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Introduction

Cells harbor an overcrowded heterogeneous population of macromolecules, which posits a great challenge for the cellular system to regulate most of the processes with uttermost accuracy. Proteins, which perform most of the cellular tasks, remain prone to misfold and aggregate inside the cytoplasm to form large inclusion bodies, as have been reported to cause a number of neurodegenerative diseases [Chhangani *et al.*, 2013]. Therefore, to restrict such unwanted misfolding events, cells invest a huge amount of energy to provide an intracellular system to monitor, identify, repair and degrade any such proteinaceous inclusion bodies, generated inside the cell. Chaperones are a class of proteins, which takes care of cellular proteins from very earlier stages of their synthesis and surveil them up to their degradation. Some of them help the nascent polypeptide chains to fold co-translationally and achieve their native conformations with the greatest accuracy [Hartl *et al.*, 2011]. But, under various kinds of intra- and extracellular stresses, the working efficiency of chaperones is compromised, which adds up to the risks of misfolding and aggregation of proteins, several folds. Under such conditions, PQC governs coordinated functions with the help of its components, that is, autophagy, chaperones, and ubiquitin proteasome system (UPS) [Amm *et al.*, 2014; Bukau *et al.*, 2006; Chen and Klionsky, 2011]. Molecular chaperones are known to be involved in folding of nascent polypeptide chains into their native three-dimensional states, while UPS and autophagy play crucial roles in the degradation of unfolded and /or aberrant proteins inside the cells to maintain the crowded cellular milieu in a healthy state [McClellan *et al.*, 2005; Wickner *et al.*, 1999].

Selective recognition and further degradation of non-functional and toxic proteins of the cell remain under tight regulation of UPS, which consists of small ubiquitin molecules, 26S proteasome and an enzyme cascade, formed of E1 (ubiquitin activating enzymes), E2 (ubiquitin conjugating enzymes), and E3 ubiquitin ligases [Goldberg, 2003a; Hershko and Ciechanover, 1998]. The energy released from ATP hydrolysis facilitates E1 activating enzyme to activate 76 amino acid long ubiquitin molecules at its C-terminal glycine by forming thioester bond. This activated ubiquitin is then transferred to a ubiquitin-conjugating domain containing E2 enzymes and further, the specific substrate recognizing ability of E3 ubiquitin ligases completes the transfer of ubiquitin molecule to substrate proteins [Hershko and Ciechanover, 1998; Nandi *et al.*, 2006]. Accumulation of aberrant proteins causes hindrance in functioning of UPS, and may ultimately cause intracellular dysfunctioning and cell death [Bence *et al.*, 2001]. Previous reports clearly indicate that impairment of this highly regulated protein degradation pathway leads to multiple disease conditions such as neurodegeneration, cancer, and aging [Ciechanover and Brundin, 2003; Low, 2011; Mani and Gelmann, 2005]. In last few years, UPS and its components have been explored for their therapeutic importance in various complex diseases [Amanullah *et al.*, 2017b; Nalepa *et al.*, 2006].

1.1 PURPOSE OF THE STUDY

Cells perform several post-translational changes in various proteins prior to allow their participation in various intracellular metabolic mechanisms. Ubiquitylation is also a post-

translational process for different cellular proteins after which modified proteins contribute their physiological functions in distinct cellular processes. E3 ubiquitin ligases are important components of ubiquitin proteasome system (UPS), which specifically recognize critical substrate proteins (abnormal, over-accumulated & old) for their intracellular elimination. Loss of cell cycle regulation is one of the chief possible causes of deregulated cellular proliferation and cancer progression. How different E3 ubiquitin ligases play essential roles in cell-cycle regulation is still one of the unsolved fundamental questions and potentially stands for the development of early diagnostic methods, which can generate new molecular strategies to treat cancer.

In neurodegenerative diseases and ageing-associated disorders, major clinical hallmark is presence of abnormal or misfolded proteins assemblies [David, 2012; Ross and Poirier, 2004]. Intracellular accumulation of misfolded proteins also represents a possible failure of protein quality control (PQC) mechanisms [Chhangani and Mishra, 2013; Goldberg, 2003b]. Previous reports indicate that the accumulation of aberrant proteins suppresses the functions of PQC machinery, but the precise molecular pathomechanism responsible for the lack of functions of protein degradation machinery is not clear [Bence *et al.*, 2001; McNaught *et al.*, 2004]. Major function of chaperones is to fold various proteins into their native structures; therefore, they can accomplish designated functions [Craig *et al.*, 1994; Hartl *et al.*, 2011]. Overall, these efforts establish the stringent functional integrity of the proteome to face the routine course of chronic cellular stress conditions [Hightower, 1991; Kültz, 2005]. Cells always try to maintain the state of proteostasis and its failure may lead to the progression of several neurodegenerative diseases and protein conformational disorders [Amanullah *et al.*, 2017b].

The major focus of the study is to understand the roles of E3 Ubiquitin Ligases linked with regulatory roles of cell-cycle transitions and finding their modulation strategies to regulate expression and function in various disease conditions like neurodegeneration. Discovery, purification, characterization, functional aspects and pathological mechanisms of Gp78, are linked with various types of cancers [Chiu *et al.*, 2008]. However, the roles of Gp78 in metastasis and tumor-progression is yet to be fully understood; therefore further research may help in understanding the unexplored molecular mechanisms so that in future it could be used as a prognostic biomarker of different cancer types and might be exploited for therapeutic purposes. Thus, available information prompt to investigate the missing link between Gp78 expression and increased cell proliferation. Similar to Gp78, there are various other cell cycle regulatory E3 ubiquitin ligases, like E6-AP, which participate in degradation of misfolded proteins accumulated in number of neurodegenerative diseases. Identification of various small natural molecules that modulate the PQC components might aid to the development of new therapeutic strategies against various diseases. Myricetin, a flavonoid was studied for multiple health benefits but not explored for modulation of PQC components and reduction of aberrant proteins accumulations.

1.2 BRIEF RESULTS AND FUTURE PROSPECTS OF THE WORK

Various research articles, books and literature have been referred for the complete research and experimental work conducted in this study. Majorly biochemical, cell culture based and molecular techniques were used in both research chapters. Two major findings obtained from current study are as follows:

Gp78 Involvement in Cellular Proliferation: Can act as a Promising Modulator for Cell Cycle Regulatory Proteins (Chapter 3 : published as Joshi *et al.*, 2018 and Joshi et al., 2017)

In the cells, protein synthesis and degradation are normal processes, which are tightly regulated by various cellular metabolic pathways. Cellular protein quality control (PQC) mechanisms take care of all proteins inside cells with the help of chaperones, autophagy and

ubiquitin proteasome system (UPS). Earlier, it has been suggested that modulating proteasomal activities can confer cytotoxicity inside the cells and may lead to apoptotic cell death [Amanullah *et al.*, 2017a; Upadhyay *et al.*, 2016]. The complex mechanistic interactions and the interplay between E3 ubiquitin ligases, the major components of UPS and their involvement in the functional regulation as well as expression of p27 are not well known.

The present study demonstrated that cell surface glycoprotein Gp78, a putative E3 ubiquitin ligase, is involved in the stabilization of intracellular steady-state levels of p27. Transient overexpression of Gp78 increases the accumulation of p27 in cells in the form of massive inclusion-like structures, which could be due to its cumulative increased stability in the cells. It has also been monitored that how under stress condition, E3 ubiquitin ligase Gp78 regulates endogenous levels of p27 inside the cells. ER stress treatment generates a marginal increase in Gp78 endogenous levels; and this elevation effect was prominent for intracellular accumulation of p27 in cells. Thus, the study explained the complex linkage of p27 regulation with PQC and its components.

Myricetin-Assisted Proteasomal Degradation Suppresses Misfolded Proteins Aggregation and Cytotoxicity (Chapter 4: under review in journal)

Formation of the misfolded proteins aggregates inside or outside the cells is the common characteristic of neurodegenerative disorders. Studies suggest that aberrant protein aggregates play critical roles in cellular homeostasis imbalance and failure of PQC mechanisms, leading to the neurodegenerative diseases, like amyotrophic lateral sclerosis (ALS), Huntington's disease and many more [Upadhyay *et al.*, 2016]. However, it is still not explored completely what are the precise mechanisms of PQC failure and cellular dysfunctions associated with the neurodegenerative diseases caused by the accumulation of misfolded protein aggregates. E3 ubiquitin ligases are the key components of UPS, and associated with multiple neurobiological mechanisms [Upadhyay *et al.*, 2017].

Previously, it has been reported that quality control (QC)-E3 ubiquitin ligases with the help of molecular chaperones suppresses misfolded proteins aggregation and cytotoxicity associated with neurodegeneration and ageing [Chhangani *et al.*, 2014]. In present study, it has been tried to find natural molecules as modulators of these E3 ubiquitin ligases and found, Myricetin, a flavonoid, which can eliminate various misfolded proteins from the cellular environment via modulating endogenous levels of Hsp70 chaperone and QC-E3 ubiquitin ligase E6-AP. It has been observed that Myricetin treatment suppresses the aggregation of different aberrant proteins. Myricetin also enhances the elimination of various toxic neurodegenerative diseases associated proteins from the cells, which can be reversed by the addition of putative proteasome inhibitor (MG132). Remarkably, Myricetin can also stabilize E6-AP and reduce the misfolded proteins inclusions, which further alleviate cytotoxicity. Hence, could be used for future therapeutic applications against neurodegenerative diseases.

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