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Introduction

Eukaryotic cells remain under a continuous threat of huge proteotoxic stress. A large number of intra- and extracellular stress-inducing factors may pose an imbalance in proteostasis conditions that can lead to a large spectrum of disorders [Fulda *et al.*, 2010]. Cancer and neurodegeneration are few of the most devastating human pathologies that present a major challenge before us to diagnose, understand and devise possible therapeutic strategies [Higuchi-Sanabria *et al.*, 2018; Levine and Kroemer, 2008]. A major hallmark of neurodegenerative diseases is the formation of insoluble misfolded intracellular inclusions of disease-linked proteins, which often are controlled by multifarious cellular factors, including transcription factors, molecular chaperones, and other essential cellular proteins [Chhangani and Mishra, 2013a; Chhangani and Mishra, 2013b; Woerner *et al.*, 2016]. Abnormal or damaged proteins are targeted by cellular protein quality control (QC) mechanism for their refolding, elimination, or deposition into distinct cellular compartments [Kaganovich *et al.*, 2008; Olzscha *et al.*, 2011]. However, when these aberrant proteins tend to accumulate due to the exponential load, the overall efficiency of QC system suffers severely [Ciechanover and Kwon, 2015]. Moreover, lack of efficiency of proteolytic machinery of the cells, e.g., ubiquitin proteasome system (UPS) and autophagy may also lead to deregulated cellular proliferation [Mishra *et al.*, 2018; Wang and Levine, 2010].

1.1 PURPOSE OF THIS STUDY

Under the stress conditions, cellular QC systems may not suffice the needs of maintaining homeostatic conditions inside the cytoplasmic *milieu* [Amanullah *et al.*, 2017]. Insufficient chaperone capacity against the massive accumulation of aberrant proteins or suppression of cellular proteolytic machinery may result in an unrecoverable damage caused by over-accumulation of several intracellular proteins inside the cytoplasm [Cuervo and Dice, 2000]. These conditions may further lead to deregulation of many other associated cellular physiological processes, including signaling, metabolism, and proliferation [Fink, 1998; Kopito, 2000]. It is of great importance to find out efficient therapeutic strategies for the elimination of cytoplasmic inclusions formed of non-native proteins.

Interestingly, pharmacological chaperone therapy, based on the use of small drug molecules, is now in clinical translational application stage for lysosomal disorders, like Fabry, Gaucher, and Pompe diseases [Parenti, 2009]. In a recent study, it has been shown that activators of small molecule nuclear factor erythroid 2-related factor 2 (NRF2) induce heat-shock protein 70 (Hsp70) in heat shock transcription factor-1 (HSF-1)-dependent manner [Zhang *et al.*, 2011]. Previously, it has been suggested that heat shock responses are elevated following the treatment of arachidonate or curcumin [Jurivich *et al.*, 1994; Teiten *et al.*, 2009]. Treatment of 4-phenylbutyrate induces Hsp70 and heat shock protein HSPH1 in IB-3 bronchial epithelial cell line [Wright *et al.*, 2004]. Based on these observations, it could be proposed that small molecule-based strategies to improve the heat shock response may be useful to target protein conformation disorders.

Induction of apoptosis by inhibition of proteasomal activities is one of the possible approaches that are currently in use to treat various cancer types [Shah *et al.*, 2001]. Synthetic molecule-based approaches to suppress the proteasome activities have been approved by Food and Drug Administration (FDA) are in wide application and currently an interesting area of research [Hideshima *et al.*, 2001]. Continuous efforts have been made in the past to identify or synthesize new proteasome inhibitors that can effectively reduce the rate of cell proliferation in different types of cancer cells [Orlowski and Kuhn, 2008]. Extensive amount of resources and enormous efforts are needed to propose and validate new drugs due to various associated toxicities or side effects. However, repurposing of older and known drugs for newer therapeutic avanues could be a wise strategy to reduce significant amount of time, efforts and cost.

1.2 AN OUTLINE OF THE RESULTS AND FUTURE PERSPECTIVES OF THE PRESENT WORK

To address the above-mentioned objectives, rigorous literature has been studied and was taken into consideration while designing research methodologies and experimental approaches. The summary of the major outcomes obtained in the presented study are as following.

1.2.1 Lanosterol Suppresses the Aggregation and Cytotoxicity of Misfolded Proteins Linked with Neurodegenerative Diseases (Chapter 3: Published as Upadhyay et al. 2018; Molecular Neurobiology)

Lanosterol is an intermediate of cholesterol synthesis and use of lanosterol induces ubiquitination and degradation of a rate-controlling enzyme of cholesterol synthesis, i.e., 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase (HMGCR); mutations in lanosterol synthase cause cholesterol deficiency in a hereditary Shumiya cataractous rat strain [Mori *et al.*, 2006; Song *et al.*, 2005]. Another report indicates that oxidosqualene: lanosterol cyclase enzyme catalyzes cyclization of the linear 2,3-monoepoxysqualene to lanosterol and its inhibition can be useful to reduce cholesterol biosynthesis and consequently in the treatment of atherosclerosis [Huff and Telford, 2005]. Interestingly, it has been shown that lanosterol is significantly reduced in nigrostriatal regions of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice and exogenous usage of lanosterol rescued dopaminergic neurons from MPTP-mediated toxicity. Collectively, this study also suggests that lanosterol treatment induces mild mitochondrial uncoupling and promotes autophagy [Lim *et al.*, 2012].

Recently, it has been shown that treatment of lanosterol dramatically decreases intracellular aggregates of different crystallin mutant proteins and also alleviates cataract severity, in vivo, in dogs [Zhao *et al.*, 2015]. However, the molecular mechanism by which lanosterol reduces abnormal intracellular protein aggregation is not well known. C-terminus of Hsp70 Interacting Protein (CHIP) is an E3 ubiquitin ligase molecule that has also been shown to function as a co-chaperone, regulating the activities of heat shock protein 70 (Hsp70); and thus play very important role in maintenance of cellular proteostasis [Joshi *et al.*, 2016]. Here, it has been demonstrated that lanosterol induces cytoprotective function of QC mechanism (UPS and autophagy) via increased expression and stabilized levels of co-chaperone CHIP; and thus also reduces aggregation of modeled and bona-fide misfolded proteins. Treatment of lanosterol diminished cytotoxic aggregation of various neurodegenerative disease-associated aberrant proteins. It was also found that lanosterol treatment not only suppresses the aggregation of ubiquitin-positive misfolded proteins, but also mitigates proteotoxicity conferred by such inclusions inside the cells. The present results suggest the role of lanosterol in cytoprotection through elimination of mutant misfolded proteins.

1.2.2 Ibuprofen Induces Mitochondrial-Mediated Apoptosis through Proteasomal Dysfunction (Chapter 4: Published as Upadhyay et al. 2016; Molecular Neurobiology)

Uncontrolled cellular proliferation and resistance towards death inducing pathways are the major hallmarks of cancer cells [Hanahan and Weinberg, 2011]. Previous studies have demonstrated that use of diclofenac and sulindac sulfide induces apoptosis in human acute myeloid leukemia cells [Singh *et al.*, 2011]. It has been observed that NSAID-mediated endoplasmic reticulum (ER) and oxidative stress events could activate the intrinsic pathways of pro-apoptosis events [Adachi *et al.*, 2007; Tsutsumi *et al.*, 2004].

Earlier studies have observed that exposure of NSAIDs and acetaminophen inhibits nuclear factor-kappaB (NF-κB) activity [Kopp and Ghosh, 1994; Ryu *et al.*, 2000] and deregulates the mitochondrial function including enhanced permeability of mitochondrial membrane [Somasundaram *et al.*, 1997; Watanabe *et al.*, 2011]. Many reports also suggest that NSAIDs disturb the proteasome function that accumulates pro-apoptotic proteasomal substrates leading to initiation of programmed cell death [Dikshit *et al.*, 2006; Huang *et al.*, 2002]. DNA fragmentation and mitochondrial abnormalities are few preliminary steps that are involved in the induction of apoptosis and it has been observed that ibuprofen treatment also induces fragmentation of nuclear DNA and promotes apoptosis in cultured neuronal cells [Berns *et al.*, 2009]. S(+)-ibuprofen treatment increases p53 expression levels which results in inhibition of cell growth and increases the unfolded protein responses in neuroblastoma cell lines [Ikegaki *et al.*, 2014].

Recently, few studies have shown the effects of ibuprofen on mitochondrial dysfunctions subjecting the cells to loss of inner mitochondrial membrane potential and release of cytochrome *c* into cytosol [Al-Nasser, 2000; Moorthy *et al.*, 2008]. However, the detailed molecular mechanisms of ibuprofen-mediated effects on mitochondrial dysfunction, oxidative and ER stress response, inhibition of cell cycle progression, and induction of apoptosis are largely unknown. This study suggests that ibuprofen treatment may disturb the proteasome function, which can induce apoptosis by altered mitochondrial permeability and cytochrome *c* release into the cytosol. Ibuprofen treatment also induces aggregation of misfolded ubiquitylated proteins and elevates aggregation of proteasomal substrates. Additionally, this study provides a better prospect to understand the molecular implications of NSAIDs in various physiological pathways and also to explore their less-explored beneficial roles, which could be effective in the treatment of a range of diseases.

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