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Introduction

Proteins are the essential biomolecules in cells involved in numerous cellular functions. New proteins are continuously synthesised, and senescent and wearied proteins are regularly degraded through proteolytic pathways. Cellular protein homeostasis (proteostasis) is a regulated process (folding, trafficking and degradation) crucial for cellular health. Disturbed proteostasis may lead to several diseases like neurodegeneration and cancer (Hohn et al., 2020;Chen et al., 2023). The accrual of damaged proteins represents possible defects in the proteostasis machinery of the cells. Removal of old or non-native proteins is a critical as well as challenging task for cells (Hanna et al., 2019); a few misfolded proteins bypass the protein quality control (PQC) mechanism of cells and do unwanted interactions with the existing protein pool (Koopman et al., 2022). Such unexpected complex conditions can lead to proteostasis failure and aggregation of abnormal proteins. Numerous neuronal degenerative conditions are known where the accumulation of proteotoxic inclusions serves as a clinical hallmark (Ross and Poirier, 2004). Despite our awareness of the existence of inclusion bodies and their linkage with proteinopathies (Sweeney et al., 2017), the precise mechanism at molecule level of how cells manage effective degradation of abnormal proteins and avoid their accumulation still needs to be determined. It is a well-known fact that the proteasome complex contributes to proteostasis and depleted proteasome functions leads to abnormal protein accumulation. We know the role of proteasomes in various cellular mechanisms and survival, but a few critical questions still need to be clarified.

How the proteasome complex and associated mechanism can sense the disturbance of proteostasis? How is proteasome machinery specifically involved in removing misfolded proteins from the cells and segregating them from the critical substrates? It is still a challenge to activate the function of proteasome under cellular stress conditions. The findings of proteasome activators can be helpful in the therapeutic interventions linked with protein conformational disorders and neurodegenerative diseases. How can cells remove the over-accumulated proteins within the physiological limits of existing proteolytic machinery? A better understanding of such questions can suggest therapeutic strategies against proteotoxic aggregate inclusions. Cancer is a significant global health burden, influencing millions of people and posing momentous challenges to global healthcare systems, economies, and societies (Siegel et al., 2023). With advancements made in the domains of cancer prevention, diagnosis, and treatment in recent times, the death rate associated with cancer has decreased in the majority of high-income nations. Despite this, there are still significant and enduring disparities in mortality, incidence, and survival rates worldwide (Santucci et al., 2020). Since disruptions in protein homeostasis networks are commonly associated with the development of tumours, therapies that focus on proteostasis regulators may be more efficient in combating cancer.

Ubiquitin-proteasome system (UPS) is critical in determining how a cell will react upon exposure to various stress signals (Figure 1.1) (Jana, 2012). In the past, major role of ubiquitin was believed to be linked with proteolysis; however, recent research has shown that it is an essential regulator of protein activity that may have an effect on signalling cascade, transcription, apoptosis, or even the development of cancer (Mata-Cantero et al., 2015).

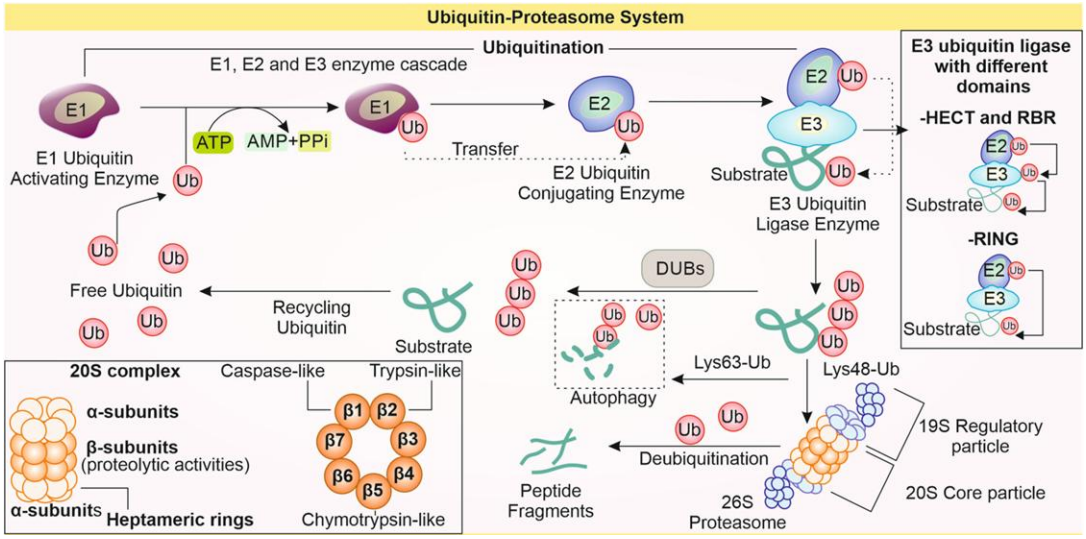

Figure 1.1: Overview of Ubiquitin-proteasome system: UPS is a well-defined proteolytic process clearing cellular proteins. The sequence of ubiquitination of a protein substrate starts with consumption of ATP for E1 enzyme for activation of ubiquitin. Ubiquitin is transported to E2 enzyme. E3 enzyme recognises the substrate, leading to ubiquitinylation of target. The substrate that has been polyubiquitinated is transported to the 26S proteasome for destruction. (*Source: Prashant Kumar and Amit Mishra* et al. (2023), Translational Medicine of Aging*)

The UPS is a proteolytic pathway dependent on proteasome, a multi-subunit proteolytic complex. 26S proteasome (~2MDa) consists of regulatory (19S) and core particle (20S). The proteolysis capability of 20S proteasome is reported via β5-subunit, β2-subunit and β1-subunit having catalytic activities chymotrypsin-like, trypsin-like, and post-glutamyl peptide hydrolase (PGPH)-like, respectively (Tanaka, 2009). The malfunction of the UPS at any level is connected with the occurrence of many illnesses, some of which include, neurodegenerative disorders metabolic diseases, immunological disorders, inflammation, and cancer (Chen and Dou, 2010). Several cancerous cells have mutations in genome and erroneous proteins that can challenge their survival. It is possible that the dependency of cancerous cells is more on proteostasis processes compared to normal cells for survival. This hypothesis presents proteasome inhibitors as potential therapeutic agents for treating cancer (Deshaies, 2014a).

1.1 PURPOSE OF THIS STUDY

UPS and autophagy are the fundamental processes that destroy the pool of aberrantly aggregated proteins (Wong et al., 2012;Amm et al., 2014a). UPS machinery targets the selective removal of aberrant proteins from the cells (Tanaka, 2009). How we can enhance the overall efficiency of the UPS process is an important question, and finding the activators of UPS can improve the proteasomal proteolysis of abnormal proteins. Synthesis and accumulation of aberrant proteins is linked to endoplasmic reticulum (ER) stress (Rao and Bredesen, 2004), oxidative stress (Malhotra et al., 2008), and other proteotoxic insults that severely impact protein synthesis (Liu et al., 2013), protein trafficking (Anelli and Sitia, 2008) as well as affect translational machinery localisation (Ghosh et al., 2020). Previous findings suggest that proteostasis failure is linked with imperfect ageing and contributes to neurodegeneration. Emerging evidence indicates that improved cellular proteolytic process functionality can ameliorate protein-conformational disease pathologies (Figure 1.2) (Thellung et al., 2018;Njomen and Tepe, 2019b). There are also evidences present suggesting oxidation of proteasome subunits can reduce its proteolytic function. Inhibiting proteasome functions may induce apoptosis in cancer cells.

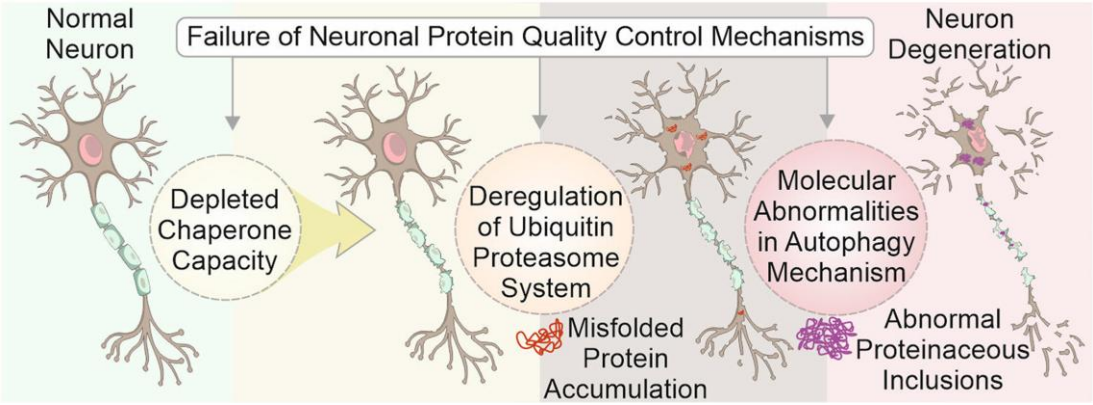

Figure 1.2: The role of PQC pathways failure in progression of neuronal degeneration: Compromised functions of molecular chaperones, UPS, and autophagy with aging are major factors for neurodegeneration. *(Source: Prashant Kumar and Amit Mishra* et al. (2023), Translational Medicine of Aging)*

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

The present study focuses on targeting the proteasome function modulation by small molecules. Enhancing or suppressing the proteasome function can provide us therapeutic strategy in complex diseases like neuronal degenerative disorders and cancer respectively. Here we have reported two small molecules which can modulate proteasomal activity. In neuronal degenerative conditions, the elevation of proteasome function may rescue neurons and provide protection from aggregation of mutant toxic proteins. Whereas in cancer cells suppression of proteasome may result in apoptosis.

1.2.1 Trehalose Promotes Clearance of Proteotoxic Aggregation of Neurodegenerative Disease-Associated Aberrant Proteins

Our current study indicates that using trehalose elevates the endogenous levels of proteasome, which seems helpful in removing bona fide abnormal proteins from the cells. Further treatment of trehalose in the cellular model of neurodegenerative diseases reveals the degradation of their improper inclusions in trehalose presence. The effect of trehalose is reverted by adding a proteasome inhibitor (MG132), which suggests proteolytic potential of trehalose is most likely mediated through the proteasome. Using trehalose elevates proteasome activities and alleviates the cytotoxic potential of various cellular stress conditions. Overall, our findings indicate that using small compounds such as trehalose can be helpful in the near future for possible therapeutic strategies against protein-conformational disorders.

1.2.2 Ketorolac Disturbs Proteasome Functions and Induces Mitochondrial Abnormalities-Associated Apoptosis

Numerous researches have concluded that the exposure of Nonsteroidal anti-inflammatory drugs (NSAIDs) in cells leads to accelerated apoptosis induction, also known as programmed cell death (PCD). The proteasome removes misfolded proteins and inhibits cell death by clearing a potentially lethal burden of protein aggregation. However, the specific process through which NSAIDs cause cell death in many types of malignant cells and prevent progression of carcinogenesis is not fully known. In present study, we observed that Ketorolac has a possible function in disruption of proteasome activities thus its exposure tends to increase ubiquitinated proteins accumulation in cells. This disruption of proteasome activity leads to Ketorolac-induced apoptosis, thus decreasing cell viability, which involves homeostasis disturbance in mitochondria and the cytochrome c release into the cytoplasm. Moreover, there was also enhanced pro-apoptotic proteins levels when cells were subjected to Ketorolac suggesting it may have a possible anti-proliferative function against cancerous cells.