Alterations In Growth Factor Receptor Protein Binding Protein 2 (Grb2) Related Signaling In Huntington's Disease Cell Model

Thesis Submitted for the Degree of

Doctor of Philosophy (Science)

In Biotechnology

By

Shounak Baksi

Department of Biotechnology

University of Calcutta

2014

Acknowledgements

This journey was so far the best in terms of discovering me, my limits and understanding what I can count on.

On the road met a few noteworthy people who made the path memorable and seamless for me. I thank Prof. Debashis Mukhopadhyay, my thesis supervisor, who made it possible for me to complete my thesis work and made me understand its value. Apart from academic advises I always admired his ever optimistic thinking. I am thankful to him for unnumbered timely helps all through the course and also for giving me independence that I enjoyed very much. Prof. Subrata Banerjee, Head, Biophysics & Structural Genomics division was the person in SINP who encouraged me the most to carry out better work during Ph.D. and in life. This thesis would have never been possible without the support of Prof. Nitai Pada Bhattacharyya, Crystallography & Molecular Biology Division. Prof. Abhijit Chakrabarti, Prof. Oishee Chakrabarti and Prof. Udayaditya Sen, members of my thesis committee were also very encouraging and helpful throughout the course. I thank Prof. Nihar Ranjan Jana, NBRC who provided us the mouse model of Huntington's disease. I am thankful to Dr. P. Lajoie, Prof. T. Johansen & Dr. L. Thompson for kindly gifting me important clones used in this study and Dr. M. MacDonald for gifting the Huntington's disease cell model. I thank Prof. Bikash Sinha, Prof. Milan Sanyal & Prof. Bikash Chakrabarty past and present directors of SINP for providing excellent working facilities.

My sincere gratitude to Dr. Mithu Raychaudhuri, I learnt most of the cell biology techniques from her. I thank Dr. Arunabha Chakrabarti, Dr. Samir Das, Kasturi Roy, Sayantani Ghosh, Mohor Sengupta and Piyali Majumdar my lab colleagues, all helped me a lot whenever necessary. I thank all the scholars of Structural Genomics lab for their support and help. I am fortunate to have with me Sibnath da, Sumanta da, Dipankar da, Madhumita di, Sutapa di, Aditi di, Anita di, Nandini di, Avik, Suchismita, Anindita, Shilpita, Madhurima, Srijan, Priyanka, Devika, Debashree, Rukmini and Zenia as colleagues. Everyone maintained a very friendly yet professional environment in the lab. I thank all staff members of Biophysics & Structural Genomics division for technical help, especially Sanjoy da, Mahuya di, Manik da, Madhu da & Raju da. I can't stop thanking the members of Prof. NP Bhattacharyya's lab; Utpal da, Saikat da, Paramita di, Mithun da, Jayeeta di, Kamalika di, Moumita di, Eashita di, Srijit and Sudha, without their constant help and support this thesis work wouldn't be possible. I can't forget they showed me a way when nothing seemed working. I thank Sayantani, Sujyoti, Ananya, Sreetama, Priya and Mohini, the trainees who worked in this project. I learnt a lot working with all these people and I wish all a very happy and successful life ahead.

I take the opportunity to thank my post M.Sc. friends who were always a happy company –Manas, Barnali, Ramanuj, Seema, Sourav, Saptaparni, Avik, Suchismita, Srijit, Kasturi and Ajoy. I was very fortunate to have some of my old buddies with me and made some new as well. Srijit, Avik, Priyanka, Neha, Eashita di and Sayantani made my stay in SINP very special. I specially thank Srijit for helping me so much. Sarit and Arghya, my friends for life, were always there, with whom I shared all the joys and frustrations. I wish they will always be with me as they are.

I would like to thank all the faculties of post M.Sc. classes, Teaching section, Accounts section, Computer section and canteen of SINP. I thoroughly enjoyed the amazing collection of books in SINP library. I thank all the members of library for maintaining it so well. I also thank the authorities of SINP for making a well equipped gym.

Lastly I thank my family who are the reason for what I am today. My parents – my late Dad and my Mom are the pride, strength and inspiration of my life and will always be. My elder brother and sister-in-law are also very loving and are closest to me. Being born and raised in a joint family at North Kolkata, I feel very fortunate and being rooted in the place. All the members of my extended family are indispensable part of me and I am thankful to them all for being there for me.

Shounak Baksi

Contents

Abbreviations	1
Synopsis	4
Chapter 1: Introduction	6
1.1. Huntington's Disease	6
1.2. Genetic Basis of HD	7
1.3. The Huntingtin Protein	8
1.4. Aggregates of Mutant Huntingtin Protein	10
1.5. Homeostatic Imbalances in The Cell Due To Presence of Mutant Htt	11
1.5.1. Apoptosis	11
1.5.2. Mitochondrial Dysfunction	12
1.5.3. Autophagy in HD	13
1.5.4. Transcriptional Deregulation in HD	14
1.6. Htt Interacting Proteins	14
1.7. Htt and Epidermal Growth Factor Receptor	15
1.8. Growth Factor Receptor Protein Binding Protein 2 (Grb2)	16
1.9. Various functions of Grb2	17
1.10. Grb2 in Neurodegeneration	21
1.11. Objective of the Thesis	23
Reference	
Chapter 2: Materials and Methods	32
2.1. Materials	32
2.1.01. Mammalian Cell Lines	32
2.1.02. Bacterial Strains	32

2.1.03. Mouse Model	32
2.1.04. Plasmids	33
2.1.05. Chemically Synthesized Peptides	33
2.1.06. Instruments	33
2.1.07. Primers	34
2.1.08. Antibodies and Chemicals	34
2.2. Methods	36
2.2.01. Bioinformatic Search for Transcription Factor Binding Site	36
2.2.02. Primer Designing	36
2.2.03. Molecular Cloning	36
2.2.04. Cell Culture and Transfection	36
2.2.05. RNA Interference	36
2.2.06. RNA Isolation from Cultured Cells	37
2.2.07. RNA Isolation from Mouse Brain Tissue Samples	37
2.2.08. CDNA Preparation from RNA	38
2.2.09. Realtime PCR	38
2.2.10. Chromatin Immunoprecipitation	39
2.2.11. Protein Extraction for Western Blot	39
2.2.12. Western Blot	40
2.2.13. Co-immunoprecipitation	40
2.2.14. Confocal Imaging	40
2.2.15. Fluorescence Lifetime Imaging	41
2.2.16. Aggregate Count	41
2.2.17. Immunocytochemistry	42
2.2.18. Fluorescence Recovery after Photobleaching	42

2.2.19. Filter Retardation Assay	43
2.2.20. In vitro Chaperone assay	44
2.2.21. In vivo Chaperone Assay	44
2.2.22. Luciferase Assay	44
2.2.23. MTT Assay	45
2.2.24. Lysosomal Inhibition	45
2.2.25. Protein Expression and Purification	45
2.2.26. Peptide Binding Assay	46
2.2.27. Data Analysis and Representation	47
Reference	
Chapter 3: Results	50
3.1 Grb2 Related Signaling pathways in HD Model	50
3.1.1. Grb2 is Upregulated in Huntington's disease Model	50
3.1.2. Search for Probable Transcriptional Regulators	52
3.1.3. Foxd3 Regulates the Transcription of Grb2	55
3.1.4. Upregulated Grb2 deviates from its normal function	57
3.1.5. Grb2 preferentially interacts with mutant Htt	57
3.1.6. Grb2 helps in Htt clearance by evoking lyso-autophagy pathway and elicits autophagosome-lysosome fusion	59
3.1.7. Endogenous mutant Htt and Grb2 colocalize with autophagosome	65
3.2. Effect of Grb2 overexpression in Neuro2A cells	68
3.2.1. Elevated level of Grb2 reduces Htt Aggregation and shows Chaperone like activity	68
3.2.2. Interaction between mutant Htt and Grb2 takes place within vesicles	72
3.3 Finding out responsible domains for Htt-Grb2 Interaction	73
3.3.1. Proline rich domain of Htt exon1 interacts with Grb2	73

3.3.2. Htt binds to C terminal SH3 domain of Grb2	76
3.3.3. Mutant Htt replaces Grb2 partner Gab1 for interaction	79
Reference	
Chapter 4: Summary & Conclusion Reference	81