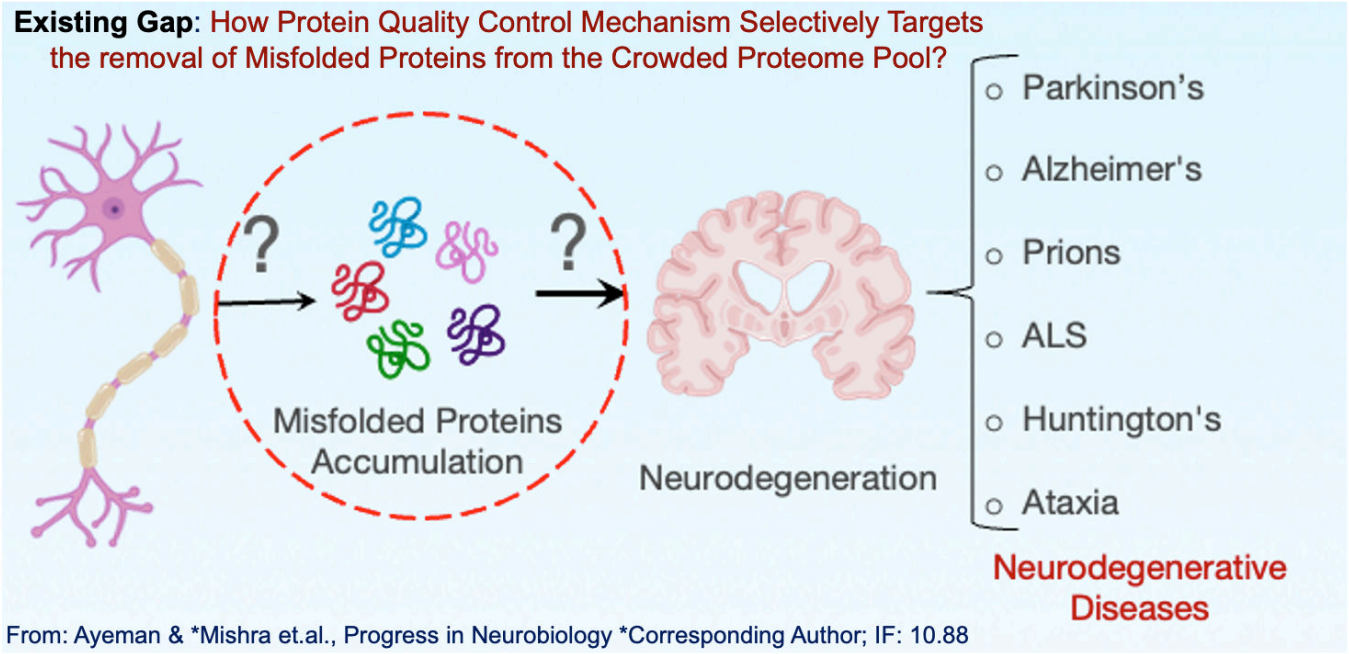
Research Work Applied for Sun Pharma Research Fellowship: Prof. Amit Mishra has done significant work in neuronal protein quality control mechanisms of neurodegenerative diseases. This has been achieved by understanding the quality control functions of selective E3 ubiquitin ligases, which barricade extreme defense against misfolded proteins aggregation. His findings provide a better understanding of this innovative concept that can develop new therapeutic targets for Neurodegeneration and Cancer.

(Date: 07-August-2024; IIT Jodhpur)

Description of Above Presented Research (Entire work was performed in India):

Professor Mishra's innovative research work on selective E3 ubiquitin ligases represents a significant therapeutic outcome in the field of neurodegenerative diseases and aging. His research has unveiled a new concept wherein these ligases serve as the frontline defense mechanism against multifactorial proteostasis failures implicated in these conditions. By elucidating the quality control functions of E3 ubiquitin ligases, Prof. Mishra has provided crucial insights into the molecular pathways involved in misfolded protein recognition and neuronal quality control. This innovative understanding offers the potential for developing novel therapeutic targets aimed at mitigating neurodegeneration and aging-related pathologies. Moreover, Dr. Mishra's studies have demonstrated how E3 ubiquitin ligases, alongside molecular chaperones, play a pivotal role in maintaining overall neuronal homeostasis.

Problem: How the Depletion of Proteostasis Causes Multifactorial Complexity In Neurodegeneration and Imperfect Aging ?



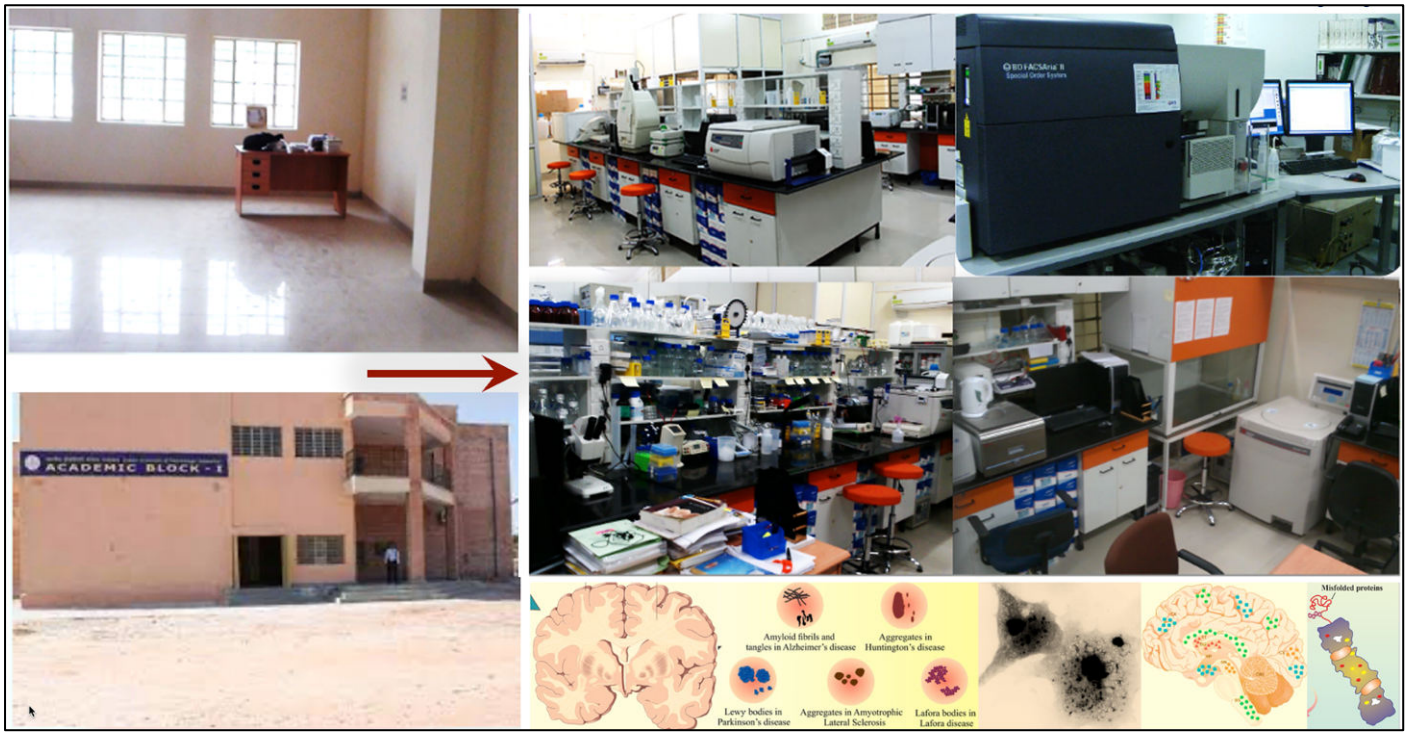
From: Ayeman & *Mishra et.al., Progress in Neurobiology *Corresponding Author; IF: 10.88

Major Contribution: Established a significant concept on selective **Quality Control E3 ubiquitin ligases** those can serve as **early first line of defense** ameliorative measures **against proteostasis failures** implicated in neurodegenerative diseases and imperfect aging.

Dr. Amit has devised unique strategies to modulate proteasomal functions, inducing autophagy pathways and serving as an anti-aggregation program in cellular proteostasis. By harnessing the molecular protein quality control system, Prof. Mishra's innovative approach inhibits aberrant protein aggregation and deregulated proliferation, offering promising avenues for therapeutic intervention. The significant contributions from Prof. Mishra's lab have significantly advanced our understanding of neurobiological approaches to address the multifaceted challenges in neurodegeneration. These findings pave the way for the development of more suitable therapeutic strategies targeting defective proteostasis events specifically linked to late-onset neurodegenerative diseases and aging. Ultimately, the outcomes of his research hold the potential to revolutionize the field, offering hope for effective treatments to mitigate the devastating impacts of neurodegenerative diseases and aging-related disorders on society.

The inherent complexity of cancer and neurodegeneration is due to the presence of multiple signalling and regulatory pathways of the cell, as well as the pleiotropic nature of many biomolecules. We have attempted to investigate and understand this complexity by focusing on the aspect of PQC. The network of E3 enzymes and chaperones interfere cellular proteome and act appropriately to recognise misfolded/unfolded proteins, directing their clearance from the cell to maintain homeostasis. The results from our collective studies indicate the involvement of multiple E3 enzymes – ITCH, MGRN1, and LRSAM1 and chaperone Hsp70 in clearing possible pathogenic misfolded proteins in neuronal degenerative conditions. The dysregulation of these PQC components in neurodegeneration can exacerbate the effect of loss-of-function or gain-of-function of misfolded proteins and aggregates. Trehalose, Itraconazole, Myricetin and lanosterol, natural compounds reported by us as PQC component modulators, can be further investigated for their therapeutic effects on neurodegeneration with the possible benefits of better pharmacokinetics.

(I) Potential Research Findings: Professor Mishra has introduced a novel and significant concept concerning selective E3 ubiquitin ligases. These ligases serve as the initial line of defence in quality control, offering remedies to mitigate multifactorial proteostasis failures linked to neurodegenerative diseases and cancer. Dr. Amit Mishra significantly advanced understanding of neuronal protein quality control mechanisms involved in neurodegenerative diseases. This understanding stemmed from elucidating the quality control functions of selective multifaceted E3 ubiquitin ligases, which actively block misfolded protein aggregation. His findings provide a clear and improved understanding of this innovative concept, paving the way for developing new therapeutic targets for neurodegeneration and cancer. Moreover, these studies clarified molecular pathways for recognizing misfolded proteins based on E3 Ubiquitin Ligases. Amit's findings shed light on the precise molecular mechanism of E3 ubiquitin ligases and molecular chaperones, their involvement in neuronal quality control pathways, and their impact on overall neuronal homeostasis. He devised a distinct mechanism to modulate proteasomal functions, inducing autophagy pathways and serving as the anti-aggregation program for affected cellular proteostasis. Research from his lab proposes that E3 Ubiquitin Ligases can act as the first line of defense against proteostasis failure under different protein conformation conditions.



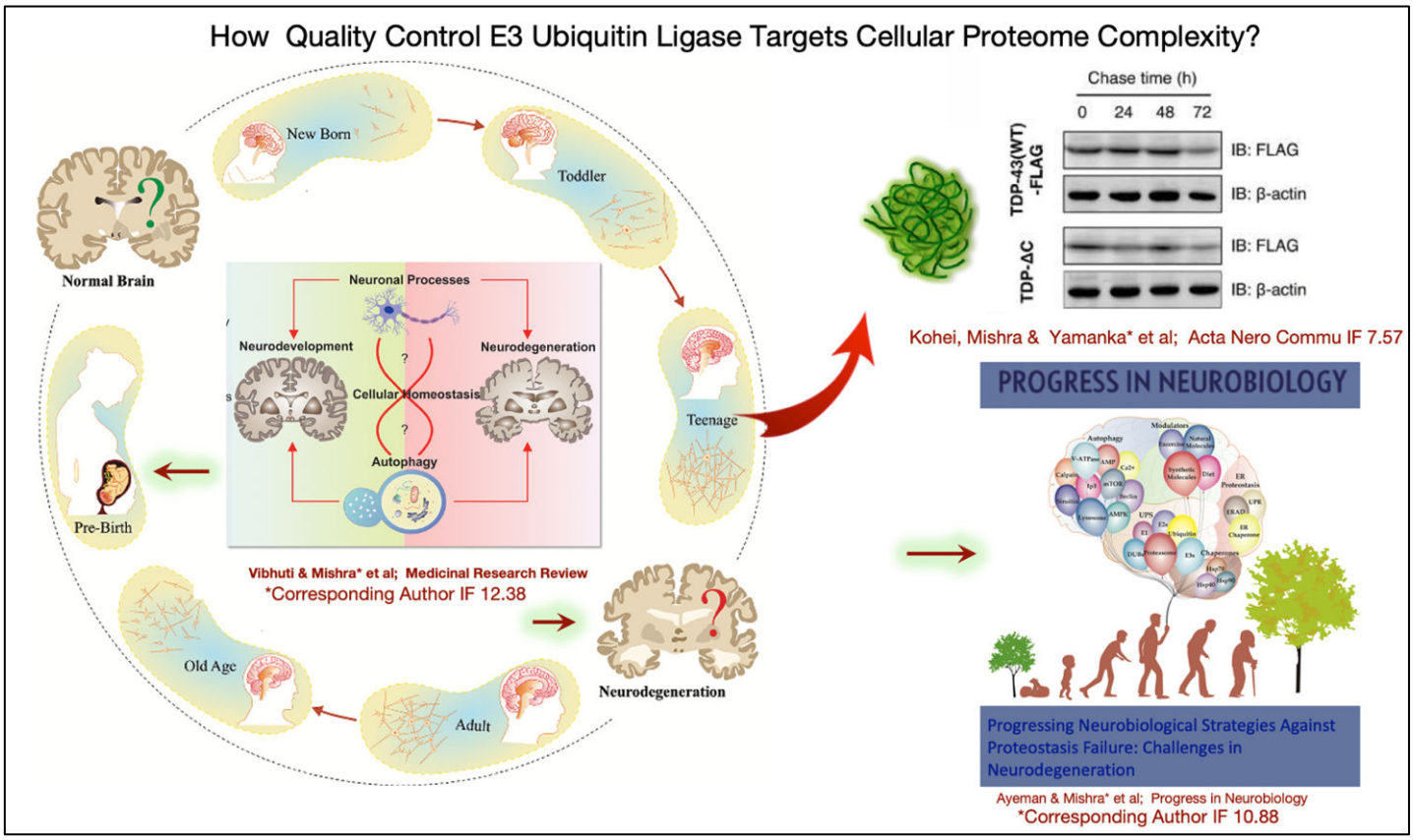
The claimed research work was performed in the Cellular and Molecular Neurobiology Unit which was established from scratch (as shown in figure) and in 2010 and two students from this lab also become Assistant Professor in another IITs.

Amit innovatively developed a method of harnessing the molecular protein quality control system to inhibit aberrant protein aggregation and deregulated proliferation. Significant contributions from his group substantially advanced knowledge of progressing neurobiological approaches against multifactorial challenges in neurodegeneration. Results of our studies may offer more suitable substitute proteolytic machinery therapeutic strategies for defective events specifically linked with late-onset neurodegenerative diseases and cancer.

(II) Descriptions of Key Research Findings: Frequently, aberrations in misfolded protein degradation pathways contribute to structural changes in cellular proteins, leading to their accumulation and the formation of inclusions. Our lab is involved in two important domains and is trying to find out how depletion of proteostasis contributes to the establishment of neurodegeneration and cancer.

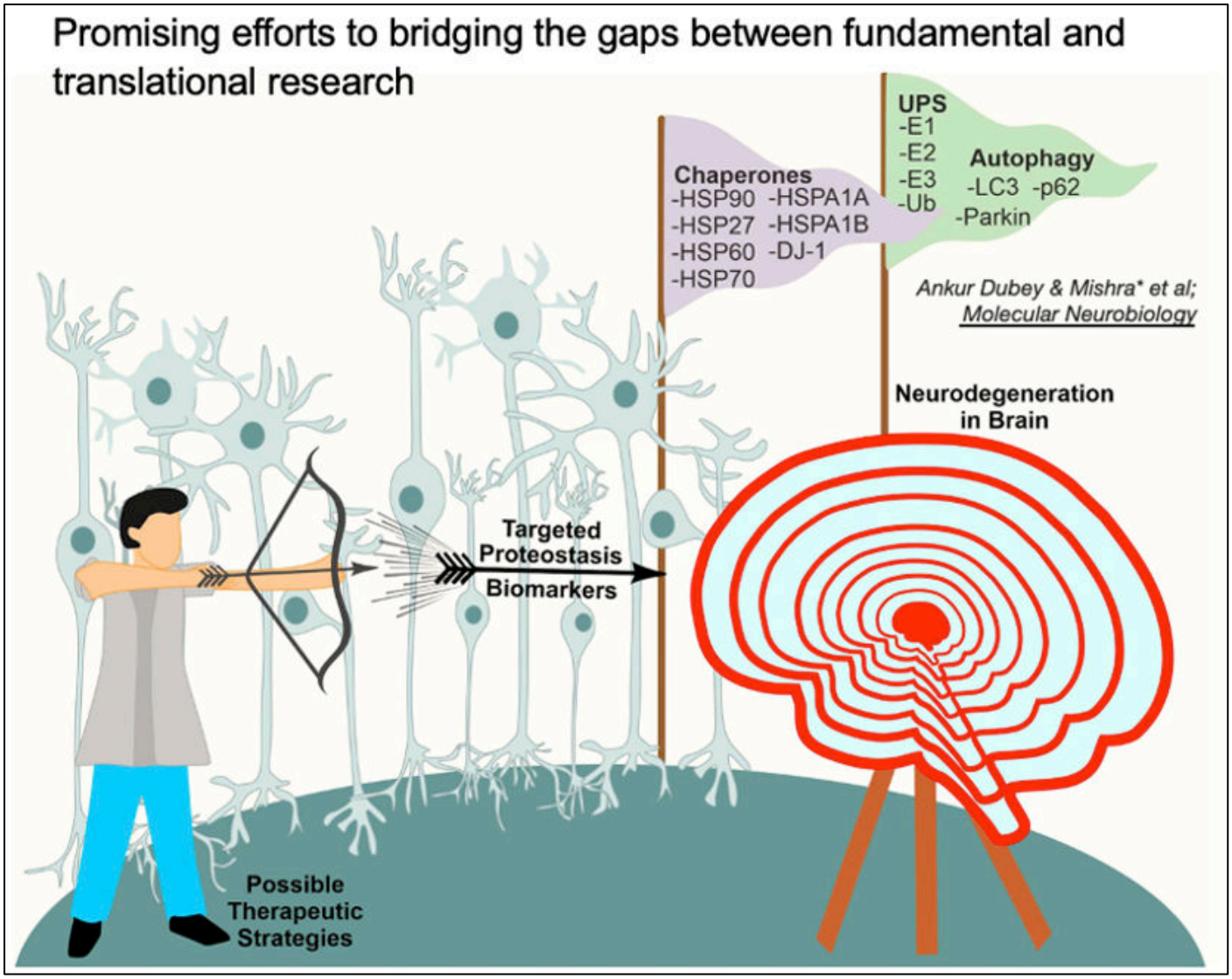
(II A) Proteostasis Failure and Neurodegeneration: Our laboratory has targeted disease-associated proteins, uncovering their potential in mitigating cellular stress conditions and preventing protein aggregation-induced toxicity, possibly averting cell death. One such protein of interest is MGRN1, an E3 ubiquitin ligase, which has demonstrated proficiency in clearing misfolded protein aggregates across various disease models, alleviating cellular stress (Reference: 5). We discovered that the Mahogunin ring finger-1 (MGRN1) E3 ubiquitin ligase, responsible for mono-ubiquitination, is dysregulated in ALS models, particularly interacting with mutant forms of SOD1 (Reference: 12). Additionally, we found that MGRN1 interacts with expanded polyglutamine proteins in models of Huntington's disease, offering cytoprotection against toxicity and potentially holding therapeutic promise for protein-disordered diseases (Reference: 13). Our study suggests that ITCH likely acts as a cytosolic quality control E3 ubiquitin ligase, targeting abnormal protein inclusions for cytoplasmic degradation, contributing to maintaining a complex quality control system (Reference: 14).

Another important study reveals that E6-AP, a C terminus-type E3 ubiquitin ligase depleted in ALS mouse models, acts as a cellular quality control ubiquitin ligase. Identified as promoting proteasomal degradation of misfolded polyglutamine proteins (Reference: 9). E6-AP suppresses polyglutamine protein aggregation and associated cell death (Reference: 16).



Interestingly we observed that E6-AP, implicated in Angelman syndrome, interacts with Hsp70/Hsc70 chaperones, promoting the degradation of chaperone-bound substrates, suggesting its role not only in Angelman syndrome but also in neurodegenerative disorders involving protein aggregation (Reference: 11). Furthermore, we report that E6-AP, a ubiquitin ligase depleted in ALS mouse models pre-neurodegeneration, might serve as a therapeutic target for eliminating mutant SOD1-mediated toxicity in ALS (Reference: 9). Our studies explore the unique characteristics and neurobiological functions of key E3 ubiquitin ligases like MGRN1, E6-AP, and ITCH, offering insights into protein degradation pathways within the cytoplasm (Reference: 5, 9, 12, 13, 14 and16).

Prof. Amit Mishra's pioneering work at the Cellular and Molecular Neurobiology Unit and Cell Culture Facility in IIT Jodhpur also sheds light on the role of modulators of protein quality control mechanism. We show that Itraconazole (Reference: 1) and lanosterol treatment reduces proteotoxic aggregation and cytotoxicity by inducing CHIP expression and autophagy (Reference: 4, 15). Trehalose and Myricetin treatment eliminate abnormal proteins by modulating Hsp70 and E6-AP levels, reducing aggregation and cytotoxicity (Reference: 2, 17). Moreover, LRSAM1 promotes E6-AP degradation, affecting cell cycle regulatory protein expression and cellular proliferation rates (Reference: 19, 20). These findings offer mechanistic and therapeutic insights into protein quality control regulation, aiding in maintaining proteostasis.



(II B) Proteostasis Failure and Cancer Progress: Our results suggest that E6-AP not only enhances degradation but also regulates p27 expression. Loss of E6-AP function in Angelman syndrome may alter cell cycle progression, leading to disease pathogenesis. E6-AP deficiency causes cell cycle arrest and apoptosis, with high p27 levels in E6-AP-deficient mouse brains, indicating disrupted cell cycle progression in Angelman syndrome (Reference: 18). We investigated E6-AP's role in p53 regulation in mouse neuro 2a cells, demonstrating its direct ubiquitylation of p53 in vitro. This research aids in understanding E3 ubiquitin ligases' oncogenic potential, facilitating the identification of diagnostic markers and anticancer drug targets (Reference: 7). We reveal Gp78's involvement in stabilizing intracellular p27 levels, uncovering a multifactorial regulatory mechanism linking p27 with the UPS pathway (Reference: 3). Identifying E6-AP as a mediator of neuronal response to misfolded proteins highlights its potential as a therapeutic target in polyglutamine and other neurodegenerative diseases (Reference: 16).

Additionally, we have screened various small molecules and identified certain nonsteroidal anti-inflammatory drugs (NSAIDs) like diclofenac (Reference: 8) and ibuprofen (Reference: 10) for their inhibitory effects on the enzymatic activities of the 20S proteasome. This inhibition disrupts mitochondrial function and may induce apoptosis, offering a potential avenue for suppressing abnormal cell proliferation observed in cancer. Our study reveals that diclofenac and Ibuprofen induce proteasome malfunction, leading to the accumulation of critical proteasome substrates and pro-apoptotic proteins, subsequently triggering apoptosis via the mitochondrial pathway. This suggests NSAIDs' potential in generating anti-proliferative effects and modulating cellular quality control, applicable in neurodegeneration and aging therapies. Similarly, indomethacin (Reference: 6) induces proteasomal dysfunction, mitochondrial abnormalities, and apoptosis. These findings may inspire the development of specific proteasome inhibitors in combination with chemo-preventive anticancer agents for therapeutic strategies.

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