Proteostasis Based Molecular Approaches Alters Proteasomal Functions Can Deplete Misfolded Proteins Aggregation and Regulate Improper Cellular Proliferation

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Conclusion

The optimum working of PQC pathways is absolutely essential for disease-free healthy quality of life. The PQC components chaperones, UPS, and autophagy systems maintain native folding of proteome and eliminate pathogenic aberrantly folded proteins and aggregates. The inevitability of aging brings with it the decline in PQC capacity of cell. The increased incidence and gradual progression of neurodegenerative and other protein misfolding-associated conditions with aging signify the systemic and global deterioration of all branches of PQC. Chaperones are the earliest responders to nonnative folding in proteins and facilitate refolding, preventing aggregation, and even interact with components of UPS and autophagy to promote their disposal. E3 ligases, proteasome, and autophagy-associated proteins are required for proteolytic removal of proteins and homeostasis of cells. Aging-associated decrease in the limited capacity of these components results not only in increased misfolded proteins and neurodegeneration but also other outcomes, such as dysregulated signalling pathways and metabolism. There are still many less understood aspects on mechanics and regulation of PQC that need to be addressed to gain insight and design better therapeutic approaches for chronic aging-related disease conditions.

Enhancing the activity of PQC components can be an effective therapeutic approach to target NDDs and senescence. For instance, the osmolytes of different chemical origin, such as trehalose and mannitol can improve the function of endogenous chaperones and themselves act as chemical chaperones as well to alleviate protein aggregation (Tanaka et al., 2004;Street et al., 2010;Shaltiel-Karyo et al., 2013). Targeting chaperones at transcription level via HSF1 can be another promising approach for diseases involving abnormal protein conformations (Westerheide and Morimoto, 2005;Thakur and Nehru, 2014). The inducers of autophagy, such as resveratrol, metformin, and rapamycin can increase the organismal lifespan (Harrison et al., 2009;Morselli et al., 2009;Anisimov, 2013). Modified Beclin-1 based peptides can also induce autophagy and have potential for decreasing load of polyglutamine-expanded protein aggregates and possibly for other proteinopathies (Shoji-Kawata et al., 2013;Majdoul et al., 2017). Upregulation of 26S proteasome activity, including by chemicals, such as quercetin, methylene blue, geldanamycin and other small molecules has implications for countering protein accumulation in aging associated diseases (Medina et al., 2011;Opattova et al., 2013;Regitz et al., 2014;Chondrogianni et al., 2015;Leestemaker et al., 2017). Inhibition of DUBs through thiol protease inhibitors (IU1) also elevates the proteasome mediated aggregated protein clearance (Lee et al., 2010). Newer alternative approaches targeting PQC elements, like engineered Hsp104 and PROTACs are also showing promise for future (Jackrel et al., 2014;Tomoshige and Ishikawa, 2021). Gaining an in-depth understanding of the connection between aging-associated decrease in PQC, factors regulating it, and progression of diseases can illuminate aging biology and give us opportunities to improve the quality of aging.

Similarly, cancer has become a formidable class of disorder affecting people worldwide. One of the most common features inherited by the tumor cells comprises the damaged genome. Irregularities in the cell's genetic material often result in the synthesis of mutant proteins possessing compromised functions (Hoeijmakers, 2009). Under normal homeostatic conditions to avoid the emergence of futile proteins, the cellular proteome is kept under check by the PQC mechanisms (Chhangani and Mishra, 2013b). The coordinated functioning of the elements of PQC enhances proteome quality and imparts cytoprotection. Despite defective proteome,

cancer cells are professed to have enhanced cytoprotective functions attributed to the upregulated functioning of such PQC elements (Deshaies, 2014b). The mutant proteins are often seen to form aggregates inside the cell or lead to improper functioning (Chiti et al., 2002). Thus to avoid this, nascent polypeptides, when translated, are accompanied by the proteins (chaperones) that help them undergo correct folding and attain a physiologically active conformation (Hartl et al., 2011b). Thus, in this condition suppressing or inhibiting the PQC elements like proteasome may prove to be a therapeutic option.

5.1 SUMMARY

In the present work (Chapter 3) we reported that subjecting the cells with a natural disaccharide Trehalose may induce the proteasome's endogenous level without affecting the overall cellular viability. The treatment of cells with trehalose also proved to clear heat-denatured misfolded protein aggregates. Moreover, the clearance of known substrate of proteasome (pd1EGFP) was also observed to be increased upon the treatment of trehalose. The exposure of trehalose was also able to increase the overall proteolytic activities of proteasome suggesting a probable inducing effect on proteasome. Furthermore, clearance of neurodegeneration-linked misfolded protein aggregates was also observed. Trehalose may also provide cytoprotection against several stress conditions.

In present study (Chapter 4), a NSAID Ketorolac is examined and observed to induce apoptosis in cells. It is also reported to reduce the cellular viability upon treatment both in concentration as well as time dependent manner. Ketorolac is also observed to reduce the proteasome's catalytic activity both chymotrypsin like and PGPH-like. The treatment of Ketorolac was seen to stabilizing several proteins linked with cell cycle and death. With reporter gene assay it was also observed that activity of NF-κB, an transcription factor, was reduced an indication of proteasome activity suppression. The effect of Ketorolac was also studied and found to disrupt the mitochondrial potential upon treatment.

5.2 CONCLUDING REMARKS

This present work reports two compounds Trehalose and Ketorolac that have potential to modulate the activity of cellular proteasome. Trehalose has an instigating effect of proteasomal catalytic activity thus enhancing the clearance of aggregated protein inclusions body. It may have therapeutic value in misfolded protein aggregation-associated disorders like NDDs. Whereas Ketorolac has an inhibitory effect on the proteasomal catalytic activity. It is observed to induce apoptosis in cells and disrupting $\Delta\psi_m$. These properties of Ketorolac may find use in diseases like cancer.

5.3 CLOSING COMMENTS AND FUTURE PROSPECTS

It is well established that the proteostasis in cells are very crucial for their proper functioning. In stress conditions, the protein misfolding and aggregation are generally observed. There are various mechanisms for dealing with these mutant misfolded proteins. These systems are pro-survival mechanisms involved in cytoprotection. In many disease conditions these PQC mechanisms are involved and their proper modulation is required. Thus, these protective mechanisms are seen as probable therapeutic targets. Exogenous modulation of PQC elements like proteasome can be achieved and many such inhibitors and activators are reported. We have also reported such molecules that can modulate the activity of proteasome upon treatment. Further these molecules need to be tested in large variety of cell systems and future experimentation can be on animal models. This study reports the probable modulation of proteosome through small molecules. Further experimentations are also required to elucidate the specific pathways through which these effects are produced. Complex diseases like cancer and NDDs can benefit from this type of study that targets crucial PQC element modulation through small molecules.

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