA binding transcription factor, which in its biologically active state is a homotetramer of 393 amino acids each. It recognizes a particular DNA sequence in case of double stranded DNA breaks and mediates either DNA repair or cell apoptosis in case of extreme severity [3] and leads to activation of effector caspases and stimulation of many downstream signaling cascades leading to recruitment of apoptosis promoting proteins like PUMA, BAX, Apaf- which leads to death of the target cell [4]. It plays a pivotal role in maintaining genome stability, regulating cell cycle, repairing double stranded DNA breaks and apoptosis under stress induced conditions. According to oncology studies p53 is one the most mutated proteins found in cancers. p53 is also observed to be upregulated in the case of neurodegenerative diseases (NDDs) like Parkinson's disease (PD). This upregulated expression of p53 can be linked to increased apoptosis rate in PD patients [5]. PD is one of the most widespread age dependent NDD which is marked by rapid pars compacta region of substantia nigra (SNpc) and can be caused by mutations in genes like LRRK2, PRKN, SNCA, GBA and protein aggregation due to protein misfolding [6]. It affects about 0.1-0.2% of the population worldwide and includes motor symptoms like resting tremors, depression, cognitive impairment, dementia and Bradykinesia [7]. Although many studies have been carried out to study p53 and its role in apoptosis, very less is known about the variants of p53 with uncertain clinical significance. Therefore, in this paper, we have tried to predict and characterize the msSNPs with the most deleterious missense mutations which occur in the DNA-binding domain of the protein p53 [8] and their probable consequences in NDDs. Among these variants using different in silico analytical tools. In total, eight variants were identified to be the most deleterious and an overall decrease in structural stability of these variants was observed. Protein-protein docking studies showed that these variants exhibit increased affinity to E3 ubiquitin ligase MDM2 which ubiquitinates p53 leading to its degradation [9] which suggests a decrease in apoptosis in the target cells. This shows that eight msSNPs namely P250S, P250T, T230P, Q167P, G154R, P98H, P98R and V97D are highly deleterious with reference to activity of p53 as a transcriptional factor and mediator of apoptosis, further studies on these variants may provide better therapeutic strategies for early diagnosis and prognosis of neurodegenerative diseases like PD and AD which are shown to have upregulated expression of p53 protein leading to neuronal cell cardiac dysfunction [10]. As p53 is a well-studied protein and is known to be responsible for activating many downstream cascades for cell apoptosis [11] which is a key characteristic of NDDs like PD and AD with this study we aimed to characterize such mutations in the p53 protein which can affect its structure and functionality and then theoretically hypothesized their possible effect on the activity of p53 which may shed light on potential therapeutic strategies that can be utilized to better treat diseases like NDDs and cancer.

Sex-determining region Y-box 2 (SOX2) which is a transcription factor and is reported to be upregulated in the nuclei of many human cancer sample cells. SOX2 is a 317 amino acid long protein that belongs to the Sox family and has three characteristic domains i.e. High Mobility

Group (HMG) which is conserved throughout the Sox family; a central dimerization domain (DIM); and a terminal transactivation domain (TAD) with which SOX2 can interact with several target genes and modulate cell physiological processes [12]. In GBMs, SOX2 is reported to be involved in several signaling pathways such as EGFR/MAPK/P13K-mTOR-AKT signaling pathway, SHH pathway, HIPPO signaling pathway, Wnt/β-catenin signaling pathway which plays a crucial role in the maintenance of cancer stem cell-like properties, tumor aggression, poor prognosis, drug resistance, invasion and migration in cancer stem cells (CSC) [12]. Therefore, we studied the different aspects of SOX2 expression and its consequences in Glioblastoma. Glioblastomas (GBM) are one of the most common forms of aggressive brain tumors affecting less than 10 individuals in a paediatric population of 1,00,000 with a survival rate of less than 5%. GBMs are characterized by their aggressive nature and resistance to chemotherapy and radiotherapy. The reason behind such poor prognosis in the case of GBMs is their extreme heterogeneity, abundant vascularization, rapid clinical evolution, and infiltrative growth. Till now several studies have shown the presence of biomarkers that can be targeted for combating Gliomas more effectively. Although SOX2 is a well-characterized transcription factor its functionality in modulating different physiological processes has been extensively studied even in GBMs along with other cancer types. Despite being a key regulator and a crucial link in GBM progression there is still more research required in the area of GBM therapeutics targeting SOX2. We have found only one profound mini-review article published in Frontiers in Oncology (2016) addressing the functional overview of SOX2 in the progression of GBM. SOX2 is very well explored in cancer stem cells (CSCs) being a part of the Yamanaka factor along with OCT4, Klf4, and c-Myc and its role in inducing pluripotency in adult stem cells but it is underexplored in terms of its role and therapeutic possibility in GSC specifically. Furthermore, although preclinical studies have demonstrated SOX2 as a potent therapeutic target for GBM progression, there are negligible clinical trials that target SOX2 in GBM therapeutics. Therapeutic strategies that are currently effective against GBM rely exclusively on chemoradiotherapy and drug-mediated inhibition of regulatory pathways but due to extreme heterogeneity and rapid clinical evolution of GBMs, resistance towards currently used drugs and chemoradiotherapy identifying specific inhibitors for downregulating the expression of transcription factors like SOX2 in such tumors can be of great interest and significance for future research there are several areas which we have identified that can be a topic of exploration.

Through this study we aimed to; (1) Characterize the clinically non-significant msSNPs of p53; (2) Analyze the structural and functional effect of the mutations on p53 protein; (3) Its binding to MDM2 and Bax gene as well as Bax protein to decipher its consequence on the MOMP and mitochondrial dysfunction; (4) Role of SOX2 in the progression of Glioblastoma brain cancer, and (5) Understanding the p53/SOX2 crosstalk in regulating cell fate.

OBJECTIVES:

(1) Characterization of p53 msSNPs and understanding its interaction with MDM2
(2) Elucidating the interaction of mutant p53 proteins with Bax gene promoter and protein to understand their effect on mitochondrial dysfunction-mediated apoptosis
(3) Understanding the relationship between p53 and SOX2 in regulating cell fate and exploring the potential effects of these mutations on p53/SOX2 axis.

2. LITERATURE REVIEW

2.1. Transcription factor p53

p53 is characterized as a transcription factor due to its structural similarity and domain composition similar to transcriptional activators. It comprises of two transcriptional activator domains (TADs) i.e., TAD1 and TAD2, at its N-terminus, which are 1-40 and 40-60 amino acid long respectively **[1]**[13]. These domains are important for recruiting modifying enzymes like histone-modifying enzymes, co-activator complexes like STAGA and Mediator, and the transcriptional machinery **[2]**[14]. On the other hand, the C-terminus domain of p53 comprises of a lysine rich region spanning from 363-393 amino acid residues which is important for stabilizing the binding of p53 tetramer to its DNA response element. In between the N and C-terminus lies a number of various domains which are responsible for different functions like the region which present between the C-terminus and the transactivation domains consists of a 60-95 amino acids residue which is a proline-rich region (PRD), and is considered important for protein-protein interaction and its function as a tumor-suppressor. The central domain of p53 is consist of 100-300 residues and is responsible of DNA binding of the protein in a sequence specific manner to the p53 response element (p53RE) in the DNA. The residues which are commonly altered in cancer cells and affect the DNA binding property of p53 are R175, G245, R248, R249, R273, and R282 **[3]**[15]. p53 also contains a tetramerization (Tet) domain between 325-356 amino acids residues which is crucial for tetramerization of p53 because p53 binds to its DNA response element as a tetramer. p53 is a widely studied protein and has many functions in maintaining cellular integrity like, apoptosis, autophagy, tumor suppression, and cell cycle arrest. p53 can induce apoptosis under conditions of stress and prolonged DNA damage. It can also cause permeabilization of the mitochondrial outer membrane (MOMP) leading to mitochondrial dysfunction mediated apoptosis under stress conditions like ROS formation. p53 also interacts with the gene promoter of Bax, a Bcl2 family pro-apoptotic protein, directly upregulating its expression in the cell. Upregulation of Bax has many significant consequences within a cell, such as mitochondrial membrane disruption and excessive release of cytochrome-c from the mitochondria, which triggers a caspase-dependent apoptotic pathway. Interestingly, the Bax/Bak axis can also function in a p53-independent manner in response to TNF-α to activate apoptosis **[1]**[13]. p53 is also involved in the process of autophagy, a cellular process which involves intracellular degradation by recruitment of lysosomal machinery. *(Fig. I depicts the various function performed by p53 in the maintenance of cellular integrity)*

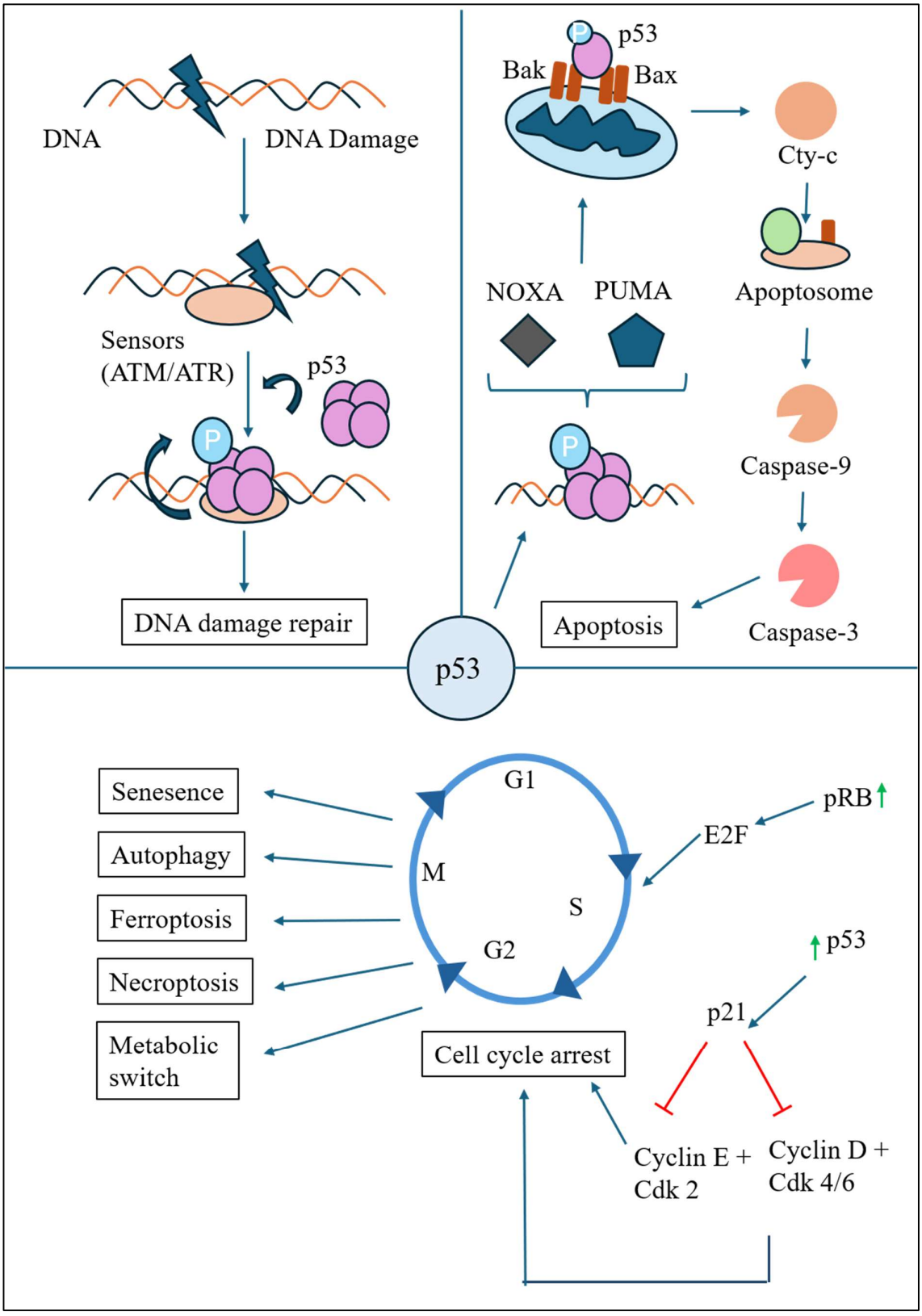


Fig I. Cellular functions of p53: (1) DNA damage repair: Under normal conditions p53 exist in its unstable form bound to MDM2 which frequently leads to its degradation via proteasomal machinery, however, under the conditions of oxidative stress or DNA damage due to any factor like UV radiation,

mutations, chemical and radioactivity exposure p53 dissociates from MDM2. During DNA damage some sensor protein like ATM/ATX sense the site of DNA damage and recruit the unbound p53 and activate it by phosphorylation. P53 then initiates the process of DNA repair and cause cell cycle to arrest at a particular stage (this mainly happens during the S phase of cell cycle where DNA starts replicating and is vulnerable to damage), if the damage is not repairable then it signals the cells for programmed cell death. (2) Apoptosis: NOXA and PUMA are the direct transcriptional targets of p53, and both antagonize the inhibitory effects of Bcl-2 family proteins on Bax/Bak leading to their activation. Activation of Bax causes MOMP and release of Cytochrome-c (Cyt-c) which acts a trigger and initiates the formation of apoptosome which is comprised of Cyt-c, APAF1, and pro-caspase 9 (inactive caspase-9). Apoptosome and addition of pro-caspase 3 leads to their conversion to their active forms i.e. caspase 9 which activates caspase 3 and ultimately leads to apoptosis; and (3) Cell cycle arrest: During the S-phase of cell cycle when DNA start replicating, prior to the mitotic phase, it unwinds from the histones and thus is prone to DNA damage due to many factors like chemical exposure, polymerase slippage, UV exposure etc. To halt the cell cycle until the damage is repaired is regulated by p53 and other proteins. Sensor proteins such as ATM/ATX identify the site of damage and recruit unbound tetrameric p53 on the site where it gets phosphorylated and initiate DNA damage repair. If cell cycle arrest persists of longer period of time it can lead to activation of apoptosis, senescence, necroptosis, ferrotopsis autophagy and metabolic switch.

2.2. P53 and Central Nervous System: Neuroprotection and Dysfunction

2.2.1. Dysregulation of p53 in Neurodegenerative Diseases

Neurodegeneration refers to the excessive loss of neuronal cells in different parts of the brain. There can be various metabolic and physiological pathways involved in the progression of such diseases which commonly arise due to mutation in the genes which are important for maintaining the normal functioning of the brain cells. Some diseases are age-related also like Parkinson's disease and Alzheimer's disease, whose pathogenesis is being studied extensively but need more research as many aspects of the disease progression remains unknown. Alzheimer's disease or AD is the most frequent form of neurodegeneration worldwide, characterized by its phenotypic symptoms like excessive memory loss, cognitive decline, difficulty in recognition, and judgement [16]. Accumulation of Amyloid-β plague and tangles of hypermethylated Tau are the hallmarks of the disease and serves as therapeutic targets. Early onset of AD, between age 30-65, is usually considered genetic whereas late onset, around age >65, is more consequential. Interestingly. High levels of p53 are reported in cases of AD, which is often associated with mitochondrial dysfunction. When AD brain was treated with Aβ, a decrease in the Bcl2 was observed with subsequent increase in the expression of Bax, suggesting a correlation between elevated p53 levels and increased apoptosis [17]. This happens due to the intervention of p53 associated miR-34a, which marks the Bcl2 for degradation. Incorporating use of anti-miR-34a drugs can help alleviate this condition by translocating the Bax protein into cytoplasm from mitochondria. Moreover, beside directly interacting with proapoptotic genes like Bax/Bak and contributing to the mitochondrial membrane permeabilization, p53 can also interact with Drp1 (Dynamin related protein 1) to cause fragmentation of mitochondrial membrane which lead to release of cytochrome c and caspase activation leading to apoptosis. p53 is also important for many cellular phenomena like, neurite outgrowth and regeneration of axon [18]. This process utilizes the acetylation of Lys-320 by CBP/p300. p53 also inhibits glycolysis which is crucial for stunting the growth of tumor cells [19]. Apart from affecting the rate of degeneration in Aβ1-42 AD brains, p53 also regulate the cell death in AD microglial cells. Studies also suggest that p53 is either misfolded or mutated in case of AD.

Another neurodegenerative disease which is age related and the second most common disease is the Parkinson's disease (PD), named after the English physician James Parkinson who firstly described it in 1817 in his famous writing, 'An essay on the shaking palsy'. The name 'shaking palsy', comes from its complex phenotypes which include tremors, bradykinesia, dementia, and cognitive impairment. In PD there is an excessive loss of dopaminergic neuron (dopamine secreting neurons) specifically which are present in the substantia nigra pars compacta (SNpc) region of the midbrain. It is more prevalent in males as compared to females, and is characterized in two types, familial PD which is the genetic form of PD and sporadic PD which manifests as later stages of life and is mainly influenced by the environment. Worldwide sporadic PD is more widespread and affect large portion of the population as compared to familial PD. PD is a result of very complex interplay between many different pathways and gene regulation but some of the key hallmarks of PD are the aggregation of a protein alpha synuclein or synuclein in the brain due to misfolding, mitochondrial dysfunction, and dysregulation of dopaminergic receptors, however, its manifestation and factors causing its onset are still under investigation but not fully understood which leads to defects in the dopamine levels and circuit of the basal ganglia [20]. Similarly, as in case of AD, the expression of p53 is highly upregulated in PD also, suggesting its role in the apoptosis od the dopaminergic neurons in the SNpc. Studies have reported that in PD, the genes which normally suppresses the activity of p53 are dysregulated like Parkin which binds to the promoter of p53 and serves as a repressor, and DJ-1 which inhibit the expression of both p53 and Bax is mutated in PD [21][22]. On the other hand, proteins like Syphilin-1, which interacts with alpha-synuclein, are also reported to inhibit the transcription of caspase-3 by repressing the transcriptional activity of p53 [23].

Huntington's disease (HD) which is also a neurodegenerative disease, caused by mutation in the HTT gene (Huntington gene) at chromosome 4 and long repeated sequences of a trinucleotide i.e. -CAG- (Cytosine, Adenine, and Guanine), normal wt-HTT contains around <35 of such repeats which becomes very high in copy number in case of HD and affects around 5-10 people in a population of 1,00,000, worldwide [24]. HD can genetically pass through from parents to progeny, whose onset is reported to be around middle age. Characteristic phenotypes of HD are behavioral dysregulation, psychiatric disturbances, and excessive choreatic movements along with dementia. First of all, like AD and PD, p53 levels are upregulated in case of HD also. Mutant HTT (mHTT) plays a role in inhibiting the activity of mitochondria which is further complemented by the overexpression of p53 as studies have suggested that p53 directly upregulate the expression of mHTT in the inclusion bodies of HD brains [25].

2.2.2. Role of p53 in Neuroprotection

In *D. melanogaster* models of Alzheimer's disease (aggregated tau protein in CNS), it has been shown that p53 plays a neuroprotective role by regulating the transcription of certain synaptic genes such as BIN1 and PICLAM which are important for maintaining synaptic integrity, synaptic vesicle exocytosis, and plasticity **[1]**[26]. The isoforms of p53 i.e., 133p53 and p53β, are reported to be involved in astrocyte mediated neurotoxicity in neurons. In AD brains, 133p53 is downregulated while p53β is upregulated contributing the pathogenesis of the disease. However, restoration of 133p53 isoform in neurotoxic or near senescent astrocytes leads to induction of neurotrophic growth factors and suppression of senescence-associated

secretory phenotype (SASP) which contribute to astrocyte mediated neuroprotection **[2]**[27]. Previous studies have also demonstrated that p53 is involved in acupuncture mediated therapy in mitigating the phenotypic motor symptoms of PD **[3]**[28]. In AD, p53 plays an important role in reducing the oxidative stress by activating certain anti-oxidant factors like MnSOD and TIGAR **[4]**[29]. In ischemic brains (low blood flow), knockdown or suppression of p53 has been suggested to be neuroprotective in nature. LncRNA-N1LR inhibits the Ser-15 phosphorylation of p53 in such brains and thus prevent its overexpression and ultimately undesired apoptosis. In subarachnoid hemorrhage (SCH) cases, when p53 levels were downregulated by using pifithrin-α, a drug which inhibit p53, an overall increase in the IL-6, and decrease in the levels of miR-22 and Bax were observed suggesting a neuroprotective effect of the p53/miR-22 axis in preventing apoptosis in SCH cases **[5]**[30]. Another drug known as pifithrin-μ, disrupts the sub cellular localization of p53 to the mitochondria which has been shown to have a greater impact on the progression inhibition of the cerebral ischemia. In recent times, the subcellular localization of p53 has been extensively targeted to develop therapeutic drugs for decreasing the rate of apoptosis.

2.3. SOX2

The SRY-box 2 (SOX2) gene is present on the 3p263-q27 chromosome and codes for a 317 amino acid protein which is essentially a well-characterized pluripotency-associated transcription factor. [12]. SOX2 belongs to an embryonically expressed SOX gene family [2][31]. The protein is known to interact with various promoters of genes as a transcription factor thus activating or repressing their expression. It mainly has three domains; an N-terminal domain which comprises of high mobility group (HMG); a central domain which is a dimerization domain (DIM); and a C-terminal transactivation domain (TAD) [1][12]. The homology between the HMG domain of SOX gene family members is what accounts for their relatedness and is conserved among mammals. [2][31]. The protein binds to the DNA sequence with the help of this TAD domain present at the C-terminus which is a serine-rich region [3][32]. The protein also has several sites for post-translational modifications like ubiquitylation, methylation, SUMOylation, phosphorylation, O-Glycosylation, acetylation, and PARPylation (whose site is not yet identified) at different sites [1][12]. Its expression is tightly regulated at transcriptional, post-transcriptional, and post-translational levels which are unique to different forms of human malignancies. SOX2 plays an imperative role in regulating several physiological processes like self-renewal, stemness maintenance, reprogramming, and homeostasis [1][12].

2.3.1 SOX2 modulate stemness in GSCs and other CSCs

Amongst the SOX family members, SOX2 can be regarded as the most important TF for regulating stemness in gliomas [33]**.** Studies have indicated that suppression of SOX2 inhibits the dedifferentiation and tumorigenicity of the HF20303 glioma models [34]. Additionally, in glioma-initiating cells (GICs), the TGF-β/SOX4/SOX2 signaling pathway plays an imperative role in maintaining stemness and tumorigenicity [35]**.** CSCs are the stem cells that are characterized by their unique property of self-renewal, high potential for differentiation, and resistance to drugs and radiation due to their mass heterogeneity and metastasis [36]**.** SOX2 is an imperative TF in sustaining the stemness and self-renewal property of the ESCs and is a crucial component of the Yamanaka factors essential for inducing pluripotency in late differentiated adult cells [37]. Elevated levels of SOX2 have been found in many cancers

including colorectal cancer, osteosarcoma, lung cancer, ovarian cancer, and pancreatic cancer which contribute to their tumorigenicity [38][39][40]**]**[41][42]**.** In pancreatic cancers especially it has been found to enhance the expression of CSC biomarkers like ESA, CD44, and ALDH1 [42]. Conversely, ectopic inhibition of SOX2 activity significantly reduces the chances of xenograft tumor formation [43] and is found to alleviate the complications of breast cancer and *vice versa* [44]. Furthermore, knock-in experiments with GFP-coated genes into the native SOX2 gene loci have showed that SOX2 positive cells showed a greater expression of stemness-specific genes and profound stem cell-like characters as compared to SOX2 negative cells which confirms the distinguished role of SOX2 in maintaining the stemness of the CSCS [45].

2.3.2 SOX2 in growth and proliferation of CSC

Human malignancies exhibit dysregulated expression of SOX2 in more than 20 different cancer types [46]**.** Although SOX2 is involved in modulating the self-renewal property in stem cells by downregulating the expression of various proliferative genes. It has been reported to promote cell proliferation in cancer stem cells. For instance, in pancreatic cancer, cyclin D3 is activated by SOX2 which drives the cell cycle whereas it downregulates p21 and p27 independent of the cell cycle [42]**.** Similarly, in the case of prostate cancers, G1/S transition is affected due to suppressed expression of SOX2 which leads to the promotion of p27 activity and inhibition of cyclin E and *vice-versa* [47]**.** SOX2 overexpression is associated with the activation of the AKT/mTORC1 pathway in a cell-cycle independent manner which results in cell proliferation in case of squamous cell carcinoma of the esophagus [48]**.** SOX2 is also involved in the progression of the cell-cycle in many cancer types. For example, in squamous cell carcinoma of the lungs, overexpression of SOX2 causes inhibition of BMP4 which is an anti-proliferative factor [49]**.** Moreover, conditional knockdown (CKD) of osteoblast-specific SOX2 in osteosarcoma mouse models has shown a decrease in the frequency of tumor phenotypes as compared to SOX2 positive models [50]**.** In breast cancers, SOX2 indirectly regulates the activity of a tumor suppressor known as TUSC3 by regulating the expression of two miRNAs, miR-1081a-5p and miR-30e-5p [51]**.** Conversely, SOX2 is found to be inhibiting the cell cycle in case of colorectal and gastric cancer by downregulating the cyclin D1, phosphorylation of retinoblastoma, promoting activity of p27 and inhibiting the mTOR pathway [52][53]**.**

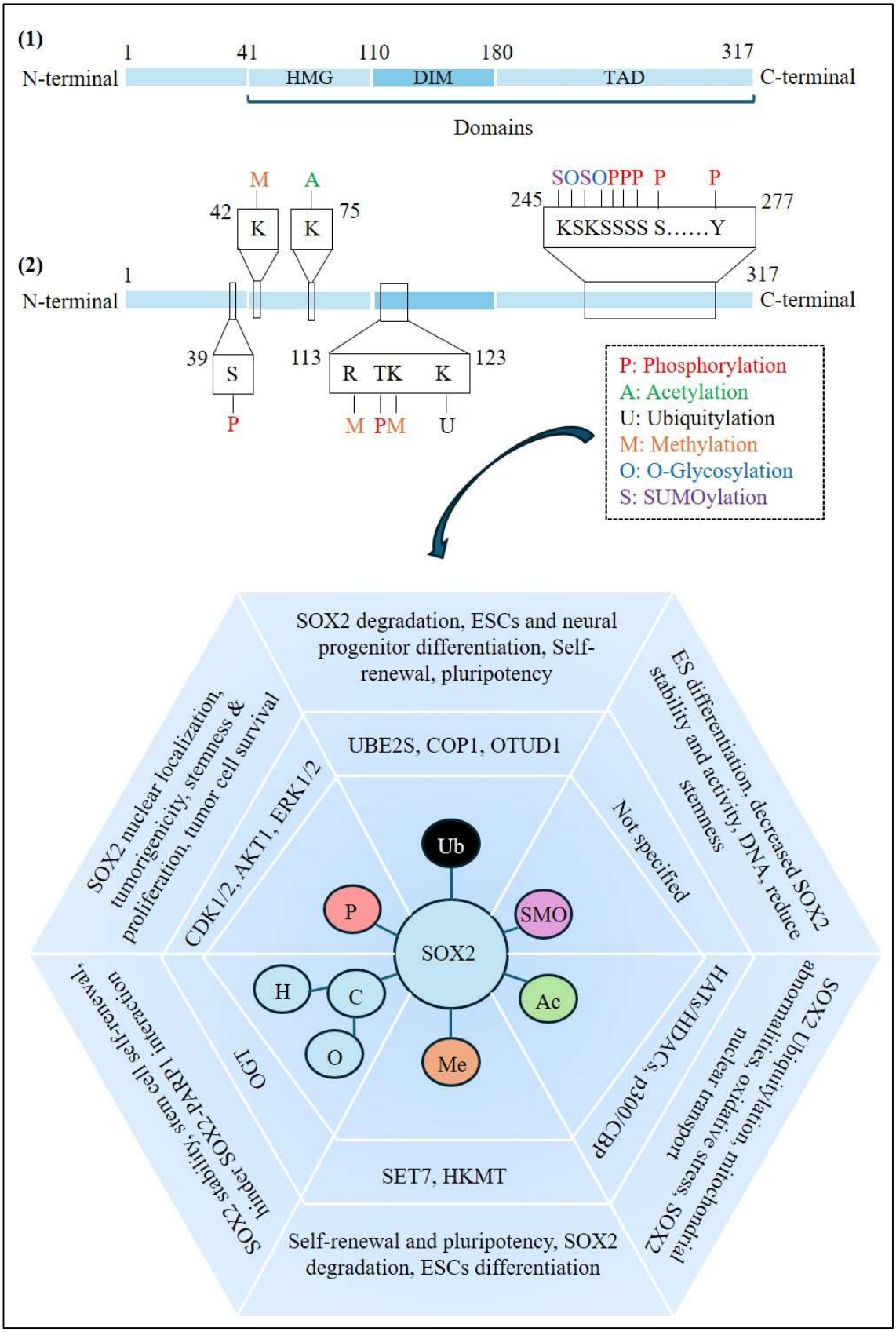
2.3.3 SOX2 and Epithelial-mesenchymal transition (EMT)

Tumor metastasis is often associated with loss of intracellular adhesion property of the epithelial cells and obtaining motility. This process is called Epithelial-mesenchymal transition [54]. There are several contrasting findings concerning the role of SOX2 in the EMT process in different cancer types. For example, SOX2 along with Twist1 is regulating the EMT process in hGBM cells [55]. *In vitro and in vivo* studies of breast and prostate cancers have revealed that SOX2 enhances the EMT process by directly influencing the Wnt/β-catenin pathway by binding to β-catenin [56]**.** In the case of lung cancer, SOX2 suppresses the expression of transcriptional intermediary factor 1 γ (TIF1 γ) which results in the promotion of TGF-β induced EMT [57]. Additionally, SOX2 serves as a joining link for EGFR-mediated EMT in lung and bladder cancer [58][59]**.** Furthermore, in lung cancer, SOX2 can promote EMT by phosphorylating the mTOR and AKT which leads to enhanced activity of matrix metalloproteinase-2 [60]. In breast and pancreatic cancer, SOX2 modulates the expression of

mesenchymal genes like Snail, Slug, and Twist along with some epithelial genes i.e. cadherin and ZO-1 which contribute to the invasiveness of the tumor cells [61][42]**.** However, it has been found in some breast cancer cells that enhanced expression of SOX2 can also inhibit the EMT process by negatively regulating the Twist1 by binding to its promoter thus inhibiting tumor-invasive properties [62]**.** In ESCC, SOX2 enhances Slug expression thus promoting EMT by negatively targeting the STAT3/HIF-1α pathway [63]**.** Evidence from studies also suggests that the EMT process is linked to promoting SOX2-mediated cancer cell stemness in the case of bladder cancer [64]**.**

2.3.4 SOX2 in chemoresistance in GSCs

The resistance to chemotherapy and radiotherapy in recurrent GBMs arises due to the heterogeneity in the tumor cells of GBM which is further enhanced by GSCs [65]. Many other factors can also contribute to this chemoresistance in the proliferating GBM cells such as enhanced expression of MGMT, a DNA mismatch repair enzyme, upregulation of drug efflux transporters, enhanced antiapoptotic signaling pathway (such as Bcl, Bcl-xL, IAPs) [66], Proneural to mesenchymal transition by TNF-α [67], Dysregulation of signaling pathways (i.e. Notch, SHH, Wnt, NF-kB), dedifferentiation of GSCs, and GSC quiescence [68][69][70][71]**.** In CD-133 positive GSCs, overexpression of SOX2 and the miR-145/OCT4/SOX2 pathway plays a critical role in chemoradioresistance of GBMs [72][73]**.** Furthermore, miR-145 mediated inhibition of SOX2 and OCT4 results in increased susceptibility of GBM patients towards Temozolomide (TMZ) and irradiation [73]. IDH4 mediated downregulation of miR-9, a SOX2 to inhibiting microRNA, leads to GBM differentiated cells conversion to GSCs and increased SOX2 expression [74]**.** The FOXM1/SOX2 axis in GBM which utilizes SOX2 as the target for FOXM1 enhances the stemness and radioresistance of tumor cells [75]**.** Studies have shown the role of the SOX2/SOX9 axis downstream to the mTOR signaling pathway and suggested effective therapeutic strategies to overcome this chemo and radioresistance in GBM cells by inhibiting the mTOR and SHH pathways [76]**.** *Fig. II is depicting the structural organization of SOX2 and its post translation modifications*


(1)
1
41
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180
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N-terminal
HMG
DIM
TAD
C-terminal
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(2)
M
A
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K
75
SOSOPPP
P
P
245
277
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317
N-terminal
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39
S
P
113
R TK K
123
M PM U
P: Phosphorylation
A: Acetylation
U: Ubiquitylation
M: Methylation
O: O-Glycosylation
S: SUMOylation
SOX2 degradation, ESCs and neural progenitor differentiation, Self-renewal, pluripotency
ES differentiation, decreased SOX2 stability and activity, DNA, reduce stemness
SOX2 Ubiquitylation, mitochondrial abnormalities, oxidative stress, SOX2 nuclear transport
HATs/HDACs, p300/CBP
SET7, HKMT
Self-renewal and pluripotency, SOX2 degradation, ESCs differentiation
OGT
SOX2 stability, stem cell self-renewal, hinder SOX2-PARP1 interaction
SOX2 nuclear localization, tumorigenicity, stemness & proliferation, tumor cell survival
CDK1/2, AKT1, ERK1/2
UBE2S, COP1, OTUD1
Not specified
Ub
P
SMO
SOX2
H
C
Ac
O
Me

Fig. II: (1) Structure of SOX2 transcription factor: SOX2 is a 317 amino acid long TF having an N-terminal domain, HMG domain, consisting of 68 amino acids (from 41 to 109) which is conserved throughout the Sox family accounting or their relatedness and similarity; a central domain, the DIM domain having 70 amino acids (from 110 to 179); and a TAD domain towards the C-terminal which is required for efficient binding to DNA consisting of 137 amino acids (from 180 to 317). (2) SOX2 is subjected to several epigenetic modifications at the different sites which have their regulatory function like Phosphorylation, Acetylation, Ubiquitylation, Methylation, O-Glycosylation, SUMOylation and PARPylation (site not identified) which are represented with a colored letter in the figure.SOX2 is involved and plays a major role in various biological processes like Embryonic development, maintenance of stemness in CSCs, regulation of multiple growth-related signaling pathways, drug resistance, autophagy, EMT, and Tumorigenicity. An increase in all these properties is reported in cancers that have an upregulated expression of SOX2. Thus, downregulating SOX2 is cancer-initiating cells is of great importance to minimize the tumorigenic phenotype and complications

2.4. Glioblastoma

Glioblastomas (GBMs) are categorized as one of the most aggressive forms of malignant primary tumors of the central nervous system (CNS). GBMs are classified as grade-IV gliomas, comparatively a higher grade, by World Health Organization (WHO) and originate from astrocytic glial cells [77]. Clinically, they are characterized by their abundant vascularization, rapid clinical evolution, and infiltrative growth which leads to their poor prognosis and a patient survival rate of 15 months. Statistically, it affects less than 10 people in a paediatric population of 100,000 individuals [52] and less than 5% patients survive upto 5 years. Still, the incident rate may vary from one demographic location to another [78]. Based on the severity of malignant properties GBMs are classified into four groups by World Health Organization (WHO), GBM-IDH WT which normally develops at the age of ~60 years (90%), GBM-IDH mutant, it has better prognosis report as compared to wildtype IDH and is observed in younger patients (10%), the two other types of IDH mutants of GBMs are not well characterized and studied due to their novelty and poor testing i.e. GBM not otherwise specified (NOS) and GBM Not elsewhere classified (NEC)][78]. Several molecular mechanisms co-dependently work and give rise to GBM-specific phenotype such as hypoxic tumor microenvironment, which plays a crucial central role resulting in oxidative stress, mitochondrial dysfunction, dysregulated apoptotic signaling, and angiogenesis. During angiogenesis, angiopoietins and angiogenic factors along with MMPs, bind to their respective receptors, leading to ECs degradation and vessel wall maturation. Cell-cycle dysregulation happens due to the downregulation of mediators of cell cycle arrest such as p53, p21, and pRB. Interestingly, TMZ drug incorporation causes double-stranded lesions and breaks in the tumor cells which is repaired by upregulated MGMT enzyme in case of GBM which prevent DNA damage mediated termination of cell cycle *(Fig. III summarizes the various factors which lead to the aggressive behavior of GBM cells).*

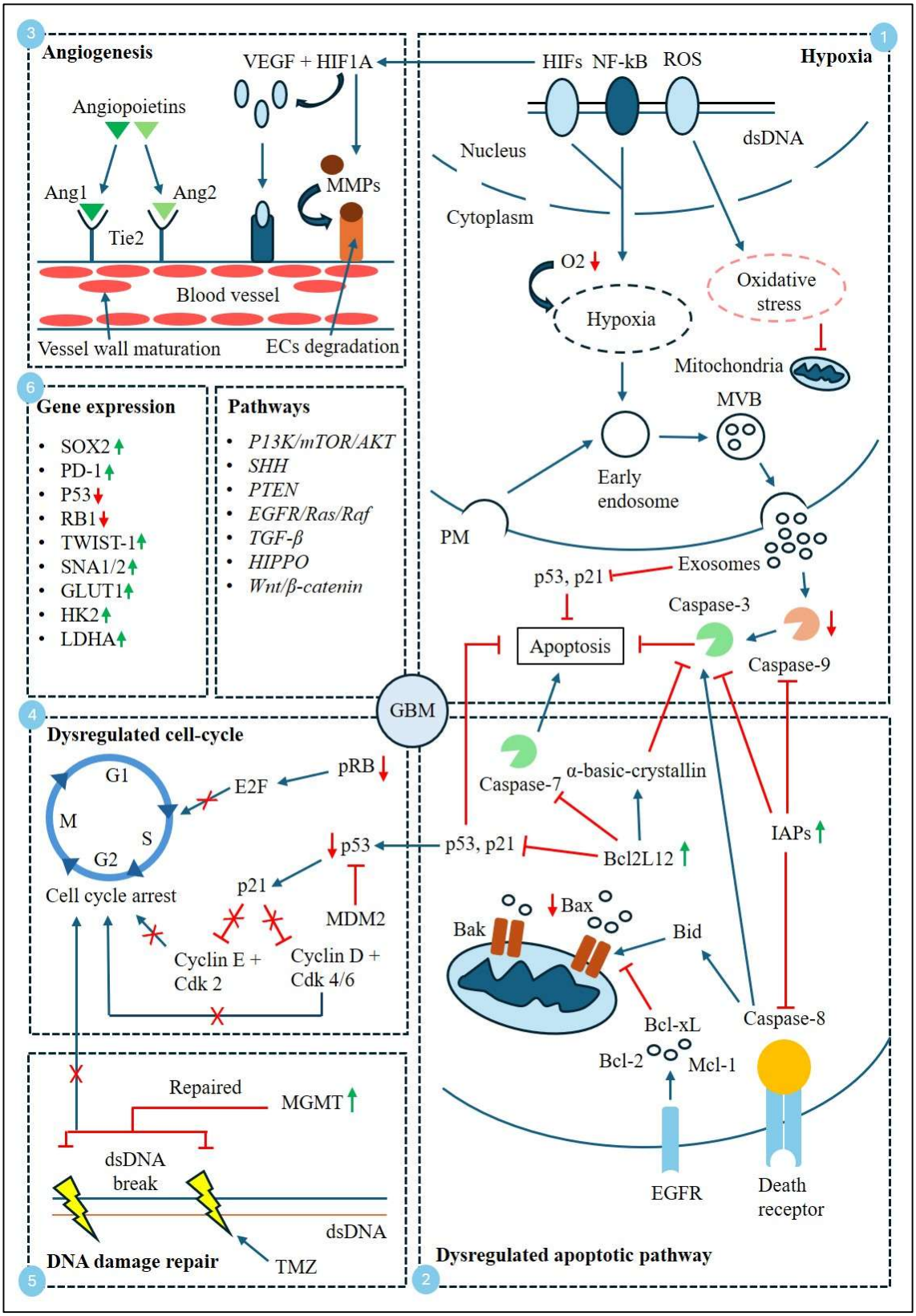


Fig. III: Molecular mechanism that contributes to the progression of GBM. (1) Hypoxic growth conditions: Hypoxia is characterized by low or negligible O2 availability to carry out cellular functions.

Transcription of genes like Hypoxia Induced Factors (HIF1A) and Nuclear Factor kB (NF-kB) activate the HIF signaling pathway associated with the hypoxia-like condition inside the cell. These hypoxic conditions lead to exosome release, which interferes with the activity of apoptosis-related genes like p53 and p21, leading to a decreased apoptosis rate in tumor cells. Furthermore, these exosomes inhibit the effector caspases like caspase-9, which in turn fail to activate the inactive procaspase-3 into active caspase-3, leading to further decreased apoptotic rate in GBM cells. On the other hand, hypoxia can also lead to hypoxia-induced ROS formation, which contributes to oxidative stress inside the cell and interferes with the activity of mitochondria. (2) Dysregulated apoptotic signaling: In GBM cells, it has been seen those anti-apoptotic proteins like Bcl-xL, Bcl-2, and Mcl-1 are upregulated (facilitated by EGFR signaling), along with a decrease in apoptosis-promoting proteins like Bax. Further, an increase in the levels of inhibitors of apoptosis proteins (IAPs) leads to the inhibition of several downstream targets like caspase-8 (associated with death receptor), whose inhibition leads to decreased levels of Bax proteins, which are responsible for activating the caspase-9. IAPs can also directly inhibit the activity of caspase-9 and caspase-3. Additionally, the upregulation of antiapoptotic proteins like Bcl2L12 directly inhibits the activity of executioner caspase-7 and p53 as well as indirectly inhibits caspase-3 via activating α-basic-crystallin. (3) Angiogenesis: Hypoxic microenvironment leads to the release to angiogenic factors such as VEGF, HIF1A, and angiopoietins like Ang-1 and Ang-2 from the GBM cells. The angiogenic factors bind to their respective receptors, while angiopoietins bind to the Tie-2 receptors on the endothelial cells (Ecs). This leads to the proliferation and migration of ECs and the degradation of the ECM (endothelial cell membrane). At last, the blood vessel walls start to mature, and the recruitment of pericytes leads to the formation of new blood vessels. (4) Dysregulated cell-cycle: Effective cell-cycle arrest is imperative to halt tumor growth, but this is compromised in GBM cancer cells. The downregulation of tumor suppressors like p53, p21, and pRB leads to the dysregulation of the cell cycle. p21 is activated by p53, which in turn inhibits the Cdk4/Cyclin D and Cdk2/Cyclin E, leading to cell cycle arrest, but as in GBM, p53 is downregulated, and this process is compromised. Further, the downregulated pRB fails to activate the E2F factor, and thus, cell cycle progression continues. (5) DNA damage repair: Double-stranded DNA breaks induced by TMZ drugs are often repaired/reversed by upregulated MGMT protein, thus preventing cell cycle arrest due to DNA damage. (6) Dysregulation of key genes and regulatory signaling pathways: In GBM, key genes like SOX2, PD-1 (high-grade gliomas), GLU1, TWIST-1, SNA1/2, HK2, and LDHA are upregulated while some genes like p53 and RB1 are downregulated along with aberrant regulation of key signaling pathways like, P13K/mTOR/AKT pathway, SHH pathway, PTEN pathway, EGFR/Ras/Raf pathway, TGF-β pathway, HIPPO pathway, Wnt/β-catenin pathway contribute to the aggressive phenotypes of the GBM.

2.5. Role of SOX2 in Glioblastoma

Upregulated expression of SOX2 has been reported in many cases of recurrent GBM which makes enhance its aggressiveness and make prognosis poor [79]**.** Studies have shown that SOX2 overexpression is seen predominantly in the nucleus [80]**.** The increased expression of SOX2 in almost every patient brain cell sample despite its gene locus been amplified in only some percentage of samples (approx. 14.4% of GBM and 11.1% of anaplastic oligodendrogliomas [33]) is due to hypomethylation of its promoter [81]. SOX2 overexpression is also found in the actively dividing GBM tumor cells which showed high levels of Ki67/MIB.1, NESTIN, CD-133 and MUSASHI-1 [33][82][83][84]**.** SOX2 plays an imperative role in maintaining stem-cell like properties of the GBM stem cells (GSC) and is found to upregulated in several GBM positive cases [79]**.** Conversely, in recurrent gliomas the expression of SOX2 was found to be downregulated as compared to its primary gliomas counterparts which is responsible of the poor prognosis of the recurrent GBM after chemoradiotherapy [85]. However, silencing of SOX2 expression can lead to cell cycle arrest

at G0 to G1 phase and promote cell senescence in GBMs [86]**.** Ectopic overexpression studies have shown that SOX2 can enhance the invasive and migration properties of the native GBM cells along with their self-renewal and proliferative properties. Additionally, SOX2 along with other transcription factors POU3F2, OLIG2, and SALL2 can convert the differentiated GBM cell into induced GSC [87]**.** Furthermore, FACT chaperone and HMGA1 mediated negative regulation of SOX2 promote asymmetric division of the GSCs [88][89]**.**

Tumor heterogeneity within the GBM malignancies which may arise due to evolutionary divergence, degree of differentiation, and local microenvironment of the tumor cells [90] pose a challenge in designing effective therapeutic strategies for the disease as it contributes to involvement of different signaling pathways and lead to radiation and drug resistance like resistance to Temozolomide [91]. Furthermore, three classes of GBM subtype signatures are classified i.e. proneural, mesenchymal and classical [92]. Additionally, it has been previously reported that two novel subclasses of high-grade GBMs cell cultures, type-A and type-B, show stem cell-like properties. The type-A glioma cell culture which is characterized by high tumorigenicity, intracranial xenograft formation, and presence of tumor spheres [90] specifically showed high level of SOX2 gene expression which may lead to non-mesenchymal subtype of GBM signature [93]**.** In GBM patients cell extract it has been seen that under depleting levels of a E3 ligase enzyme which belong to the TIF (transcription intermediary factor) family, Tripartite motif-containing 26 *(TRIM26)*, SOX2 expression levels also get significantly depleted suggesting that TRIM26 is associated with SOX2 stability in GBM. TRIM26 negatively interacts with another E3 ligase of SOX2, WWP2, and inhibit it from ubiquitinating SOX2 which protect it from proteasomal degradation in a catalytic activity independent manner and provide stability to SOX2 expression level which accounts for self-renewal and tumorigenicity of GBMs stem cells [94]. Another member of the TIF family which is also the founding member, TRIM24, has been found to be overexpressed in GBMs. It binds with chromatin via its bromodomain leading to activation of SOX2 and contribute to CSCs stemness [95]. Furthermore, SOX2 can potentially contribute in inducing GBM stemness and tumor proliferation by inhibiting the TET2, a member of ten-eleven translocation (TET) family which converts 5mC DNA methylation into 5hmC, thus negatively regulating the 5hmC DNA methylation leading to poor disease prognosis [96]. Deletion of SRR regions and silencing of SOX2 gene in GBMs models using gene editing techniques like CRISPR/Cas9 and RNAi has shown decreased SOX2 activity which correlates with the poor initiation and progression of tumor cells in vivo [97][81]. Dedifferentiation of GBM cells to CSCs phenotype was restricted in SOX2 knockdown murine models suggesting its role in modulating stem cell like phenotype in GBMs while maintaining plasticity to modulate between differentiated and stem cell-like properties [34]**.** Ectopic expression of SOX2 in GBMs is enough to result in their invasion and migration [81]**.** SOX2 in combination with other transcription factors like FOXG1 extensively contributes to the proliferation of mutated cells and restricts differentiation of astrocytes in GBMs [93]**.** In U87 and U118 GBMs, elevated levels of SOX2 are identified to be associated with poor progression of the disease by disrupting their proliferation**.** Furthermore, negative regulation of SOX2 by siRNA mediated gene silencing in GBMs resulted in increased susceptibility towards PDGF- and IGF-1-receptors inhibitors [93]. miRNA mediated silencing of the coding SOX2 gene in transplanted xenograft mouse model from neural cells of human patients suffering from GBM shows that its silencing ceases the GBM tumor initiating cells from differentiating and proliferating [98].

2.6. Key Signaling Pathways affected by SOX2

SOX2 is associated with various signaling pathways like EGFR/MAPK/P13K-mTOR-AKT signaling pathway, SHH pathway, HIPPO signaling pathway, and Wnt/β-catenin signaling pathway (In Fig. IV, various pathways associated with SOX2 are depicted in pictorial representation).

2.6.1 SOX2 and EGFR/MAPK/P13K-mTOR-AKT signaling pathway

Various physiological processes like cell proliferation, migration, survival, and differentiation are regulated by the binding of EGF family ligands to their EGFR-specific receptors present on the cell surface which are the members of tyrosine kinases. These interactions between ligands and receptors of the EGFR family result in several mitogenic reactions in target cells. In lung stem-like cells, the expression of SOX2 is suppressed by pharmacological or genetic inhibition of the EGFR signaling pathway which results in the aberration in the self-renewal property of the stem-like cells[12]. Interestingly, the EGFR-mediated self-renewal ability of the lung stem-like cells can be significantly enhanced by ectopic expression of the EGFR mutant or ligand, which helps in the accumulation of the SOX2 protein [12]. The SOX2 protein can enhance the carcinogenic phenotype of lung cancer stem cells by directly interacting with the EGFR receptor promoter region at position 389-383 base pair upstream to the transcriptional start site, which transcriptionally upregulates the expression of EGFR receptor [12]. It has been shown in the pathogenesis of papillary craniopharyngioma (PCP), which is a condition marked by abnormal morphogenesis and hyperplasia in the pituitary gland, that overexpression of SOX2 is often associated with gain of function mutation in the BRAF-V600E and KRAS-G12D in the MAPK signaling pathway which results in the disruption in pituitary differentiation and its proliferative capacity [99]. Activation of SOX2 expression can also be seen in skin keratinocytes, where the EGFR/MEK/ERK pathway is affected leading to the promotion of cutaneous wound healing and angiogenesis [100]. In vitro and in vivo studies have revealed that SOX2 expression is upregulated in the case of liver tumor-initiating cells (T-ICs) due to intentional overexpression of cyclin-G1 in AKT/mTOR signaling which led to increased self-renewal, tumorigenicity and drug resistance in hepatoma cells [101]. Additionally, mice SOX2 is stabilized by AKT1-mediated phosphorylation at position K119 whereas interestingly, it is destabilized and marked for ubiquitin-mediated degradation by mono-methylation at position T118 by Set7 protein, this phosphorylation-methylation works as a switch and is crucial for maintaining SOX2-associated embryonic stem cells (ESCs) fate [102]. SOX2 has also been identified to be associated with P13K signaling mediated squamous cell carcinoma progression in tracheobronchial basal cell lines which leads to dysplasia[103].

2.6.2. SOX2 and Wnt/β-catenin signaling pathway

Wnt signaling pathway is evolutionary conserved in the animal kingdom and plays crucial roles in maintaining tumorigenesis and correctly shaping tissues during development [104]. Previously studies have shown that SOX2 is associated with the Wnt signaling pathway in guiding cell lineage proliferation during the development [12]. SOX2 acts as a marker for dental epithelial stem cells and its temporal knockdown is associated with dysregulation of the Wnt/β-catenin pathway in mice which leads to abnormal tooth development from the dental epithelium cells [105]. Conversely, SOX2 has been shown to directly regulate the self-renewal property in osteoblast cell lineages by interacting with β-catenin with its TAD domain present at the C terminus and inhibiting the Wnt/β-catenin pathway [106]. The tumor-forming ability

of the SOX2-compromised osteosarcoma cells in mice was found to be impaired due to dysregulation in the Wnt signaling pathway [107]. Furthermore, the regulation of osteosarcoma cells to be maintained in the osteoblast-like state is often associated with suppression of SOX2 expression by activated Wnt pathway [108]. SOX2 has also been studied in vertebrate retina development where it plays two specific roles i.e. (a) maintain optic cup in a neurogenic fate via Wnt/β-catenin dependent manner and (b) ensures cycling of optic cup progenitors via a Wnt/β-catenin independent manner [108]. Consequently, SOX2 can regulate the Wnt/β-catenin signaling cascade by directly or indirectly engaging with the components of the pathway it has been shown that tumor metastasis can be promoted by direct binding of SOX2 with the promoter of β-catenin leading to its overexpression and translocation from cytosol to nuclei activating the Wnt/β-catenin pathway [108]. Indirectly, SOX2 plays an imperative role in the activation of several inhibitors of the Wnt/β-catenin pathway like GSK3β, dickkopf-1, and adenomatous polyposis coli, so as to keep the Wnt/β-catenin pathway activity in check [106].

2.6.3. SOX2 and TGF-β signaling

TGF-β is a superfamily comprising a variety of cytokines including BMPs, GDFs, activins, inhibins, TGF-β isoforms, nodal, and AMH. The superfamily comprises 33 genes in total having a secretion signal peptide, a pro-domain of ~250 residues, and a growth factor domain of ~110 residues [109]. These cytokines regulate various biological processes like cell proliferation, differentiation, migration, apoptosis, embryonic development, and tissue homeostasis [109]. Dysregulation of TGF-β superfamily members is associated with many diseases like cancer, inflammation, and fibrosis [109]. TGF-β is produced in an inactive form, for activation a proteolytic cleavage is required after dimerization. The cleavage results in the formation of two molecules, latency-associated peptide and mature TGF-β [109]. TGF-β family members are receptor-specific, they either bind to type-I (TβR II) or type-II (TβR II) receptors which is crucial for their respective functions [109]. TGF-β/Smad signaling is associated with tumor cell proliferation, morphogenesis, and pathogenesis. In GSCs, it has been reported to be associated with SOX2 in maintaining stemness while its repression can lead to cell differentiation by decreasing the expression of SOX2 [6]. TGF-β can lead to SOX2 accumulation in case of melanomas [110].

2.6.4. SOX2 and SHH signaling pathway

Hedgehog signaling is an evolutionarily conserved signaling mechanism in mammals and plays important role in embryonic and CNS development, cell proliferation, differentiation, and tissue polarity [111]. The Hedgehog protein family is comprised of three main classes namely, Sonic Hedgehog (SHH), Indian Hedgehog (IHH), and Desert Hedgehog (DHH) [111]. Hedgehog signaling predominantly occurs in the primary cilia (PC) of mammals. The signaling starts when the Hedgehog protein bind to its respective receptor i.e. PTCH1/2, liberating the SMO protein. After this, a downstream cascade is activated involving proteins like KiF7, SuFu, and GLI2/3 which leads to GLI activation [111]. Several target genes are transcribed after that like PTCH1 and GLI1. SMO is inhibited by PTCH in the absence of hedgehog protein which leads to cleavage of GLI protein after phosphorylation. This cleavage of GLI protein reduces it to its repressor form (GLIR) and thus transcription of target genes is inhibited [111]. The non-canonical hedgehog signaling operated independtly of GLI transcription factors and is of two major types, type-I (SMO-dependent) and type-II (SMO-independent) [111]. SHH or the sonic hedgehog protein plays imperative roles in the development of CNS [80]. Enhanced

SOX2 activity has been reported in SHH-associated medulloblastoma cells [112]. SHH being a downstream target of SOX2 is important for maintaining stem-cell-like properties and the development of the hippocampus [112]. Deletion studies have shown prominent evidence suggesting that removal of SOX2 leads to a similar neurogenesis phenotype as observed in SHH-depleted cells, whereas replenishing the normal SHH levels can alleviate such complications [112].

2.6.5. SOX2 and Hippo signaling pathway

The effect of environmental cues on the growth of cells, tissues, and organs and their response to it is regulated by the Hippo signaling pathway which is evolutionarily conserved [113]. The Yes-associated protein (YAP) which act as a transcriptional co-activator is a Hippo pathway effector molecule which in airway development regulates the cell fate of the epithelial progenitor cells and modulate their morphogenesis by interacting with the SOX2 protein [12]. It forms a boundary between SOX9 associated distal and SOX2 associated airway areas during branching of epithelial tubules. Furthermore, the absence of YAP can cause failure in the airway epithelial cell precursor specification because it regulates the expression levels of the SOX2 protein which is essential for specification process to occur whose imbalance can cause failure in the specification [114]. SOX2 can directly regulate the Hippo pathway as seen in adipogenesis where SOX2 directly transactivates the expression of YAP. Additionally, it directly supresses the Hippo signaling pathway by interacting with activators of the pathway like WWCI and NF2 which leads to enhanced YAP expression. SOX2 can also regulate the CSCs in osteosarcomas by inhibiting the Hippo pathway which act as a tumor suppressor [115]

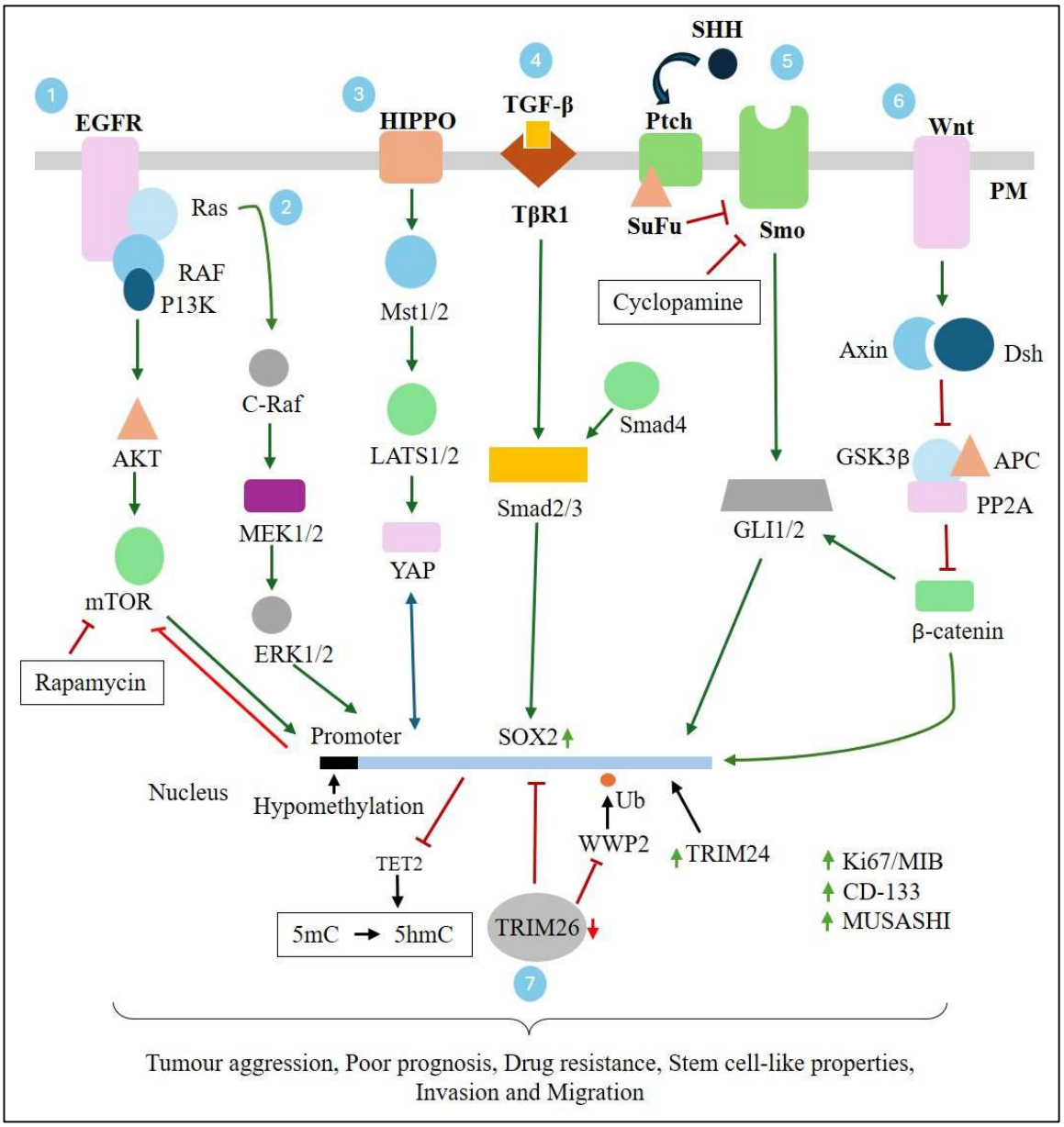


Fig. IV: Pathways and proteins which interact with SOX2 in GBMs and give rise to the cancer phenotype. (1) EGFR/MAPK/P13K-mTOR-AKT signaling pathway: EGFR signaling plays important role in maintaining self-renewal property in stem cells by upregulating the expression of SOX2 via downstream activation of intermediates like AKT and mTOR. Rapamycin is a drug which affect the EGFR pathway by inhibiting mTOR. (2) The EGFR-mediated Ras pathway promotes SOX2 expression in the nucleus via activation of c-Raf, MEK1/2 and ERK1/2. (3) HIPPO pathway is responsible for developing multidrug resistance towards TMZ in GBM by overexpression of TAZ protein. (4) TGF-β pathway is associated with tumor cell proliferation, morphogenesis and pathogenesis in GBM and upregulate SOX2 expression in Smad2/3 and Smad4 dependent manner. (5) SHH pathway has a key role in the development of CNS and an upregulated expression pattern of SHH is seen in brain tumors as it is also a downstream target gene of SOX2, Cyclopamine drug inhibits the interaction between SuFu and Smo. (6) The Wnt/β-catenin pathway is a major dysregulated signaling pathway observed in GBM which is involved in determining the differentiation of cell lineages during development. The intermediate β-catenin directly interacts with SOX2 and also activates GLI1/2 in the SHH signaling

pathway. (7) SOX2 is upregulated in the nucleus with other marker proteins like Ki67/MIB, CD-133, and MUSASHI in GBM along TRIM24, which are responsible for maintaining stem-cell-like properties in GSCs. On the other hand, low levels of TRIM26 have been reported in GBM patient cells which suggests that TRIM26 is involved in stabilizing the SOX2. Its normal levels inhibit E3 ligase of SOX2 i.e. WWP2 which prevents Ubiquitin-dependent SOX2 proteasomal degradation. SOX2 also inhibits TET2 protein which is responsible for converting 5mC methylation to 5hmC. SOX2 promoter hypomethylation is responsible for its overexpression in patient cells.

2.7. SOX2 interacts with a variety of downstream targets in GBMs

Fang et al. have reported 17 genes to be possible downstream targets of SOX2 in GBM from a pool of 59 genes associated with cellular differentiation [116] The majority of these are listed in *Table I*. SOX1 serves as an oncogene in GBM and contributes extensively to tumor proliferation, neural cell differentiation, and plasticity. No evidence has been reported of SOX1 being involved with any pathway in GBM but it regulates a variety of other proteins like SOX2, Cyclin-D, PML, and p27 [117]. BEX-1 activates the YAP/TAZ pathway in GBM via F-actin polymerization which leads to tumor aggression and poor clinical outcomes [118]. SHH or the sonic hedgehog protein plays an imperative role in the development of CNS [112]. SHH being a downstream target of SOX2 is important for maintaining stem-cell-like properties and the development of the hippocampus [119]. Additionally, IGFBP3 regulate PD1 expression via JAK2/STAT3 pathway in GBM which enhances immune evasion [120]. ETS1 plays an important role in endothelial cell (EC) differentiation and a dual role in angiogenesis. In GBM, it regulates several genes like VEGFA, KDR, ANGPT2 SOX4, and MCAM resulting in the elevation of tumor angiogenesis, the two main regulatory pathways in this regard are VEGF and TGF-β signaling pathway [121]. SOX18 belongs to the SoxF group of Sox family and is reported to be downregulated in GBM but exact role and pathway is not identified for SOX18 in GBM, further research is required [122]. Although BMPR1B is associated with embryogenes (mendeley) (mendeley)is and astroglial differentiation in TICs, its exact function in GBM needs further exploration we only know that BMPR1B is downregulated in most of the GBM samples whose significance is yet to deduced but we can conclude that overexpression of BMPR1B can potentially reduce GBM phenotype [123][124]. RUNX1 plays a crucial role in hematopoiesis and neural cell differentiation, In GBMs, it acts as an oncogene and modulate expression of MMPs and VEGFA via p38/MAPK pathway to enhance tumor invasion and angiogenesis. Over expression of MMPs help glioma cells to invade neighboring healthy cells via invadopodia. Overexpression of RUNX1 in GBM is reported to be associated with tumor invasion to healthy cells in a TGF-β dependent manner. RUNX1 also plays an important role in glioma cell migration and proliferation by activating the JAK/STAT pathway. Furthermore, downregulation of RUNX1 in GBM is associated with increased sensitivity of cancer cells towards TMZ treatment [125][126][127][128][129]. CDC20 is a cell cycle protein but is also involved in inducing TMZ resistance in GBM cells. Studies has shown that repressing CDC20 enhances TMZ sensitivity in Bim-compromised GBM cells [130]. It is also involved in dendritic trimming during neuronal development and serves as a biomarker [11]. FGF13 is responsible for microtubule stabilization via intracellular tubulin dynamics and sodium channels which contribute to glioma invasion and provide resistance against bevacizumab drug [131]. UTF1 acts in dual nature by either being a tumor proto-oncogene or tumor suppressor gene in many cancer types but its precise role in GBM progression needs further research [132].

Table I: Downstream target genes of SOX2 in GBM

Gene	Family	Location	Function	Pathway	Reference
Cellular differentiation, Proliferation, Apoptosis, Angiogenesis and Tumorigenesis					
SOX1	Sox	13q34	Modulation of Neural cell differentiation	Single Administration	[117]
BEX-1	Bex	Xq22.1	Tumor suppressor	YAP/TAZ signaling	[118]
SHH	Hedgehog	7q36	Embryogenesis	HH signaling	[112]
IGFBP3	IGFBP	17p12.3	Recruit IGFs, Role in cell proliferation, differentiation, and apoptosis	JAK2/STAT3 signaling	[121]
ETS1	ETS	11q24.3	Transcriptional activators or repressors, angiogenesis, tumorigenesis, apoptosis	TGF-β and VEGF signaling	[121]
SOX18	SoxF	20q13.3	Single Administration	Single Administration	[122]
BMPR1B	BMPR	4q22.3	Endochondral bone formation, embryogenesis	BMP signaling	[124][123]
RUNX1	RUNX	21q22.12	Invasion, migration, Angiogenesis, drug resistance, and Hematopoiesis	P38-MAPK, JAK/STAT and TGF-β pathway	[125][126][127][128][129]
CDC20	Cell cyclin	1p34.2	Regulation of cell cycle, TIC proliferation, drug resistance	Single Administration	[130][11]
FGF13	FGF or FHF	Xq26-28	Tumorigenesis, cell invasion, embryogenesis, tissue repair	FGF signaling	[131]
UTF1	UTF	10q26.3 (H) and 7F5 (M)	Embryonic development	Single Administration	[132]
BMPR1B	BMPR	4q22.3	Endochondral bone formation, embryogenesis	BMP signaling	[124][123]

RUNX1	RUNX	21q22.12	Invasion, migration, Angiogenesis, drug resistance, and Hematopoiesis	P38-MAPK, JAK/STAT and TGF-β pathway	[125][126][127][128][129]

3. METHODOLOGY

3.1. Retrieval of p53 msSNPs

The NCBI ClinVar database [133] was used to retrieve missense SNPs of p53 and only p53 specific variants were selected for further study. NCBI (National Centre for Biotechnology Information) is a genomic and proteomic database under the National Health Institute (NIH) which can be used to retrieve, store, analyze and interpret the information related to biochemistry, molecular biology, and genetics. NCBI ClinVar stores data concerning the information about the clinical variants of a particular gene or protein which are investigated, under investigation and not explored at all.

3.2. Variant annotation

The variants obtained from NCBI ClinVar were further compared with UniProtKB [134] protein specific variant data and those variants which were common in both NCBI and UniProtKB, along with those present only in UniProtKB with SIFT scores were further selected. SIFT software is used for analyzing the effects of missense point mutations. It provides output in terms of deleterious or tolerated mutational effects. Only deleterious mutations were chosen. UniProtKB is a universal database which holds information specifically about proteins and their variants. It helps in exploring the protein sequences and characterization of their functional information.

3.3. Selection of deleterious msSNPs

Three in silico analysis tools namely MutPred2 [135], PANTHER [136] and SNP&GO [137]were used to carry out the predictions. MutPred2 is a Machine learning based software which provides information about the pathogenicity of the mutant and its effect on the phenotype. PANTHER utilizes evolutionary and phylogenetic relationships to classify proteins and their genes for better comprehensive analysis. SNP&GO is used to predict the disease association of missense mutations in a protein based on its gene ontology (GO) annotation.

3.4. Analyzing structural stability

Missense mutations cause changes in protein structure. The I-Mutant 2.0 tool [138] was used to study the stability of these structural changes. Effect of mutations on structural stability is imperative in protein engineering and thus needs to be analyzed properly. I-Mutant tool is a vector machine and predicts the effect of mutated sequence in a protein on its stability. FASTA sequences of the variant proteins of p53 were submitted in the tool.

3.5. Analysis of conserved sequences

Conserved sequence analysis within the wildtype p53 protein amino acids was done with ConSurf server [139]. It provides information about the conserved sequences present within a protein based on similarity between the homologous sequences and their phylogenetic relation. Only Conserved sequences were selected for further study.

3.6. Interaction with other proteins

Hub genes such as p53 form complex intricate network with other genes and show a diverse range of protein-protein interaction which can be studied by utilizing multiplex network approach [140][141]. STRING database [142] was used to obtain the data about protein

interactions of wildtype p53. STRING provides information about the physical or direct as well as functional or indirect interactions between proteins. 10 proteins were found to be interacting with p53 from which only MDM2 was further used for docking, to study the effect of variations on the binding properties of these variants with inhibitors like MDM2.

3.7. Introduction of mutations

Mutations at specific sites of wildtype p53 structure were induced according to the desired variant using PyMol software and were downloaded in format of PDB for docking. Mutational changes in the amino acid sequence of native p53 allowed us to analyze the structural changes in the protein.

3.8. Dock-prep

AlphaFold software [143] which is an AI based server was used to obtain PDB structures from the amino acid sequence of the proteins to be docked. Ftsite software [144] was then used to identify ligand binding sites within the p53 protein structure.

3.9. Docking analysis

Wildtype and variants of p53 were docked with MDM2 on HDOCK server [145] which provides a docking score based on the binding affinities of the two proteins.

4. RESULTS

4.1. Data retrieval

Variants of human tumor suppressor gene p53 with uncertain clinical significance containing missense mutation were identified using ClinVar NCBI database. A total of 5472 SNPs were identified containing mutation at specific sites in the amino acid chain out of which 5455 were associated with germline variation while 107 with somatic variation. Out of these germline variations 2193 were identified to be of uncertain clinical significance from which 1461 missense mutations and 1429 single nucleotide variations were further used for carrying out the study. 546 SNPs which were related to p53 were screened from these variants for.

4.2. Deleterious msSNPs characterization

Out of 546 msSNPs 57 which were not reported to be associated with any disease on NCBI were selected and consecutively compared on the UniprotKB database. 23 variants were found to be present in both NCBI and UniprotKB amongst which 12 were somatic and 11 were germline. 37 variants were present in Uniprot among which 13 were somatic and 24 were germline. Uniprot also provided the information about the SIFT predictions of these 60 variants which were found to be deleterious. We only selected 34 variants with germline mutations. Germline variations are associated with inheritance of the disease and thus these mutations were chosen for the study.

4.3. Prediction of deleterious msSNPs

Three different in silico prediction tools for msSNPs like MutPred2, PANTHER, and SNP&GO were used for further studies. Amongst 34 variants only 11 msSNPs were chosen for analysis whose MutPred2 score was above 0.5 which is considered to be deleterious the msSNPs were R283P, P250S, P250T, T230P, Q129P, Q167P, G154R, F109C, P98H, P98R, V97D. Out of these, 3 were benign i.e. R283P, Q129P, F109C and 8 were identified to be damaging in nature amongst which P98R had the maximum score of 0.699 while G154R obtained the minimum score of 0.548. PANTHER and SNP&GO predicted all of them to be damaging and associated with diseases (but the proof of them being associated with a particular disease is still unknown) (Table II).

Table II: Deleterious Mutations of p53

dbSNP ID	Mutation	SIFT	MutPred2	PANTHER	SNP &GO
rs371409680	R283P	Deleterious	0.8	Probably benign	Disease
rs2151020578	P250S	Deleterious	0.593	Probably damaging	Disease
	P250T	Deleterious	0.604	Probably damaging	Disease
rs1597365431	T230P	Deleterious	0.615	Probably damaging	Disease
rs730882002	Q192P	Deleterious	0.698	Probably benign	Disease
rs1319163924	Q167P	Deleterious	0.591	Probably damaging	Disease
	G154R	Deleterious	0.548	Probably damaging	Disease
rs587781371	F109C	Deleterious	0.787	Probably benign	Disease
rs1245723119	P98H	Deleterious	0.638	Probably damaging	Disease

rs1597374015	P98R	Deleterious	0.699	Probably damaging	Disease
rs730881995	V97D	Deleterious	0.585	Probably damaging	Disease

4.5. Structural stability analysis of variants

Stability analysis of these variants was carried out using the I-Mutant tool and a decrease in overall structural stability was observed in the case of all variants (Table III).

Table III: Showing I-Mutant Results

dbSNP ID	Mutation	I-Mutant	RI
rs2151020578	P250S	Decrease	8
	P250T	Decrease	8
rs1597365431	T230P	Decrease	3
rs1319163924	Q167P	Decrease	5
	G154R	Decrease	8
rs1245723119	P98H	Decrease	8
rs1597374015	P98R	Decrease	8
rs730881995	V97D	Decrease	5

4.6. Evolutionary conservation analysis of proteins

Using ConSurf, evolutionary conserved amino acids were identified. The software analyses the homologous sequences and their phylogenetic relationship between protein sequences to give the result. All msSNPs were found to be conserved (Fig. V), which signifies that these amino acid positions are highly conserved throughout the evolutionary history of the protein and is crucial in maintaining its structural and functional integrity and thus, any mutation in these positions will result in aberrant protein stability and function.

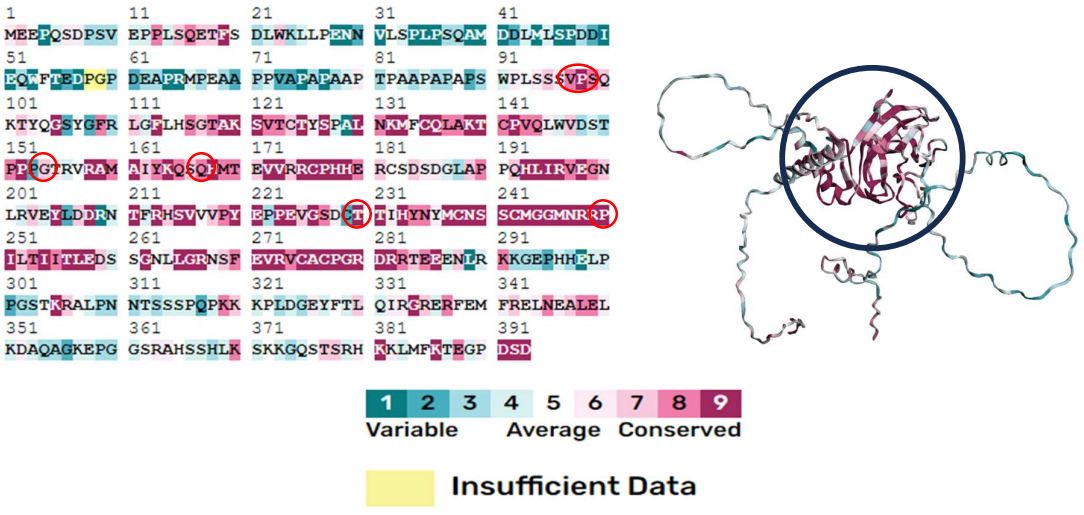


Fig. V: ConSurf results of wildtype p53 protein conserved regions

4.7. Protein-protein interaction data

The STRING database revealed information about the proteins interacting with p53. RPA1, SFN, ATM, MDM2, DAXX, CREBBP, HSP90AA1, EP300, SIRT1, and p53BP2 were shown to be associating with p53 (Fig. VI). RPA1, SFN, ATM, CREBBP, HSP90AA1, TP53BP2, and EP300 were involved in activating and stabilizing the p53 structure either directly or indirectly, thus enhancing its activity while MDM2, DAXX were inhibiting its activity. SIRT1 can act as an activator or inhibitor depending upon the conditions. MDM2 which is an inhibitor of p53 was selected to study the effects of structural changes on binding affinities towards such proteins. Additionally, Bax gene promoter and protein were used to analyze the potential effects of the mutation on mitochondrial integrity.

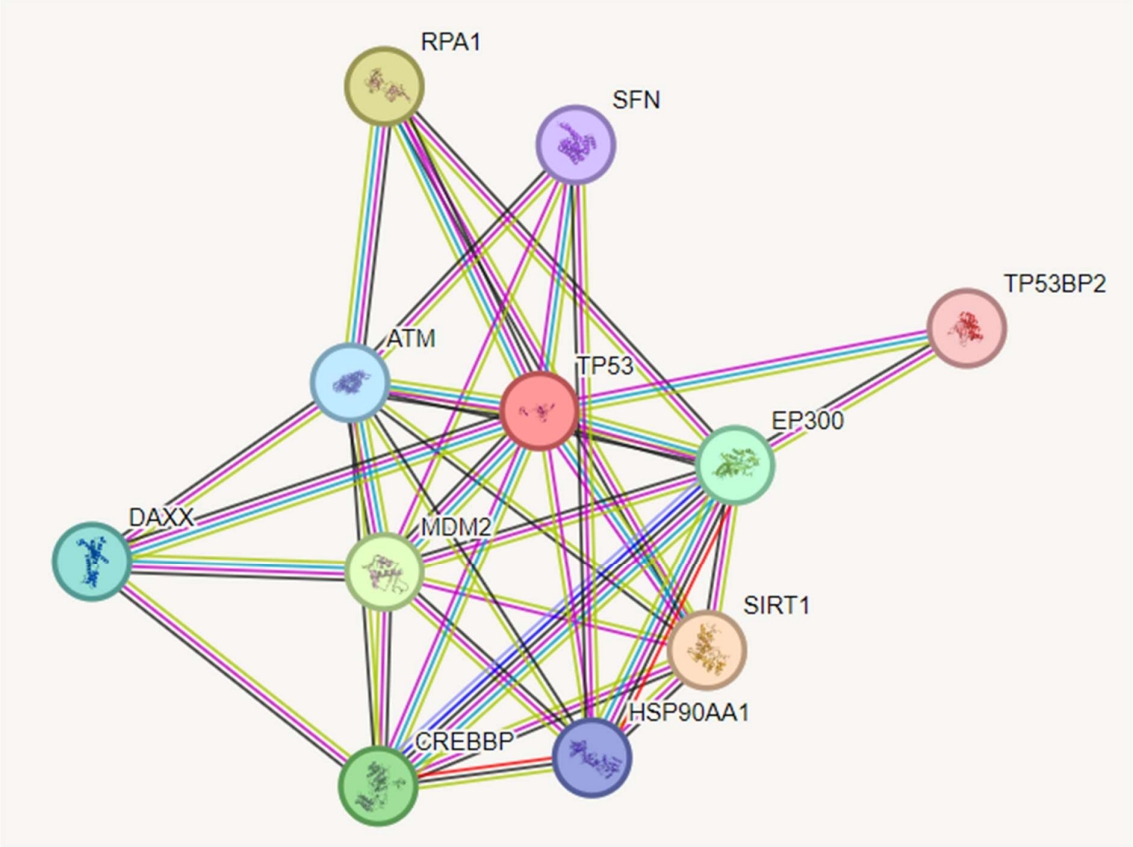


Fig. VI: STRING database results demonstrating pro-pro interaction of p53 with other proteins

4.8. Protein-protein docking

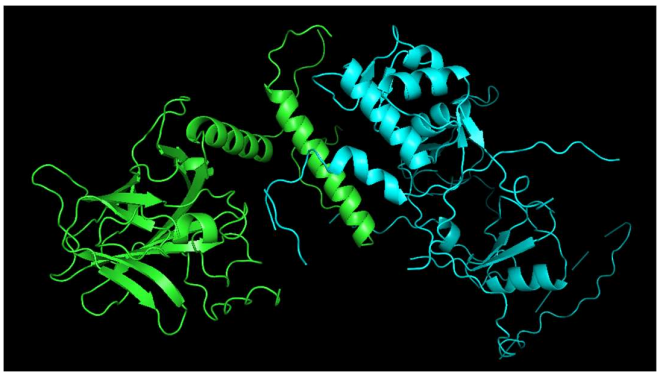
Docking results were analyzed to infer the most deleterious single nucleotide missense mutations in the wildtype p53 amino acid sequence. PDB structures of the protein were retrieved from AlphaFold software and ligand binding sites of the p53 protein were obtained through Ftsite software. Three binding sites were identified. PyMol software was then used to visualize the sites. HDOCK server was used to obtain data on docking scores of SNPs with MDM2. Results showed an increase in the binding affinities of all the variants with MDM2. Wild type p53 showed a docking score of -226.64 while variant Q167P showed highest score of -278.21 and P98R showed lowest score of -229.92 (Table IV). The top three docked structures are depicted in Fig. VII. The same was performed for p53 variants and dsDNA of the

p53RE in Bax gene promoter, however, variants showed very less devation in the binding affinity as compared to the wildtype p53. To further test our results, docking was performed with Bax protein also as p53 can interact with both (1) Bax gene promoter, and (2) Bax protein, p53 variants showed varied affinity with Bax protein as compared to WTP53 which showed a affinity of -243.58, while the highest affinity score was obtained for variants G154R (-250.46), P250T (-250.15), T230P (-249.73), and P98H (-249.17) and the lowest for variant V97D (-234.76).

OBJECTIVE-1

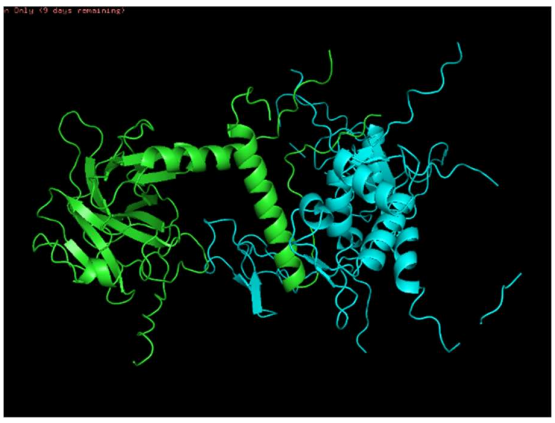
Table IV: Docking Score of p53 Variants with MDM2

dbSNP ID	Mutation	Docking score with MDM2
WT		-226.62
rs2151020578	P250S	-233.58
	P250T	-247.96
rs1597365431	T230P	-234.81
rs1319163924	Q167P	-278.21
	G154R	-246.82
rs1245723119	P98H	-234.09
rs1597374015	P98R	-229.92
rs730881995	V97D	-233.75

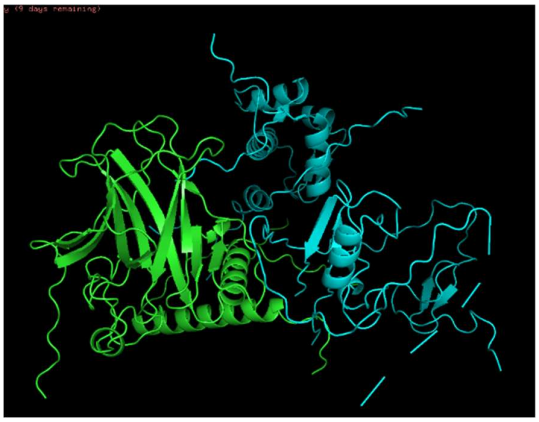


(A) WTp53

(B) Q167P



(C) T230P



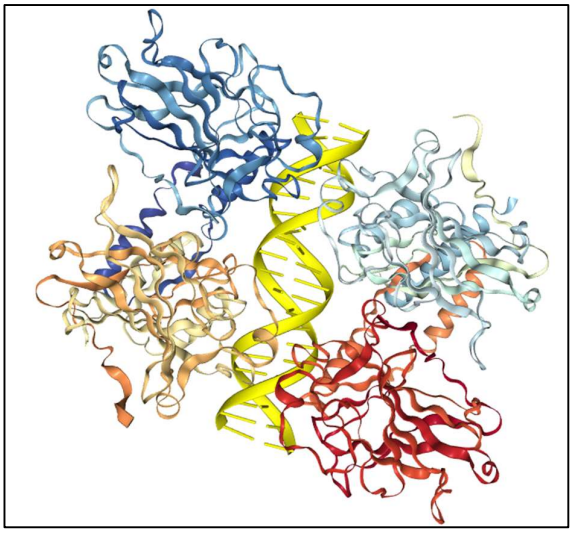
(D) G154R

Fig. VII: Showing top three docking results (A) WT p53-MDM2 (-226.62) (B) Q167P-MDM2 (-278.21) (C) T230P-MDM2 (-247.96) and (D) Q154R-MDM2 (-246.82). Green is depicting p53 variant while blue MDM2

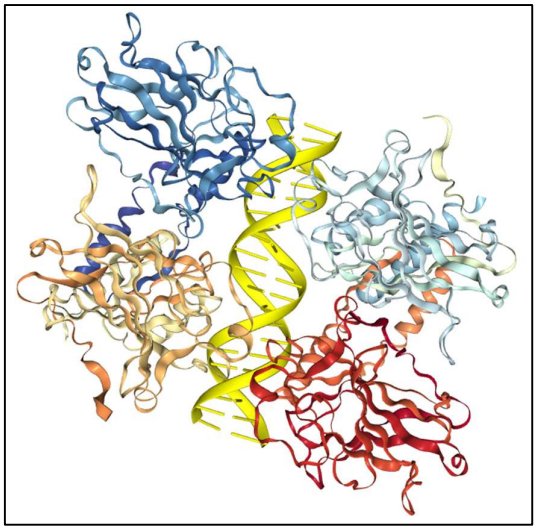
OBJECTIVE-2

Table V: Docking score of p53 variants with Bax gene promoter

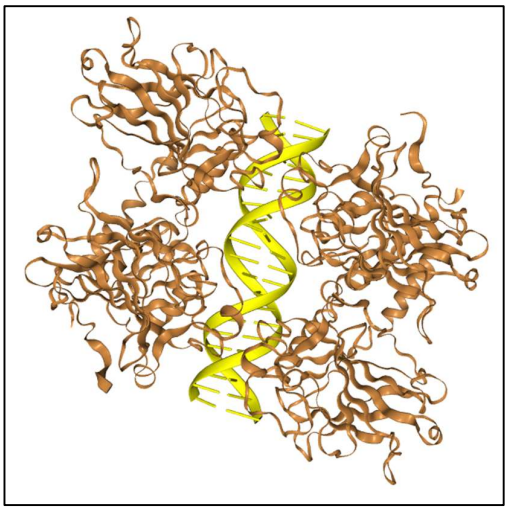
dbSNP ID	Mutation	Docking score with Bax gene promoter p53RE
WT p53-Bax		-416.66
rs2151020578	P250S	-412.58
	P250T	-412.72
rs1597365431	T230P	-416.68
rs1319163924	Q167P	-414.37
	G154R	-417.76
rs1245723119	P98H	-418.38
rs1597374015	P98R	-418.38
rs730881995	V97D	-416.77



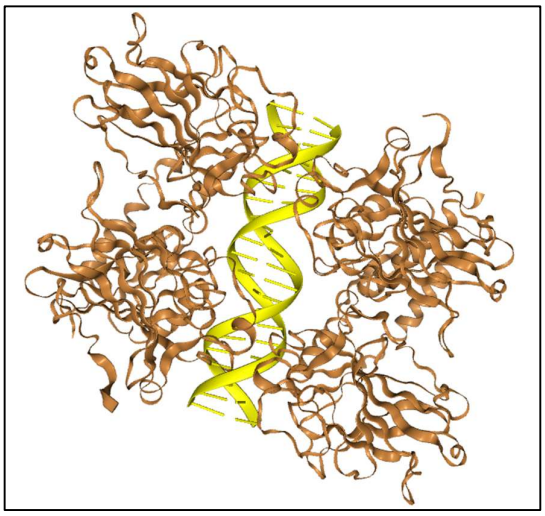
(A) WTP53



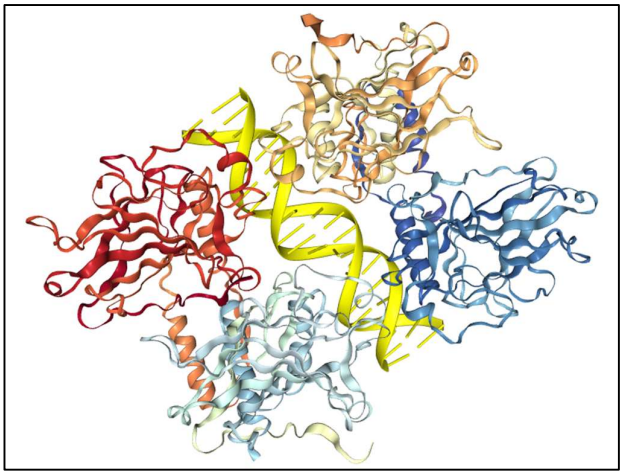
(B) P250T



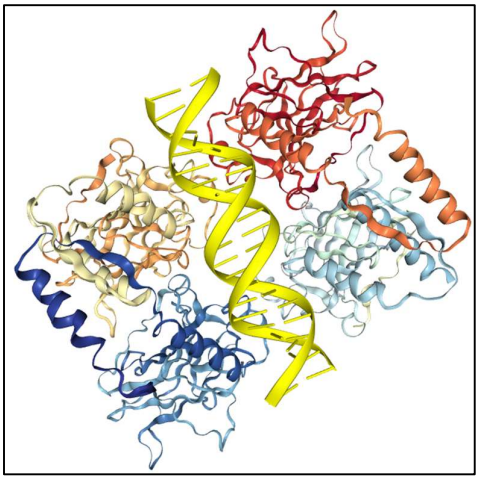
(C) P250S



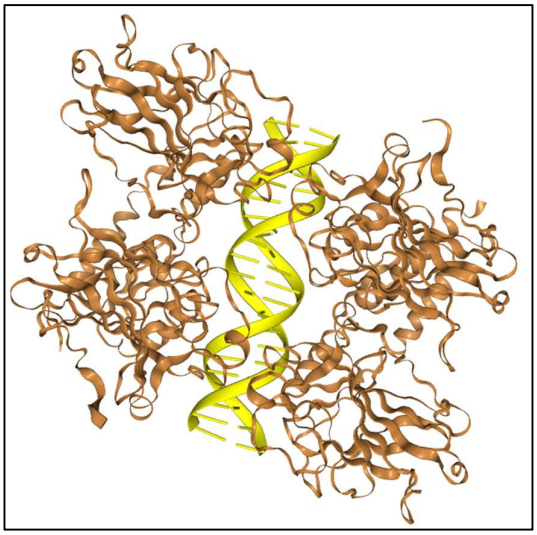
(D) Q167P



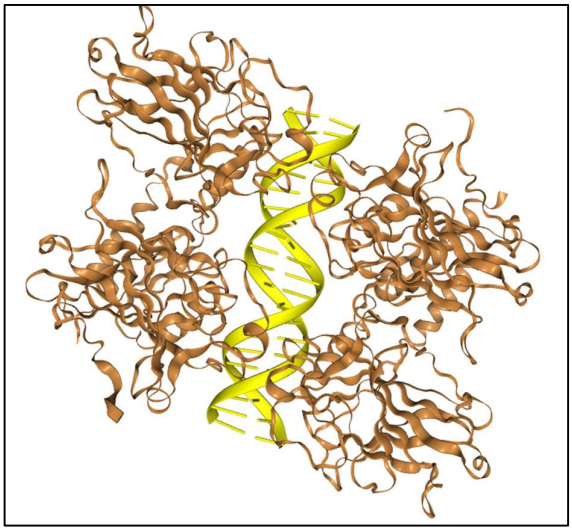
(E) P98H



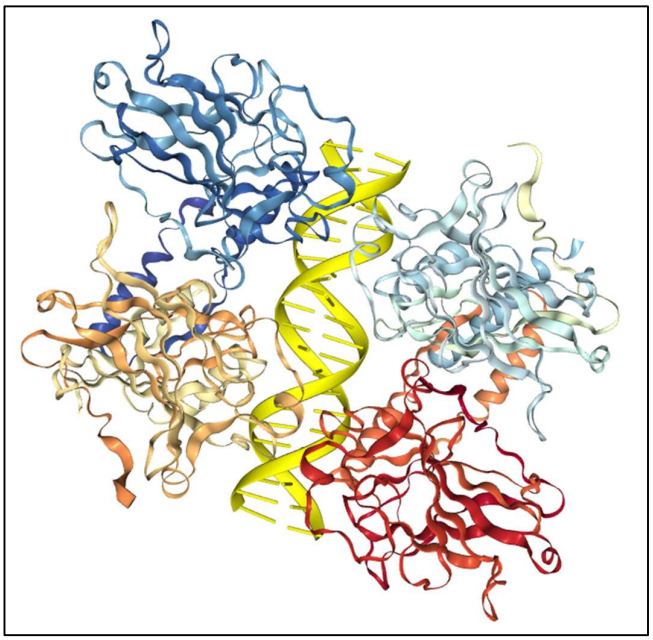
(F) P98R



(G) G154R



(H) V97D

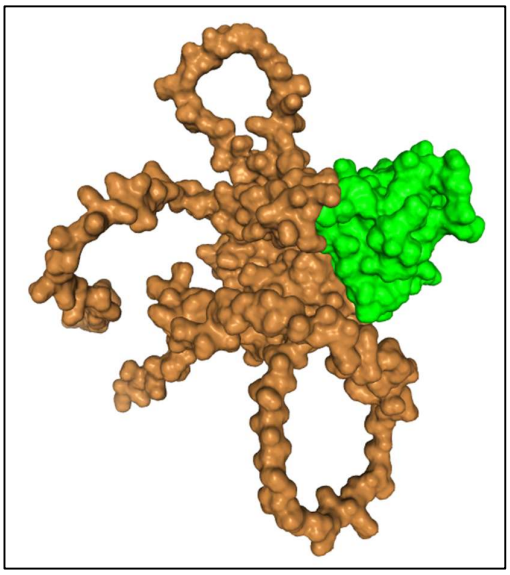


(I) T230P

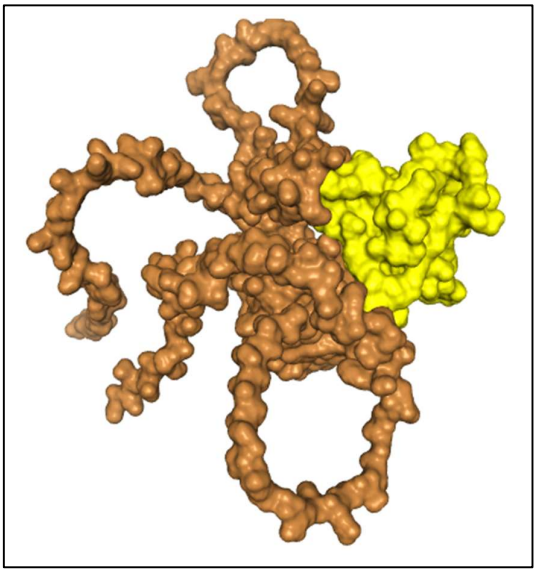
Fig, VIII: Results of HDOCK server showing binding affinities of the p53 variants with p53 response element of Bax gene promoter. WTp53 showed a binding affinity of -416.66 while variants P98H and P98R showed a slightly higher binding of -418.38 whereas, variant P250S and P250T showed lowest affinity of -412.67 based on the docking score.

Table VI: Docking score of p53 variants with Bax protein

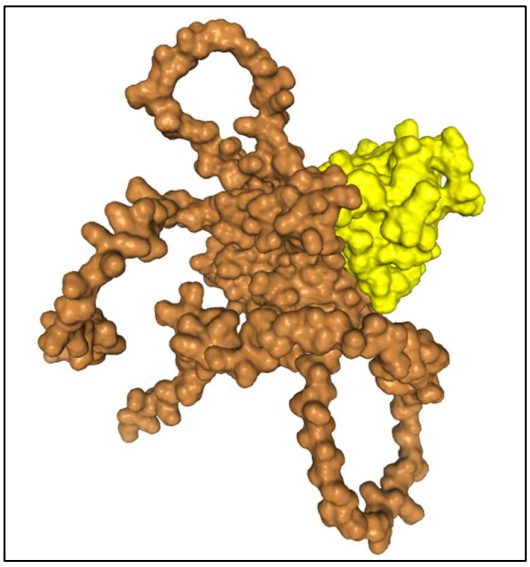
dbSNP ID	Mutation	Docking score with Bax protein
WTp53		-243.58
rs2151020578	P250S	-248.99
	P250T	-250.15
rs1597365431	T230P	-249.73
rs1319163924	Q167P	-244.41
	G154R	-250.46
rs1245723119	P98H	-249.17
rs1597374015	P98R	-246.17
rs730881995	V97D	-234.76



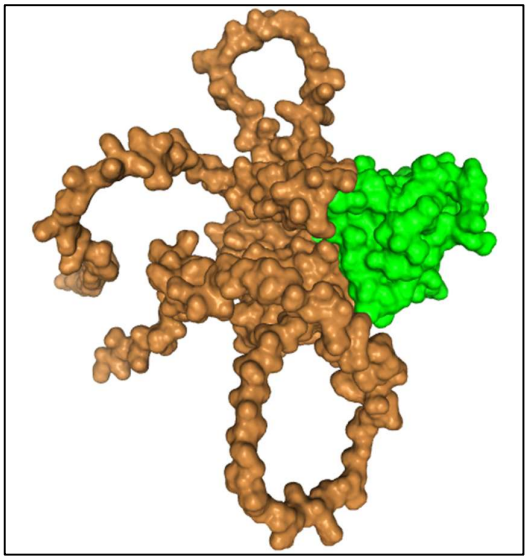
(A) WTP53



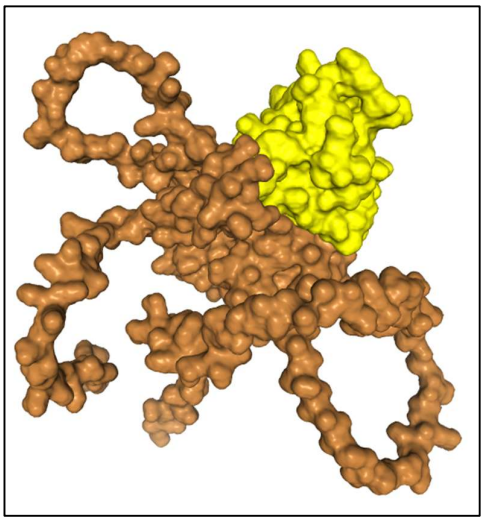
(B) P250S



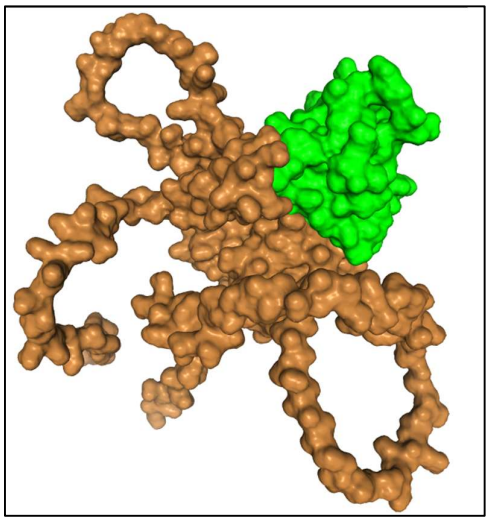
(C) P250T



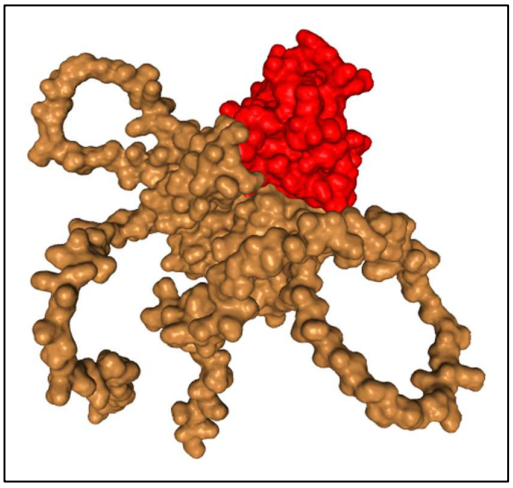
(D) G154R



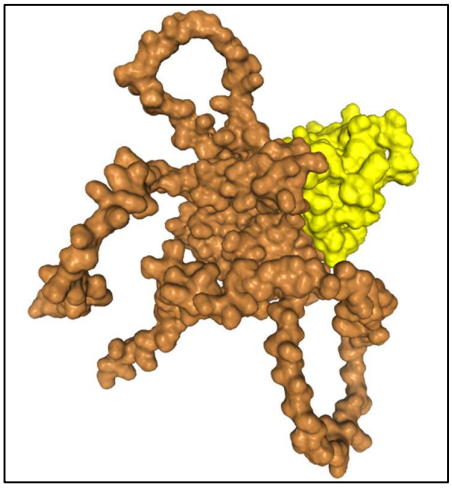
(E) P98H



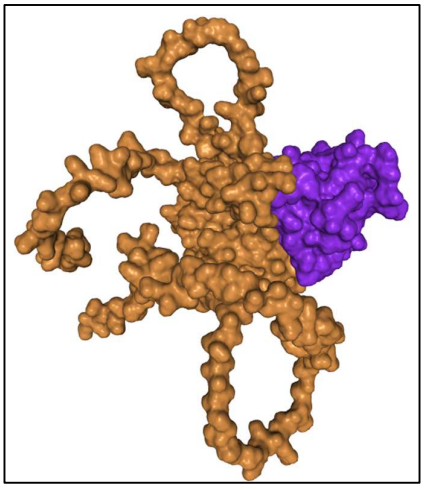
(F) P98R



(G) Q167P



(H) T230P



(I) V97D

Fig. VIII: HDOCK server results depicting the docking interface between p53 variants and Bax protein. As per the docking score WTP53 showed a affinity of -243.58, while the highest affinity score was obtained for variants G154R (-250.46), P250T (-250.15), T230P (-249.73), and P98H (-249.17) and the lowest for variant V97D (-234.76).

4.9. Result interpretation

Table IV depicts the docking score of the eight p53 variants with MDM2 which is an inhibitor of p53 and mark it for proteasomal degradation under normal conditions. It can be inferred from the results that all the variant showed slightly higher affinity towards MDM2 as compared to WTp53 and thus bind more firmly with MDM2 making them more unstable in the normal conditions. However, some variants like P250S, T230P, P98H, P98R, and V97D showed a relatively low binding with MDM2 as compared to other variants.

On the other hand, table V summarizes the results of HDOCK between p53 variants and Bax gene promoter, however, there is no significant difference between the binding score of WTP53 and the variants. This observation can be explained by assuming that the selected mutations may cause a decrease in the overall stability of the protein but they do not affect the DNA binding capability of the protein as they may not play a significant role in the protein-DNA binding. In p53 DNA binding domain there are only certain amino acid residues which participate in the protein binding with the DNA i.e. His193, Lys120, Asp210, Ser240, Ser241, Arg248, Arg273, and Ala276.

Docking studies of the p53 variants with the Bax protein which are summarized in the table VI revealed that WTp53 showed an affinity of -243.58, while the highest affinity score was obtained for variants G154R (-250.46), P250T (-250.15), T230P (-249.73), and P98H (-249.17) and the lowest for variant V97D (-234.76). It has been reported in past studies that p53 transiently bind with the Bax monomer and leads to activation and oligomerization which lead to mitochondrial membrane dissolution and cytochrome-c mediated apoptosis. It can be concluded from the data in table IV, V and VI that variants like P98H and P98R which has comparatively low affinity towards the inhibitors like MDM2 and high affinity towards pro-apoptotic proteins like Bax can influence the P13K/AKT pathway and regulate properties like apoptosis, stemness, and proliferation as p53 is reported to be involved in direct repression of the SOX2 transcription by binding to its promoter and SRR2 enhancer which can be further enhanced by mutation like P98H and P98R which needs to be further tested in vitro and in vivo.

OBJECTIVE-3

4.10. p53/SOX2 Crosstalk: Potential Regulator of Cell Fate

In conditions of DNA damage, it has been reported that p53 suppresses the expression of stemness and pluripotency regulating genes like, SOX2 and Nanog. The P13K/AKT/mTOR pathway also serves as a central link between p53 and SOX2 in regulating stemness, cell cycle, and apoptosis [146]. In case of adult spinal cord injuries, SOX2 plays an important role in reprogramming these cells into induced adult neuroblasts (iANB) which are capable of transforming into mature neurons exclusively into glutaminergic neurons and hold potential to be used as agents of regenerative medicine in case of brain injuries. However, p53 dependent pathways has shown to be involved in inhibiting this process, knock out or knock down of p53 in damages spinal cord cells increased the production of these iANBs by almost two-folds [147]. In cancer cells, p53 suppresses the expression of SOX2 by transcriptionally binding to its promoter and SRR2 enhancer, thus leading to decreased stemness and pluripotency of cancer cells. Conversely, downregulating the activity of p53 significantly enhances the expression of SOX2 in metastatic tumors of different origins [148]. Studies have suggested that p53 can directly influence the expression of SOX2 by binding to its promoter and enhancer regions, however, it can also regulate its expression in an indirect manner by modulating the miRNA biogenesis via DICER and DROSHA, as revealed by competing endogenous RNA (ceRNA) analysis, forming a regulatory loop [149]. The P13K/AKT signaling pathway is a conserved yet sophisticated pathway which processes the signals from external stimuli to downstream target proteins giving rise to a cascade which is responsible for regulating stemness and tumorigenicity [146]. The pathway is activated when external stimuli like metabolic (i.e. amino acids, insulin etc.) and survival signals (i.e. FGF, IGF etc.) bind with the respective receptor and initiate the conversion of PIP2 to PIP3. PDK1 is then recruited by the newly formed PIP3 on the plasma membrane and activates it [146]. PDK1 then activates AKT or PKB, a Ser/Thr kinase, which plays central role in the signaling by regulating various cellular functions like glucose metabolism, cell-cycle regulation, anti-apoptotic pathway, and stemness. AKT further stimulate another important protein complex known as mTORC1 which phosphorylates the 4E-BP1, which is translational co-factor, and acts a multifunctional unit in regulating protein synthesis [146]. An antagonist to this P13K/AKT pathway is a phosphatase i.e. PTEN, which helps in keeping the activation of the pathway in check and controls the turn over number of AKT. Two of the most frequently mutated proteins in the human cancers are PTEN and p53. In mice embryonic fibroblasts, it has demonstrated that p53 can directly bind to the p53 response element in the PTEN promoter and upregulate its expression and activate p53-mediated apoptosis. PTEN protein is also known to bind with tetrameric p53 and stabilize it while promoting its transcriptional activity which can potentially repress the transcription of SOX2 as p53 can directly bind to p53RE in SOX2 promoter and one its enhancer regions i.e. SRR2. Additionally, AKT facilitates the nuclear entry of MDM2 by activating it via phosphorylation which mark p53 for proteasomal degradation, whereas p53 promote degradation of both AKT and MDM2 in a caspase-dependent manner [146]**.** This interplay between the two signaling pathways mediate the balance between proliferation and programmed cell death. In dormant cells when cell mass needs to be minimum to support survival over growth and proliferation, the P13K/AKT pathway is downregulated. On the other hand, in stem cells where regeneration is required with growth and proliferation the pathway is upregulated which helps in wound healing.

5. CONCLUSION AND FUTURE PERSPECTIVE

p53 is a tumor suppressor and said to be associated with various cellular and biological processes like cell cycle, DNA repair, Apoptosis and regulating genome stability. It has been observed that its mutation is involved in the induction and progression of many types of cancers. Reportedly cancers like lung cancer, breast cancer. Despite extensive research being done to study the molecular mechanism of p53 activity and its association with other protein mediators of programmed cell death and DNA repair, certain variants of p53 and their structural properties are not well explored. Here, we have tried to investigate such variants of p53 and P250S, P250T, T230P, Q167P, G154R, P98H, P98R and V97D were found to be most deleterious amongst the others. It is shown through past studies that p53 expression is upregulated in case of PD which leads to rapid death of nerve cells which secrete Dopamine in SNpc part of the midbrain which is a hallmark of the disease. Thus, the result of this study theoretically suggests that such mutations may be potentially introduced and targeted for minimizing the motor symptoms of PD as the mutants showed increased binding affinity towards MDM2 that can retain p53 in its inactive state. It can be concluded that there would be a decrease in the degree of apoptosis in the neuronal cells if these specific mutations are introduced within the p53 amino acid sequence. Docking scores of p53 with p53 response element in the promoter of Bax gene did not show much difference between the wild type and mutants despite mutants having low structural stability. We hypothesize that this was observed because p53 bind to its response element with a conserved set to sequences present in the tetramerization and DNA binding domain i.e. 120-280 amino acids which do not have the identified mutations and thus do not significantly affect the binding affinity of the protein to DNA. However, it has been shown through biochemical studies that p53 can directly bind to Bax protein also, rather than binding to its promoter. The results showed that the binding varied among the mutants and the Bax protein, suggesting that certain p53 mutation, not all, may cause its increased affinity towards Bax protein in the cytoplasm leading to its excessive activation and ultimately permeabilization of the mitochondrial membrane and release of cytochrome c, activating a cascade of apoptosis.

SOX2 expression is important for carrying out biologically essential processes like embryogenesis but its overexpression has also been reported to be involved in progression of many cancers like GBMs, lung carcinoma, breast cancer etc. In GBM, SOX2 overexpression results in various cancer phenotypes like cancer stem cell-like properties, tumor aggression, poor prognosis, drug resistance, invasion and migration which lead to poor prognosis and low survival rate of patients. Thus, developing effective therapeutic strategies which target SOX2 are of great significance in combating gliomas. Small molecules which can directly interact with SOX2 and inhibit its expression and activity are yet to explored which promises great outcome. One such approach could be use of PROTACs (Proteolysis targeting chimeras) which act as linker and mark a particular protein for proteasomal-dependent degradation by ligating it with cullin-RING ligase [1][12]. In recent times, non-invasive peptide-based immunotherapy for treatment of GBM are being explored but there is still need to find such peptides which can be effectively utilized as biomarkers for the cancer. Furthermore, SOX2 is localized and differentially expressed in the nucleus and evidence shows that its overexpression in the nucleus along with some other proteins like Ki67/MIB, CD-133, and MUSASHI has been reported in many cancer types including gliomas. Thus, identification of antagonistic molecules which can restrict the nuclear import of SOX2 by inhibiting the SOX2-importin interaction can

be a novel therapeutic approach against GBM. Previous literature has also suggested that the role of SOX2 in CSC should be studied based on specific SOX2 positive cancer cell rather than overall elevated levels of SOX2 in different cells [1][12]. There are other questions also which can be of interest for exploration and answering which can interesting results like why SOX2 is differentially expressed only in nucleus of cancer cells including GBM? What is the role of SOX2 in transdifferentiation of cell lineages especially cancer cells and how knocking down SOX2 will affect this process? [79] Additionally, SOX2 targeting therapeutic strategies can be explored in this regard we can emphasize on (1) identification of potent SOX2 inhibitors; (2) Designing drugs targeting the various pathways affected by SOX2, currently Rapamycin and Cyclopamine are being used; (3) SOX2 has many downstream target genes which are associated with regulating glioma cancer phenotype but inhibitors targeting those genes are not being explored; (4) Exploring SOX2 crosstalk with other transcription factors and drug repurposing strategies; (5) Identifying non-invasive peptides for immunotherapy; (6) Utilizing gene knockdown molecular techniques like CRISPR, RNAi etc. to downregulate overexpressed SOX2 in gliomas; and finally, (7) SOX2 is overexpressed and localized in the nucleus in many CSCs and GSCs thus identifying antagonistic molecules to inhibit the Nucleus import of SOX2 by exploiting the SOX2-importin interaction. Although preclinical studies have demonstrated SOX2 as potent therapeutic target for GBM progression, there are negligible clinical trials which target SOX2 in GBM. Finally, role of p53/SOX2 axis should be explored in different neurological diseases like brain cancer and neurodegeneration to better understand the pathophysiology of brain tumors. The axis shares the P13K/AKT which can serve a crucial link in developing therapeutic molecules and drug to regulate the cell fate in Neurons and help in tackling the diseases which arise from the dysfunction of this pathway.

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LIST OF PUBLICATIONS

1. Conference paper:

H. Vardhan and P. Kumar, "In silico analysis of deleterious p53 SNPs in Parkinson's Disease," 2025 International Conference on Ambient Intelligence in Health Care (ICAIHC), Raipur Chattisgarh, India, 2025, pp. 1-5, doi: 10.1109/ICAIHC64101.2025.10957514.

2. Poster:

Harsh Vardhan[1], Pravir Kumar[1], "P53 msSNPs: A Computational Analysis of Bax-dependent Apoptosis in Neurodegeneration"

Presented at: SNCI, Jamia Hamdard, New Delhi

Harsh Vardhan

+91 7206803205
vardhanharsh0323@gmail.com

EDUCATION

Master of Science in Biotechnology *2023-Present*
Delhi Technological University (Formerly DCE), New Delhi CGPA: 8.39/10

Bachelor of Science in Life Sciences *2020-23*
Zoology, Botany, Chemistry CGPA: 8.721/10
Hansraj College, University of Delhi, New Delhi

Senior Secondary (10+2), CBSE *2020*
Biology, Physics, Chemistry, English, Hindi Percentage: 93.2%
Kendriya Vidyalaya Jhajjar, Haryana

Marticulation (10th), CBSE *2018*
Science, Mathematics, Social Science, English, Hindi Percentage: 78.6%
Kendriya Vidyalaya Jhajjar, Haryana

RESEARCH EXPERIENCE

Two-months internship at DSKC, Miranda House, University of Delhi *June-July, 2020*

- Worked with Groundnut bud necrosis virus (GBNV), isolated from infected cowpea plant leaf
- Isolated N-gene fragment and cloned in E.coli bacteria for expression analysis

Skills obtained:

- RNA isolation and preparation for cDNA synthesis
- Utilizing computational tools to retrieve gene data, primer designing, and data analysis
- Techniques like: plasmid isolation, PCR, colony PCR, transformation, primer designing, restriction digestion, gel electrophoresis, autoclaving

15 days hands on training at Environmental Biotechnology laboratory, DTU *December, 2023*

- Used E.coli bacteria to learn about different microbial culturing and maintenance techniques
- Learned about different protein analysis techniques and instruments like HPLC, distillation protein analyzer, spectrophotometry for proteomics studies

Skills obtained:

- Microbial culturing techniques: plating, streaking, transformants selection
- Instruments: HPLC, Laminar air flow, autoclave, protein analyzer, spectrophotometer

2 days workshop on Computational Biology tools and techniques at DTU *2024*

- Utilized computational tools for genomic data analysis, BLAST, homology modeling, sequence analysis, protein mutation, protein structure prediction and stability analysis
- Learned about different genomic and proteomic databases for information retrieval and application in research and mutational studies

10 days hands on Workshop and Symposium at SNCI, Jamia Hamdard, New Delhi *April, 2025*

- Theme: "Translational Neurochemistry: Bridging Basic Science and Clinical Application"

Learned about:

- Neural toxicity assessments for nanomaterial used in different model organisms in vivo and in vitro
- Behavioral aspects of neurodegenerative diseases and current therapies
- Brain stem cells and cell/organoid cultures as models for disease assessment
- Different techniques used in neurological research like, Silver staining, Y2H, CHiP, Western blotting, ELISA, AI empowered microscopes
- Model systems used in neuroscience like, Mice/rodents, *C. elegans, Drosophila, Chick embryo for drug testing, and Zebrafish*

PUBLICATIONS

- *Published*

***Conference paper*:** H. Vardhan and P. Kumar, **"In silico analysis of deleterious p53 SNPs in Parkinson's Disease,"** 2025 International Conference on Ambient Intelligence in Health Care (ICAIHC), Raipur Chattisgarh, India, 2025, pp. 1-5, doi: 10.1109/ICAIHC64101.2025.10957514.

Poster presentation: **"p53 msSNPs: A Computational Analysis of Bax-dependent Apoptosis in Neurodegeneration"**

Society of Neurochemistry India (SNCI), Jamia Hamdard, New Delhi

- *Unpublished*

Research paper: **"Unraveling the enigma of Lipoxygenase in Glycine max (soybean) during plant defense against herbivory"**

Department of Botany, Hansraj College, University of Delhi, New Delhi

Submitted for publication in: Physiology and Molecular Biology of Plants

Research paper: **"DFT investigation on theoretical spectroscopic, ELF, MEP, Hirschfeld Surface, and Molecular Docking Studies of Dibenzothiophene"**

Poster presented to NAAC committee

Department of Chemistry, Hansraj College, University of Delhi, New Delhi

Review paper: **"Unraveling the role of SOX2 transcription factor in Glioblastoma progression: From Cancer Stem Cells to Therapeutic strategies"**

Molecular Neuroscience and Functional Genomics Laboratory, Delhi Technological University, New Delhi

Additional Information

Skills: Molecular and Computational Biology, Critical thinking and Analytical skills

Languages: English and Hindi

Awards and Honors: Member of the Society for Neurochemistry India (SNCI), served as vice-president of Life Sciences Society Hansraj College, GATE (2023 and 2024), CSIR-UGC NET (Category II)(2023)

Interests: Badminton, Poetry, Sketching (Art)

DELHI TECHNOLOGICAL UNIVERSITY

(Formerly Delhi College of Engineering)

Bawana Road, New Delhi, 110042

PLAGIARISM VERIFICATION

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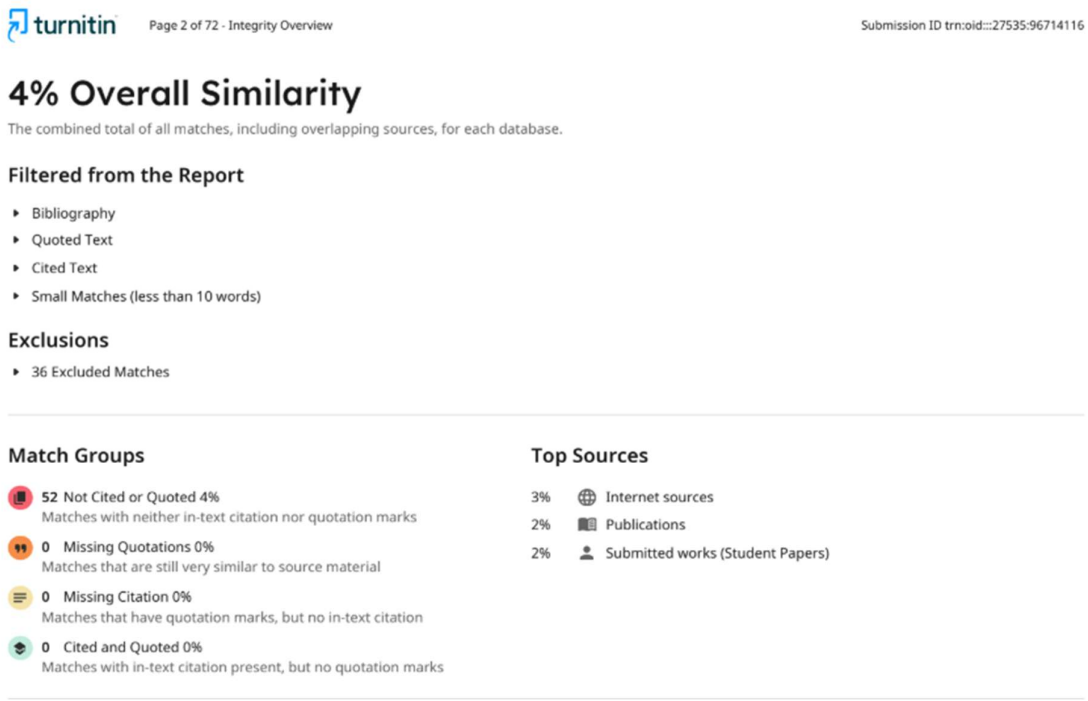

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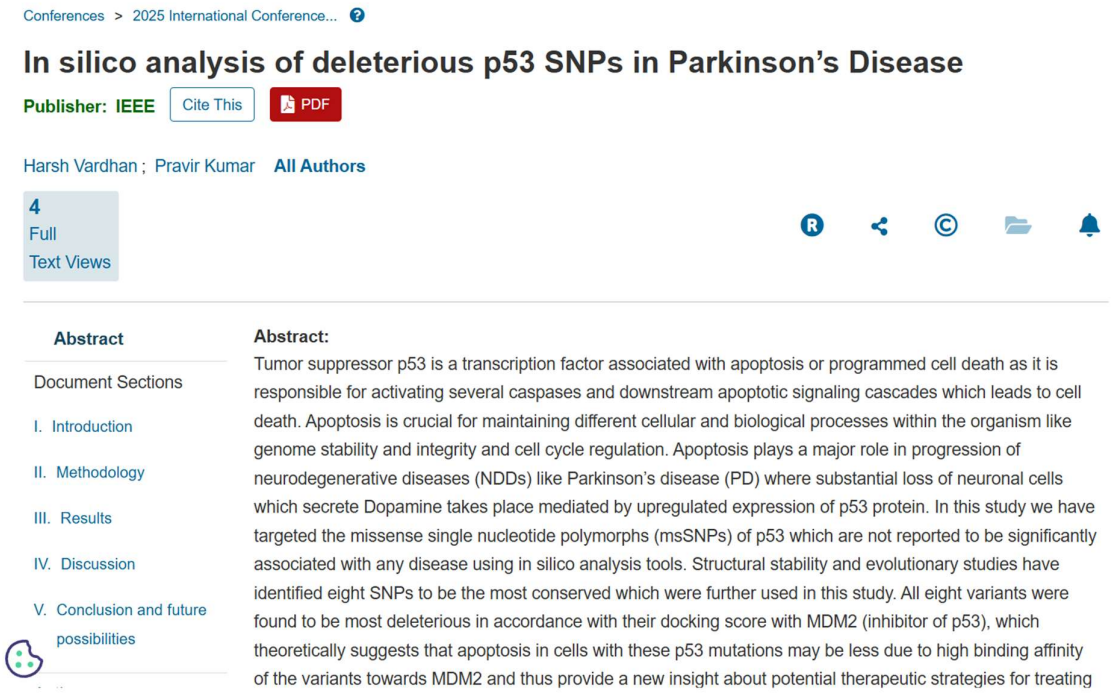
Software used: Turnitin



Conference paper:

H. Vardhan and P. Kumar, "In silico analysis of deleterious p53 SNPs in Parkinson's Disease," 2025 International Conference on Ambient Intelligence in Health Care (ICAIHC), Raipur Chattisgarh, India, 2025, pp. 1-5, doi: 10.1109/ICAIHC64101.2025.10957514.

Certificate of participation in the workshop and symposium

CBME, IIT-Delhi, New Delhi

January 2025

Jamia Hamdard

SNCI Workshop

Translation Neurochemistry: Bridging Basic Science and Clinical Application

Certificate of Participation

This is to certify that Dr./Ms./Mr. *Mr. Harsh Vardhan*

of **Delhi Technological University** has

contributed as **Participant** in SNCI Workshop on **"Translation Neurochemistry: Bridging Basic Science and Clinical Application"** held from 7th April 2025 to 15th April 2025 at Jamia Hamdard, New Delhi

Prof. Suhel Parvez
Organizing Secretary

Prof. Mohammad Akram
Organizing Chairperson

Prof. (Dr.) M. Afshar Alam
Vice- Chancellor

Certificate of participation in the workshop

SNCI, Jamia Hamdard, New Delhi

April 2025

Society for Neurochemistry, India (SNCI) Delhi Local Chapter
&
Department of Toxicology, School of Chemical and Life Sciences
Jamia Hamdard, New Delhi

Certificate of Appreciation

This is to certify that Prof./Dr./Ms./Mr. Mr. Harsh Vardhan
of Delhi Technological University has
Participate as **Delegate** in the Two Days National Symposium on **"Neurochemistry and Emerging Therapeutics: Challenges and Opportunities in Neuroscience"** held from 16th April 2025 to 17th April 2025 at Convention Centre, Jamia Hamdard, New Delhi. He/She has also presented in Young Investigator/Poster Session.

Prof. Mohammad Akram
Organizing Co-Chairperson

Prof. Suhel Parvez
Organizing Chairperson

Prof. Prakash Babu Phanithi
Secretary General (HQ), SNCI

Certificate of participation in the poster presentation

SNCI, Jamia Hamdard, New Delhi

April 2025

Undertaking

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