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Introduction

Proteins comprise one of the most common biomolecules in the cellular system and their appropriate structures and functions are essential to homeostasis. Proteostasis, that is, the protein homeostasis network of cells, covers all the aspects of a protein's lifespan—from synthesis to the maintenance of native conformational landscape to, finally, degradation upon failure to attain native structure, loss of native structure and aggregation or completion of their functional role in cells [Labbadia and Morimoto, 2015]. A coordinated network of proteins is at the core of proteostasis in cells. Protein quality control (PQC) machinery in cells primarily comprises of three branches: chaperone proteins, for native folding and stabilization of their client proteins; ubiquitin-proteasome system (UPS), involved in regulated degradation of proteins; and autophagy, which responds to nutrient stress and facilitates removal of damaged proteins and organelle [Chen et al., 2011]. The chaperones are required for maintaining the integrity of protein conformations via prevention of misfolding and aggregation and guide the ones with abnormal conformations to degradation. UPS and autophagy are major cellular pathways for degrading short-lived and long-lived proteins, respectively [Pohl and Dikic, 2019]. A failure or decreased functioning of components of this network can lead to proteotoxicity and disease, like neuron degenerative diseases, though the underlying factors are not completely understood.

1.1 PURPOSE OF THIS STUDY

Several critical findings suggest that a portion of newly synthesized proteins can be rapidly eliminated by UPS [Duttler et al., 2013; Wang et al., 2013b]; however, still, it is a challenge to understand how cells deal with the improper functions of UPS and avoid different translational errors. Ubiquitination of nascent polypeptides and simultaneous clearance of over accumulated misfolded proteins is a critical task for the UPS [Ciechanover and Kwon, 2015], specifically when cells face various stress conditions such as proteasomal dysfunction.

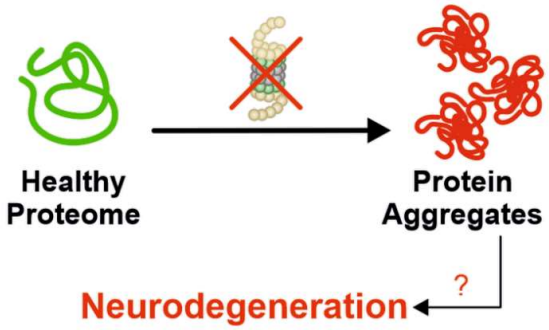

Figure 1.1: Decreased protein quality control function in neurodegeneration: Diminished function of protein quality control (PQC) machinery, including proteasome can disturb the healthy cellular proteome, leading to formation of cytotoxic protein aggregates, which are implicated in neuronal degenerative condditions. (*Source: Sumit Kinger and Amit Mishra* et al. (2024), Advances in Clinical Chemistry*)

An important and still not completely addressed question is to understand and find various molecular strategies, which can improve the suppressed functions of UPS pathway caused by overburdening accumulation of aberrant proteins. The current understanding of dysregulated functions of UPS associated with misfolded protein formation and pathomechanisms of neurodegeneration requires more knowledge for developing effective therapeutic solutions [Bedford et al., 2011] (Figure 1.1). Compelling evidence from various studies suggest impairment of PQC mechanisms as one of the key factors that can lead to protein misfolding and, consequently, generate the accumulation of aberrant proteins linked with the pathology of neurodegenerative diseases [Sweeney et al., 2017]. Improving the function of proteasome and other PQC components is a plausible therapeutic strategy for such disorders.

In contrast to neurodegenerative disorders, cancer cells are highly dependent on many PQC components, such as proteasome and chaperones, which can facilitate their growth and survival [Masdehors et al., 2000; Arlt et al., 2009; Calderwood and Gong, 2016; Rodina et al., 2016]. The proteasome multi-protein complex exhibits multiple types of proteolytic activities via its β catalytic subunits, namely, β5 (chymotrypsin-like), β2 (trypsin-like), and β1 (caspase-like) subunits. Proteasome inhibitors have been classified into major chemical classes of peptide boronates (e.g., bortezomib, ixazomib), peptide aldehydes (e.g., MG132), peptide epoxyketones (e.g., carfilzomib, epoxomicin), and β-lactones (e.g., lactacystin), which covalently inhibit proteasome active sites [Kisselev et al., 2012] (Figure 1.2). Inhibition of proteasome can induce apoptotic cell death via multiple mechanisms, like pro-apoptotic proteins stabilization, increased endoplasmic reticulum (ER) stress and unfolded protein response (UPR) and decreased activation of transcription factor NF-κB [Almond and Cohen, 2002]. Till now, three proteasome inhibitors have been FDA-approved for cancer therapy – Bortezomib, Carfilzomib, and Ixazomib Citrate [Park et al., 2018]. The potential effect of these proteasome inhibitors depends on enhanced stabilization of proteins associated with cell cycle control and apoptosis induction. However, the emerging resistance in cancer against these drugs and their usage limited primarily to multiple myeloma, underscores the need of investigation for novel UPS modulators targeting diverse tumor-associated mechanisms with promising effects in combination therapies. Inhibition of proteasome and chaperones to target cancer cells can be an efficient strategy for targeting cancer cells.

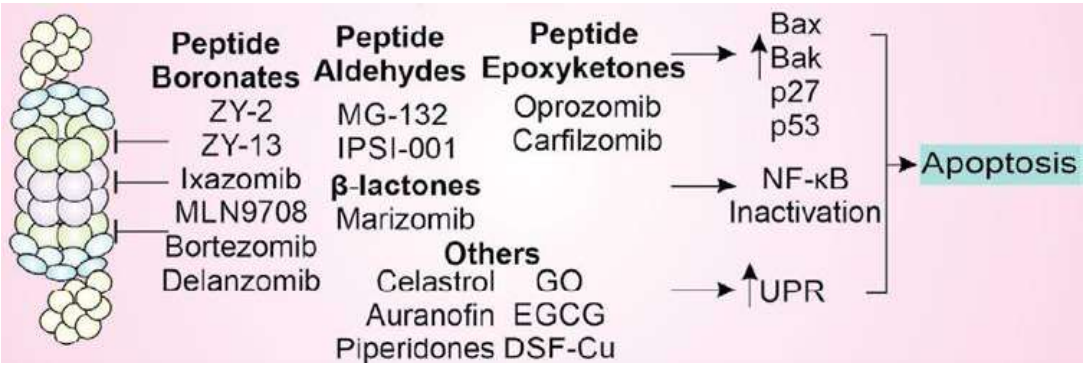

Figure 1.2: Proteasome inhibitors as inducers of apoptosis: Several inhibitors of multi-subunit proteolytic machinery proteasome belonging to different chemical classes have been reported, which can induce apoptosis via different mechanisms, including stabilisation of proapoptotic proteins, decreased activity of transcription factor NF-κB, and increased ER stress and UPR. *(Source: A.R. Dubey, Sumit Kinger# and Amit Mishra* et al. (2021), Cellular Physiology and Biochemistry)*

1.2 A BRIEF OUTLINE OF THE RESULTS AND FUTURE OUTLOOK OF THE PRESENT WORK

The current work has reported a natural compound lanosterol and an FDA-approved drug valproate as inducer and inhibitor of proteasome function, respectively. This work has implications for diseases with depleted PQC function, such as neuron degenerating conditions as well as the conditions with higher expression of PQC components is critical for disease progression, such as cancer [Chhangani and Mishra, 2013; Dubey et al., 2021].

1.2.1 Lanosterol Elevates Cytoprotective Response through Induced-Proteasomal Degradation of Aberrant Proteins

Lanosterol is an endogenous sterol molecule intermediate in the cholesterol synthesis pathway that is reported to promote clearance aberrant protein aggregates [Zhao et al., 2015]. In the current study, we observed treatment with lanosterol improves the clearing of aberrant proteins from cells. Exposure of lanosterol also promotes the proteolysis of both bona fide misfolded proteins and various neurodegenerative diseases associated proteins. Our finding suggests that this is achieved by treatment with lanosterol by elevating the endogenous proteasome activities, also aiding in alleviating cytotoxicity. A better understanding of how small molecules and their functional principles lead to inhibition of protein misfolding may open new therapeutic insights against protein conformational disorders, like neurodegeneration and other aging associated conditions.

1.2.2 Valproate Mediated Proteasome Dysfunctions Induce Apoptosis

Valproate is a salt of valproic acid (2-Propylpentanoic acid), a short (branched) chain fatty acid, originally approved as an anti-convulsant [Johannessen, 2000]. Valproate has also exhibited anti-cancer potential in several studies [Gottlicher et al., 2001]. Moreover, the low dose of valproate is suggested for increase N-terminal acetylation of proteasome subunits and their proteolytic activity in mice myocardium [Wang et al., 2013a]. The mechanistic understanding of any direct relation between valproate and proteasome is lacking. In current study, we noted valproate to impair proteolytic activity of proteasome and enhanced ubiquitinated proteins accumulation in cells. Moreover, valproate treatment also decreased cell viability and elicited disturbances in mitochondrial homeostasis, such as changes in its membrane potential and cytochrome *c* release into cytosol. Furthermore, we observed an elevation in proapoptotic and cell cycle regulator proteins in cells upon exposure to valproate, indicating its possible potential for limiting proliferation and inducing apoptosis.

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