

Contents

	Page
Abstract	i
Acknowledgements	iii
Contents	v-vi
List of Figures	vii-viii
List of Tables	ix
List of Symbols	x
List of Abbreviations	xi-xii
Chapter 1: INTRODUCTION	
1.1 Purpose of the Study	1-2
1.2 Brief Results, Scope and Future Prospects of the Work	2-3
Chapter 2: Review of Literature	
2.1 E3 Ubiquitin Ligases and Protein Quality Control Mechanism	5
2.2 Endoplasmic Reticulum Stress and E3 Ubiquitin Ligases	6-8
2.2.1 Gp78	7
2.2.2 Doa10	7
2.2.3 HRD1	7-8
2.2.4 Rfp2	8
2.2.5 RMA1	8
2.3 Oxidative Stress and E3 Ubiquitin Ligases	8-10
2.3.1 CHIP E3 Ubiquitin ligase	9
2.3.2 Keap1–Cul3–Rbx1 E3 Ligase Complex	9
2.3.3 Park–PINK–PARK7 E3 Ligase Complex	10
2.3.4 Cul2–VHL E3 Ligase Complex	10
2.4 E3 Ubiquitin Ligases Implicated In Neurodegeneration	10-12
2.4.1 Parkin	11-12
2.4.2 Malin	12
2.4.3 E6-AP	12
2.5 Global Impairment due to Protein Aggregation	13-14
2.6 Misfolded Proteins Recognition Tactics of E3 Ubiquitin Ligases	14-18
2.6.1 Conformational Plasticity of Disorder	14
2.6.2 Ribosomal Association of Quality Control Mechanism	15-16
2.6.3 Harmonious Interaction of E3 Ubiquitin Ligases with Chaperones	16
2.6.4 Disposal of Endoplasmic Reticulum-Anchored Misfolded Proteins	16-17
2.6.5 Hand to Hand Coordination	17
2.6.6 Modulated Recruitment with Misfolded Proteins	18
2.6.7 Sugar Chains Recognition	18
2.7 Model Misfolded Proteins	19-20
2.7.1 Heat-denatured Luciferase Protein	19
2.7.2 Expanded Polyglutamine Proteins	19-20
2.8 Mahogunin Ring Finger-1 E3 Ubiquitin Ligase	20-23
2.8.1 MGRN1 Gene and its Discovery	20
2.8.2 MGRN1 Protein and its Biochemical and Physiological Functions	20-23
Chapter 3: Mahogunin RING Finger-1 (MGRN1) Suppresses Chaperone Associated Misfolded Protein Aggregation and Toxicity	
3.1 Results	25-40
3.1.1 MGRN1 is Induced under Various Cellular Stress Conditions and Interacts with Hsp70	25-30
3.1.2 Recruitment of MGRN1 to Components of Inclusion Bodies Following Inhibition of Autophagy	30-31
3.1.3 MGRN1 is Colocalized with Hsp70-anchored Misfolded Luciferase Inclusion Formations	32-33
3.1.4 MGRN1 Alleviates Cellular Insults Generated by Various Stress-inducing Agents	34-35

	page
3.1.5 <i>MGRN1 Overexpression Induces the Degradation of Misfolded Luciferase Protein and Knockdown of MGRN1 Leads to Mitochondrial Membrane Depolarization and Cytochrome c Release</i>	35-38
3.1.6 <i>MGRN1 Protects against Cell Death mediated by ER and Oxidative Stress</i>	38-41
3.2 Discussion	41-43
3.3 Concluding Remarks	43
Chapter 4: Mahogunin RING Finger-1 Suppresses Misfolded Polyglutamine Aggregation and Cytotoxicity	
4.1 Results	45-65
4.1.1 <i>Misfolded and Ubiquitinated Expanded Polyglutamine Proteins Dysregulated MGRN1 Expression</i>	45-48
4.1.2 <i>MGRN1 Interacts with the N-terminal of Truncated Misfolded Huntingtin and Ataxin-3 with Expanded Polyglutamine Tract</i>	49-51
4.1.3 <i>Association and Recruitment of MGRN1 with Polyglutamine Aggregates</i>	51-54
4.1.4 <i>Recruitment and Colocalization of MGRN1 with p62 and Ubiquitin into Neuronal Aggregates of Mutant Huntingtin in the Brains of R6/2 Transgenic Mice</i>	55-58
4.1.5 <i>MGRN1 Promotes the Ubiquitination of Polyglutamine-expanded Proteins</i>	58-62
4.1.6 <i>MGRN1 Suppresses Aggregate Formation and Polyglutamine-induced Cell Death</i>	62-65
4.2 Discussion	65-67
4.3 Concluding Remarks	67
Chapter 5: Conclusion	69
Annexure A: Materials and Methods	
A.1 Materials	71-72
A.1.1 Cell Lines	72
A.1.2 Huntington's Disease (HD) Transgenic Mice Brain Samples	72
A.2 Methods	72-74
A.2.1 Large scale Plasmid Extraction	72
A.2.2 Cell culture, Transfection, Cell Viability Assay and Counting of Aggregates	72
A.2.3 Co-immunoprecipitation	72
A.2.4 Immunofluorescence and Immunohistochemical Techniques	72-73
A.2.5 MTT Assay	73
A.2.6 JC-1 Staining	73
A.2.7 Protein Estimation	73
A.2.8 SDS-PAGE and Immunoblotting	73
A.2.9 RNAi Experiments	73-74
A.2.10 TUNEL Assay	74
A.2.11 Filter Trap Assay	74
A.2.12 RT-PCR Analysis	74
A.2.13 Quantification of Agarose Gels and Immunoblots	74
A.2.14 Statistical Analysis	74
References	75-88

List of Figures

Figures	Title	page
1.1	Sequestration of various cellular components with protein aggregates	3
2.1	Cellular and molecular steps of protein quality control mechanism primarily implicated in various neurodegenerative diseases	6
2.2	Proposed diagrammatic representation of comprehend cellular tactics adopted by various E3 Ubiquitin ligases implicated in the clearance of misfolded and other client proteins	15
2.3	MGRN1 gene and its isoforms	21
3.1	MGRN1 mRNA levels are elevated under various stress conditions	26
3.2	MGRN1 protein levels are increased after cellular stresses	27
3.3	MGRN1 redistribution in cells with stress	27
3.4	MGRN1 gene contains an HSF1 binding site and CMA targeting motif	28
3.5	MGRN1 interacts with Hsp70 protein	29
3.6	Hsp70 interacts with MGRN1 protein	29
3.7	Negative control for MGRN1 and Hsp70 interaction analysis	30
3.8	MGRN1 is recruited to accumulated p62 after Bafilomycin treatment	30
3.9	Hsp70 colocalizes with p62 aggregates followed by Bafilomycin treatment	31
3.10	MGRN1 gets colocalized with Ubiquitin positive aggregates after Bafilomycin treatment	31
3.11	MGRN1 is recruited to accumulated Luciferase protein after heat stress	32
3.12	MGRN1 is colocalized with accumulated Hsp70 protein after heat stress	32
3.13	MGRN1 is recruited to heat-denatured Luciferase protein aggregates and Hsp70 protein followed by heat stress and Bafilomycin treatment	33
3.14	MGRN1 overexpression confers cytoprotection under various stress conditions	34
3.15	MGRN1 knockdown makes cells more vulnerable under stress conditions	35
3.16	MGRN1 promotes heat-denatured Luciferase protein degradation	36
3.17	Catalytically inactive MGRN1 does not promote Luciferase degradation	37
3.18	MGRN1 Knockdown Leads to Mitochondrial Dysfunction	38
3.19	MGRN1 overexpression representation	38
3.20	Immunocytochemistry and representative blot and RT-PCR analysis for MGRN1 knockdown in cultured A549 cells	39
3.21	MGRN1 leads to relatively increased cell viability under endoplasmic and oxidative stress conditions	40
3.22	MGRN1 knockdown decreases cell viability under stress conditions	41
4.1	MGRN1 dysregulation in expanded polyglutamine-expressing cells	46
4.2	Expression and immunoblotting of polyglutamine proteins	47
4.3	MGRN1 protein levels are decreased after overexpressing polyglutamine proteins	47
4.4	Ubiquitination of expanded polyglutamine proteins depletes the endogenous level of MGRN1 protein and interaction of MGRN1 with soluble misfolded normal and expanded polyglutamine proteins	48
4.5	MGRN1 interacts with polyglutamine proteins	49
4.6	Negative controls for interactions of MGRN1 and polyglutamine proteins	50
4.7	Polyglutamine proteins interact with endogenous MGRN1 protein	51
4.8	MGRN1 associates with Huntingtin expanded polyglutamine aggregates and recruitment of MGRN1 to Ataxin-3 aggregates	52
4.9	MGRN1 colocalizes with expanded polyglutamine protein aggregates in cells	53
4.10	MGRN1 colocalization with Huntingtin expanded polyglutamine protein, p62 and Ubiquitin-positive aggregates in cells	54
4.11	Redistribution and association of MGRN1 with p62-positive aggregates in R6/2 transgenic mice cerebellum and cortex	55
4.12	Colocalization of MGRN1 with Ubiquitin-positive aggregates in the neurons of the brain cerebellum and cortex of transgenic R6/2 mouse model of Huntington's Disease.	56
4.13	Recruitment of p62 and MGRN1 with Huntingtin-positive aggregates in the neurons of the transgenic R6/2 mouse model of Huntington's Disease	57

4.14	<i>Colocalization of MGRN1 with Huntingtin and p6- positive aggregates in model mice</i>	58
4.15	<i>MGRN1 overexpression induces the ubiquitination of expanded polyglutamine proteins</i>	59
4.16	<i>Expanded polyglutamine ubiquitination is promoted by MGRN1</i>	60
4.17	<i>MGRN1 promotes degradation of expanded Huntingtin polyglutamine proteins</i>	61
4.18	<i>Degradation of expanded polyglutamine protein by MGRN1 is suppressed by autophagy inhibition</i>	62
4.19	<i>MGRN1 reduces aggregation of expanded polyglutamine protein</i>	63
4.20	<i>Representative blots to confirm knockdown of MGRN1</i>	63
4.21	<i>MGRN1 suppresses aggregation of Huntingtin expanded polyglutamine protein</i>	64
4.22	<i>MGRN1 suppresses the expanded polyglutamine aggregation mediated cytotoxicity</i>	65