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Research Article

BMI1: A Potential Biomarker for Alzheimer's Disease

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Abstract

Aging elevates the risk of developing various neurodegenerative diseases, although the mechanisms of neurodegenerative diseases are poorly understood. Alzheimer's disease (AD) is the most common neurodegenerative disorder and it is associated with the selective damage of brain regions and neural circuits which are critical for memory and cognition. Although AD was discovered many years ago pharmacological therapies are still not very effective in preventing disease progression. Inactivation of the Polycomb group gene BMI1 in mice resulted in growth retardation, cerebellar degeneration, and development of a premature aging-like phenotype.

In this study, we induced AD in a rat model by $AlCl_3$ and thereafter checked the expression of BMI1. Intriguingly, protein and gene expression assays showed upregulation in BMI1 expression in the induced AD model. The study indicates that BMI1 could serve as a valuable target for therapeutic intervention in Alzheimer's Disease (AD). Besides that, it can be considered as a potential biomarker for early AD diagnosis, as early detection could significantly impact treatment outcomes.

Keywords: *β-Amyloid; Tau; BMI1; p53; Alzheimer's Disease; Neurodegenrative Disease*

Abbreviations

AD: Alzheimer's Disease; Aβ42: Amyloid Beta 42; PRC1: Polycomb Repressive Complex 1; H2Aub; DAPI: 4'.6-Diamidino-2-Phenylindole; IHC: Immunohistochemistry; PCR: Polymerase Chain Reaction; $AlCl_3$: Aluminum Chloride

Introduction

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder [1]. Old age people are mostly affected by AD. According to US Census Bureau data, between 2000 and 2020, the number will be raised by over 200% and the number of people between 90 - 95 years will be double [2]. Accumulation of β-amyloid results in the formation of Aβ oligomers and fibrils, which are the principal components of the plaque [3]. The symptoms of Alzheimer's disease are memory problems, linguistic problems, psychological and psychiatric changes, and difficulties that occur in daily life activities. Aged people's brains are more vulnerable to amyloid beta 42 (Aβ42) accumulation than young ones [4]. Aging causes amyloid β accumulation in the brain [5]. Specific genes are also downregulated in the brain through the accumulation of oxidative DNA damage at their promoters [6]. So it may be possible that epigenetic gene silencing could trigger AD.

BMI1 is a component of the polycomb repressive complex 1 (PRC1), which promotes chromatin compaction and gene silencing through its E3-mono-ubiquitin ligase activity mediated by Ring1a/b on histone H2A at lysine 119 (H2Aub) [7]. BMI1 knockout in human post-mitotic neurons resulted in amyloid beta peptide secretion and deposition, p-Tau accumulation, and neurodegeneration. BMI1 is involved in embryonic development, cell cycle regulation, DNA damage response, senescence, stemness, and cancer [8-11]. BMI1 encodes a 37 kDa protein composed of 326 amino acids with a highly conserved structure. Regulation of BMI1 expression includes numerous transcription factors and miRNAs, well summarized in Bhattacharya., *et al*. 2015 [8]. Although several correlative relationships have been established, generally the mechanism of action is not well explored.

It has been reported that BMI1 showed reduced mRNA and protein expression levels in AD brains compared to controls, maybe suggesting potentially epigenetic down-regulation [12,13]. Analysis of publicly available RNA-seq data of AD brain samples showed that BMI1 reads were significantly lower in AD compared to controls, vascular dementia, and mixed dementia samples [14].

p53 activity is amplified by BMI1 deficiency during aging or in pathological conditions such as AD. In aged people, at the promoter of antioxidant response (AOR) genes, p53 accumulates, corresponds to a repressed chromatin state, down-regulates the AOR genes, and augments oxidative damage to lipids and DNA [15]. It is reported that knockout BMI1 in human post-mitotic neurons resulted in β-amyloid and tau accumulation, and which causes neurodegeneration. BMI1 activity against neuronal mediated by p53 apoptosis may be closely related to AD. p53 expression is regulated in AD brains, and intracellular levels of Aβ1-42 are activated by its transcription, inhibition of p53 resulted in Aβ1-42- induced neuronal cell death [16,17].

Aim of the Study

In this study, we aimed to investigate the role of BMI1 in Alzheimer's disease development.

Materials and Methods

This study was conducted at Interdisciplinary Brain Research Centre, J. N. Medical College, Faculty of Medicine, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

Animals: In this study, we selected 200-250g weight Wistar rats. Rats were obtained from Central Animal House Facility, Jawaharlal Nehru Medical College, Faculty of Medicine, Aligarh Muslim University (A.M.U) one week before the experiments and housed individually in cages maintained under suitable conditions with temperature (25 ± 1°C), humidity (60 ± 10%) in a well-ventilated room with 12-h light/dark cycle with ad-libitum diet and water. All experimental animal procedures were approved by the Institutional Animal Ethics Committee and carried out as per CPCSEA guidelines India (Reg no. 401/GO/Re/S/2001/CPCSEA).

Rats were randomly assigned into two groups: the control group and the AD group. Each group contained six rats. The control group received saline for 28 days, while the AD group received $AlCl_3$. $AlCl_3$ was dissolved in water and injected into rats through the i.p. (intraperitoneal) route at a dose of 0.71 mg/kg/day for 28 days [18].

Brain isolation of rats: After 28 days of $AlCl_3$ dosing, a behavioral study was done and thereafter, rats were subjected to surgical procedures for the estimation of different parameters. Animals were anesthetized using 75 mg/kg of ketamine (i.p.) and sacrificed using cervical dislocation. Brains were immediately isolated in ice-cold phosphate buffer (0.1 M, pH 7.4). The isolated tissue samples were kept in RNAlater (RNA stabilization reagent, Qiagen) and stored at -20°C for mRNA expression analysis, and in 4% paraformaldehyde (PFA) for histopathological experiments.

Hematoxylin and Eosin (H&E) staining: Brain tissue samples were embedded in paraffin and then this embedded tissue was sectioned into 4 μm thin layers and placed on slides. Slides were dewaxed in xylene for 20 minutes and rehydrated in a series of alcohol-distilled

water solutions (100% - 50% ethanol) for a time interval of 5 minutes and then dipped in distilled water. After this, the sections were stained with hematoxylin and eosin (H&E) for 15 and 5 minutes, respectively. Thereafter, sections were dehydrated by repeating the rehydration steps in the reverse order.

Immunohistochemistry (IHC): Tissue samples were fixed in 4% PFA. After the fixation tissues were dehydrated (in series of ethanol 50% to 100% ethanol xylene, for 30 minutes), and embedded in paraffin wax. The paraffin-sectioned tissues were dewaxed, rehydrated, and then incubated with primary antibodies (0.1 µg/100 µL) against BMI1 and p53 for 30 minutes (Novus mouse monoclonal and Thermo Fisher Scientific polyclonal). The non-covalently bound antibodies were washed out with PBS-T buffer. Then the samples were incubated with secondary antibody (anti-mouse secondary antibody-fluorescein isothiocyanate tagged (Novus biologicals) 0.05 µg/100 µl per sample for 30 minutes and then the washing steps were repeated. The ibidi-mounting medium was used for mounting and images were taken by a fluorescent microscope (Nikon, Tokyo, Japan) [22].

DAPI staining: For DAPI (4′.6-diamidino-2-phenylindole) staining, tissues were embedded in paraffin and cut into 4 µm paraffin sections. The paraffin-sectioned samples were dewaxed, rehydrated, stained with DAPI for 5 minutes, and then washed with PBS buffer. The slides were mounted and images for DAPI were acquired by a fluorescent microscope (Nikon, Tokyo, Japan).

Confocal microscopy: The tissue samples were prepared for immunohistochemistry and examined under a confocal microscope (Zeiss, LSM-780, Germany). Confocal microscopy was done at the University Sophisticated Instrumentation Facility (USIF), Aligarh Muslim University, Aligarh.

Reverse transcriptase PCR: RNA from tissue samples was isolated and purified by the Tri-reagent (Sigma-Aldrich, St Louis, MO, USA). RNA concentration and quality were determined with the help of a spectroscope (Shiamdzu, UV-1800, Japan) by measuring the absorption at the A260/A280 ratio. A total of 1µg of RNA was used to prepare cDNA. Synthesis of cDNA was done by M-MLV reverse transcriptase (Invitrogen, California, USA). After the cDNA synthesis Reverse transcriptase PCR (RT-PCR) (Thermal Cycler, TCS) and Real-time PCR (qRT-PCR) (Applied Biosystems) were done. Beta amyloid and Tau were used as positive biomarkers for AD while BMI1 and p53 were amplified. We used β-actin gene as a housekeeping gene.

Quantitative real-time PCR (qRT-PCR): Real-time quantitative PCR was done by Step-One (Applied Biosystems) with a SYBER green PCR master mix (Thermo-Scientific, Waltham, MA, USA). The reaction mixture contained 100 ng of a cDNA sample and specific PCR primers. The cycle profile included an initial denaturation at 95°C for 10 minutes, followed by a 40-cycle amplification consisting of denaturation at 95°C for 15s, annealing at 60°C for 30s, and extension at 72°C for 30s. Each sample was run in triplicate and the means and standard deviations were calculated. We checked and compared the mRNA expression of the beta-amyloid, Tau, BMI1, and p53 genes in tissue samples. All transcript expressions showed the relative level of the target transcripts to reference transcript was calculated by 2 (^{Ct}ref - ^{Ct}test) [23].

Statistical analyses: All the quantitative analyses were performed using GraphPad Prism 7. The results are expressed as mean± SE by oneway ANOVA calculation.

Results and Discussion

Effect of $AlCl_3$ on neuronal cell morphology: To determine the morphological changes in AD-induced rats, paraffin-sectioned samples on slides were stained with H&E. Nuclei were showing blue and cytoplasm in pink color (Figure 1). In addition, the nuclear DAPI stain showed abnormal nuclei in AD rat brain tissue as depicted in figure 2.

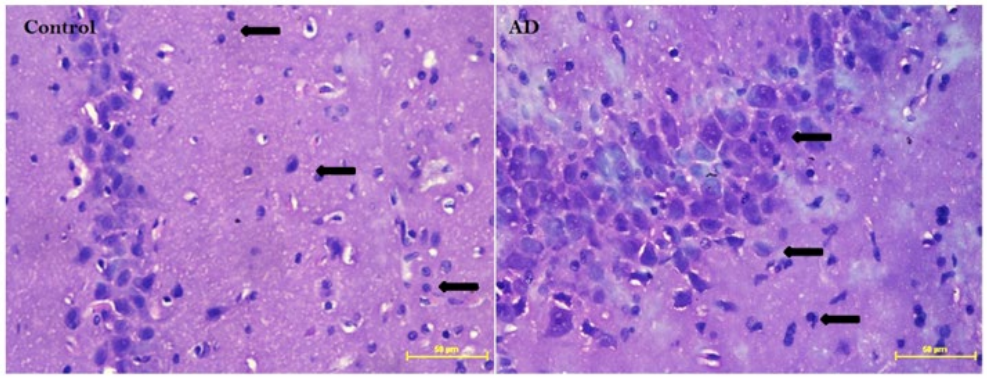


Figure 1: *Effect of AlCl3 on neuronal cell morphology. Control rat tissue (Left) exhibits normal nuclear morphology, appearing round and blue-stained. AD rat tissue (Right) shows abnormal nuclear morphology, indicating cellular damage.*

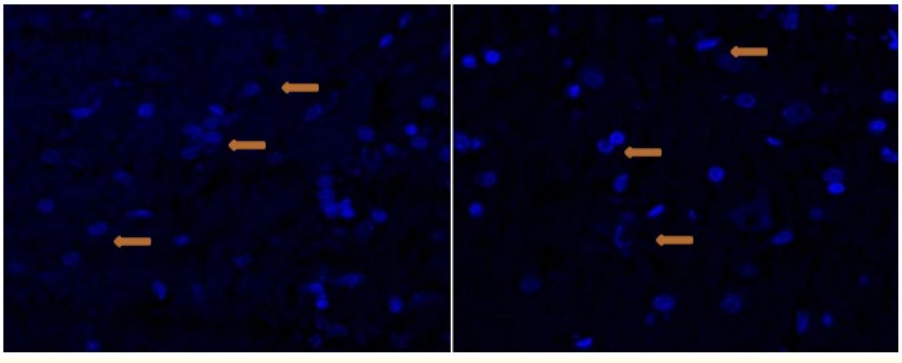
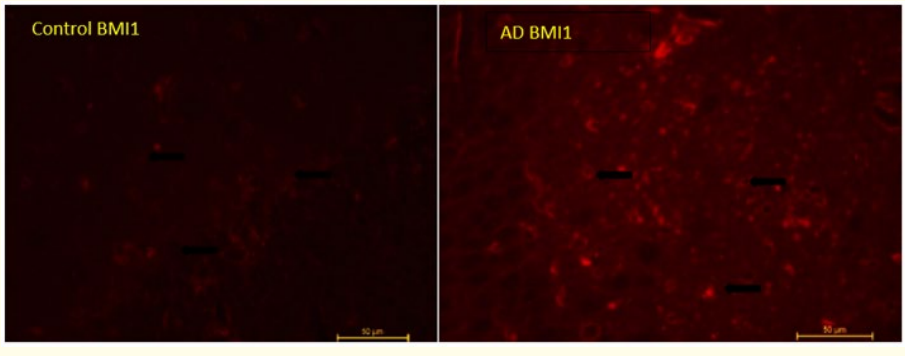


Figure 2: *DAPI: In DAPI stained sections damage and shrinkage nuclei are showing in AD rat tissue samples compared to control rat tissue samples.*

Effect of AlCl3 on neuronal cell BMI1 expression:

The protein expression of BMI1 was assessed by immunofluorescent. The result showed an increase in the expression of BMI1 in AD rat model as compared to control group (Figure 3). In addition, the p53 expression showed low expression in rat AD group compared to control group (Figure 4).



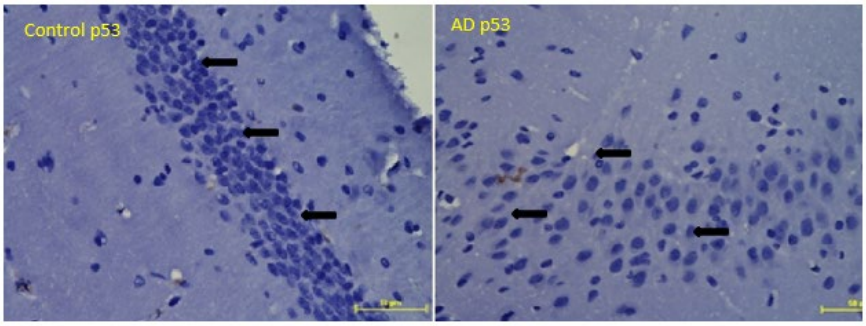


Figure 3: *IHC: In IHC images the AD group showing high expression of BMI1 compared to control and p53 showing low expression in rat AD group compared to control group*

Confocal microscopy: Under a confocal microscope the antibody incubated against the BMI1 and counterstained with 4,6-diamino-2-phenylindole (DAPI) stains were examined (Figure 5).

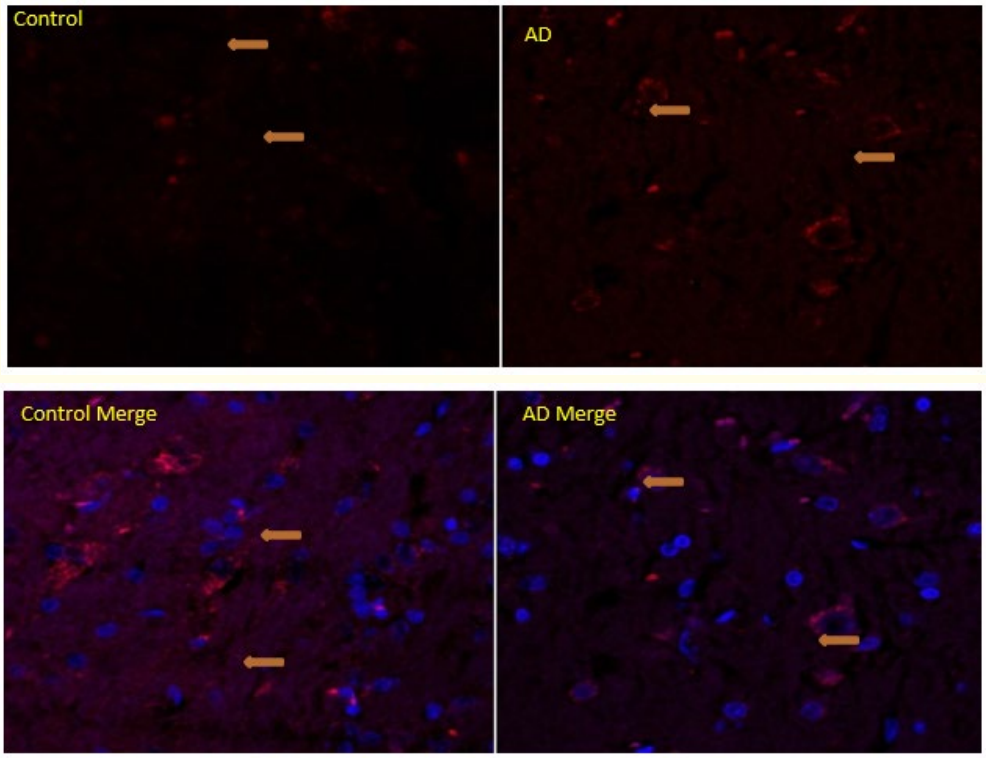


Figure 4: *Confocal microscopy: Under confocal microscopy low expression of BMI1 is showing in AD group compare to control group rat.*

Reverse transcriptase PCR and Real time PCR: In our real time results, we found that BMI1 was highly expressed in AD group compare to control group.Although, it has been reported that BMI1 showed low expression in in AD group. Intriguingly, our results showed high expression of BMI1. However, we did not find any significant cahnges in p53 expression pattern. p53 activity is amplified by BMI1 deficiency but in our results BMI1 expression was not reduced in AD group therefore it might be the the reason for no significant changes in p53 expression in AD group (Figure 6).

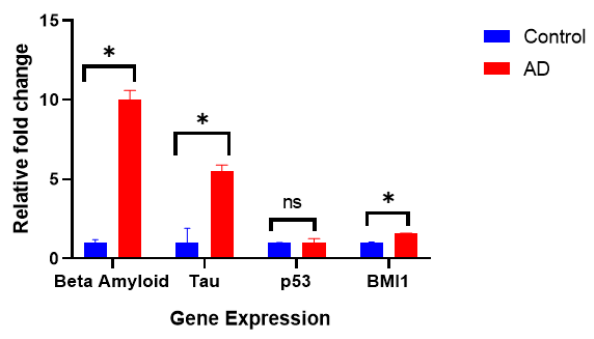


Figure 5: *Real Time PCR: Expression pattern of β Amyloid, Tau, BMI1 and p53 in rats Control and AD Groups. We have found high expression of BMI1 in AD group compared to control. Although we did not find any significant chnages in p53 expression in AD group compared to control group. Results mean ± S.D. analysed by one-way ANOVA.*

In this study, we attempted to explore the role of BMI1 and p53 in AD induced rat model. $AlCl_3$ model is widely used to induce AD in rats. In this study, the AD rat model was induced by $AlCl_3$. After 28 days of dosing, we performed the behavioral test and also checked the expression of β-amyloid and Tau to confirm the effect of $AlCl_3$.

AD is associated with impaired memory and learning so we performed the Morris water maze (MWM) test. The MWM test is widely used for the evaluation of spatial learning and memory [24]. In MWM results we found that $AlCl_3$ caused significant impairment of memory and learning in rats. Our behavioral results were matched with previous studies reported in the $AlCl_3$ model [25].

Previous studies reported low expression of BMI1 in AD patients compared to control. Low BMI1 expression was found in some aging tissues and AD [26]. Likewise, BMI1-deficient models, both *in vitro* and *in vivo*, replicate the important hallmarks of AD. At both mRNA and protein levels, epigenetic deregulation BMI1 reduction seems likely, however, the mechanism remains unknown [14]. A study reported that the central dogma (DNA to RNA to proteins) is not always the case for some genes, mainly those genes which are regulating complex cellular processes. Intriguingly, our IHC and mRNA expression showed high expression of BMI1 in AD group as compared to the control group. Neurogenesis is a complex cellular process. Henceforth, posttranscriptional modifications may be responsible for discrepancies between mRNA and protein expression [27].

AD is accompanied by neurodegeneration and neuronal loss in the frontal cortex, leading to cognitive impairment and dementia [28]. Several factors are thought to trigger neurodegeneration in AD, including cytoplasmic accumulation of β-amyloid proteins [29,30]. p53 appears to mediate apoptosis in primary human neurons expressing A 1-42 [31]. Microglial apoptosis is also mediated by p53 in AD [32]. It has been reported that p53 is downregulated in AD. Similarly, our histopathological results showed low expression of p53 in AD group compared to the control group but real-time RT-PCR results showed no significant changes in AD group compared to the control group. p53 expression is amplified due to BMI1 deficiency but in our results, BMI1 expression was not reduced in AD group. Henceforth, this might be the reason that resulted no significant changes in p53 expression in AD group [33].

Additionally, secretion and deposition of Aβ species, p-Tau accumulation causes inactivation of BMI1 in human post-mitotic neurons and results in neurodegeneration [34]. BMI1 activity against neuronal apoptosis is mediated by p53 and it may be closely connected to AD; while p53 expression is augmented in AD brains, and its transcription is activated by the intracellular levels of Aβ1-42, p53 inhibition impedes Aβ1-42- induced neuronal cell death [35,36]. To prevent neurodegeneration BMI1 is mechanistically required because it represses the microtubule-associated protein tau (MAPT) transcription and obstructs the stabilization of p53 [37].

On the sites of DNA breaks BMI1 is recruited in order to promote DNA damage response (DDR) and repair [38,39]. According to previous studies, BMI1 plays various biological functions in the maintenance of both epigenetic and genetic integrity. BMI1 expression is reduced in neurons but not observed in astrocytes [40]. Notably, the mechanism by which BMI1 regulates p53 activity remains unknown [41].

Conclusion

In this study, we tried to check the role of BMI1 in AD so that it can be used as a biomarker for AD diagnosis. We hypothesize that abnormal BMI1 regulation in the aging human brain may be in result of AD. Further work will be required to decode the mechanism by which BMI1 is regulated and whether this anomaly occurs before or at the time of AD onset. BMI1 repairing activity could represent a therapeutic modality for AD.

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Conflict of Interest

The authors declare that they have no competing interests.

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