

LIST OF FIGURES

CHAPTER 1

Figure 1.1.1: Hallmarks of neurodegenerative diseases.

Figure 1.1.2: Path to cognitive decline in neurodegeneration.

Figure 1.2.1: Correlation between the length of polyQ and the age of onset.

Figure 1.2.2: Immune activation, induced by mutant huntingtin, occurs both peripherally and centrally in HD.

Figure 1.3.1: Schematic diagram of huntingtin amino acid sequence.

Figure 1.5.2: The schematic representation of Polycomb repressive complex 2 (PRC2) suppression of neurodegenerative transcription process in neurons.

Figure 1.5.1: The schematic representation showing interactions of PRC2 with chromatin.

Figure 1.6.1: Pathogenetic cellular mechanisms in Huntington's disease.

Figure 1.6.2: Model of mHTT aggregation, generation of ER stress and the consequent UPR protective and later pro-apoptotic responses.

CHAPTER 3

Figure 3.2.12.1: A schematic representation illustrating the generation process of Epstein Barr Virus (EBV) from B95-8 cells.

CHAPTER 4

Figure 4.1.1: 0.8% Agarose gel showing pFastBac vectors (HttQ23, HttQ46 and HttQ78).

Figure 4.1.2: 1.2% Agarose gel showing PCR confirmation of plasmids.

Figure 4.1.3: Blue-white Colonies observed upon transformation of plasmids into *E. coli* DH10Bac.

Figure 4.1.4: 0.8% Agarose gel showing bacmids (HttQ23, HttQ46 and HttQ78).

Figure 4.1.5: 2% Agarose gel showing PCR verification of HttQ23, HttQ46 and HttQ78 bacmids.

Figure 4.1.6: 0.8% Agarose gel showing site directed mutagenized PCR products for HttQ23.

Figure 4.1.7: 0.8% Agarose gel showing site directed mutagenized PCR products for HttQ23 as a result of gradient PCR.

Figure 4.1.8: 0.8% Agarose gel showing site directed mutagenized PCR products for HttQ46.

Figure 4.1.9: 0.8% Agarose gel showing site directed mutagenized PCR products for HttQ46 as a result of gradient PCR.

Figure 4.1.10: 0.8% Agarose gel showing *DpnI* digested site directed mutagenized PCR products for HttQ23 and HttQ46.

Figure 4.1.11: 0.8% Agarose gel showing recombinant mutagenized plasmids for HttQ23 and HttQ46 isolated using Plasmid Miniprep Kit.

Figure 4.1.12: 2% Agarose gel showing PCR confirmation for HttQ23 recombinant plasmids using HD1 and HD2 primers.

Figure 4.1.13: 2% Agarose gel showing PCR confirmation for HttQ46 recombinant mutagenized plasmids using HD1 and HD2 primers.

Figure 4.1.14: 2% Agarose gel showing PCR confirmation for mutagenized HttQ23 plasmids using Htt SQ primers.

Figure 4.1.15: 2% Agarose gel showing PCR confirmation for mutagenized HttQ46 plasmids using Htt SQ primers.

Figure 4.1.16: Blue-white Colonies observed upon transformation of HttQ23 recombinant plasmids into *E. coli* DH10Bac cells.

Figure 4.1.17: Blue-white Colonies observed upon transformation of HttQ46 recombinant plasmids into *E. coli* DH10Bac cells.

Figure 4.1.18: 0.8% Agarose gel showing HttQ23 S13A, HttQ23 S13D and HttQ23 S16D bacmids.

Figure 4.1.19: 0.8% Agarose gel showing HttQ46 S13A, HttQ46 S13D and HttQ46 S16D bacmids.

Figure 4.1.20: 2% Agarose gel showing PCR confirmation of HttQ23 and HttQ46 recombinant bacmids.

Figure 4.2.1: Western Blot image showing huntingtin expression at P1 stage.

Figure 4.2.2: Western Blot image showing huntingtin expression at P2 stage.

Figure 4.2.3: Western Blot image showing huntingtin expression at P3 stage.

Figure 4.2.4: Representative microscopy images showing *Sf9* cells after 72 hours post-incubation with HTT Q23, Q46 and Q78 P3 virus.

Figure 4.2.5: Western Blot image showing huntingtin expression after 48 hours post-incubation with HTT Q23, Q46 and Q78 P3 virus.

Figure 4.2.6: SDS-PAGE gel showing purified huntingtin proteins by FLAG affinity chromatography.

Figure 4.2.7: Western Blot image showing confirmation of huntingtin expression by FLAG affinity purification.

Figure 4.2.8: SDS-PAGE gel showing purified huntingtin proteins with SA or SD mutation by FLAG affinity chromatography.

Figure 4.2.9: Western Blot image showing confirmation of recombinant huntingtin expression with SA or SD mutation by FLAG affinity purification.

CHAPTER 5

Figure 5.1.1: 0.8% Agarose gel showing pFastBac vectors of PRC2 subunits (*Ezh2*, *EED*, *Suz12* and *RbAp48*).

Figure 5.1.2: Blue-white Colonies observed upon transformation of plasmids of PRC2 subunits into *E. coli* DH10Bac cells.

Figure 5.1.3: 0.8% Agarose gel showing bacmids of PRC2 subunits.

Figure 5.1.4: 0.8% Agarose gel showing PCR confirmation of bacmids of PRC2 subunits.

Figure 5.1.5: Western Blot image showing EZH2 expression at P2 stage.

Figure 5.1.6: Western blot image showing EZH2 expression at P3 stage.

Figure 5.1.7: Representative microscopy images showing *Sf9* cells after 48 hours post-incubation with EZH2, EED, SUZ12 and RbAp48 P3 virus.

Figure 5.1.8: SDS-PAGE gel showing purified PRC2 by different purification methods.

Figure 5.1.9: Western Blot image showing purified PRC2 expression by different purification methods.

Figure 5.2.1: Pictorial representation showing *in-vitro* Assay for PRC2 mediated H3K27me3.

Figure 5.2.2: Western Blot image showing *in-vitro* Assay for PRC2 mediated H3K27me3.

Figure 5.2.3: (A) Western Blot image and (B) Graphical representation for densitometric analysis showing *in-vitro* Assay for PRC2 mediated H3K27me3 with purified huntingtin proteins

Figure 5.3.1: (A) Western Blot image and (B) Graphical representation for densitometric analysis showing pSer13-pSer16 level in Kinetin, BMS 345541, and Bay 11-7082 treated *STHdh*^{Q7/Q7} and *STHdh*^{Q111/Q111} cells.

Figure 5.4.1: (A) Western Blot image and (B) Graphical representation for densitometric analysis showing Histone H3K27me3 activity in Kinetin, BMS 345541, and Bay 11-7082 treated *STHdh*^{Q7/Q7} and *STHdh*^{Q111/Q111} cells.

CHAPTER 6

Figure 6.1.1: Western Blot images (A, C) and graphical representations for densitometric analysis (B, D) show pSer13-pSer16 levels in HD150Q cells treated with Kinetin (A, B), BMS 345541, and Bay 11-7082 (C, D).

Figure 6.2.1A: 40X Fluorescence microscopic images showing GFP tagged mHTT aggregates in HD150Q cells after Pon A treatments for 24, 48, 72 and 96 hours, respectively.

Figure 6.2.1B: Graphical representation showing average number of puncta/field in HD150Q cells after Ponasterone A treatment for 24, 48, 72 and 96 hours, respectively.

Figure 6.2.2A: 40X Fluorescence microscopic images showing GFP tagged mHTT aggregates in HD150Q cells after different concentrations of Kinetin with/without Ponasterone A for 24 hours.

Figure 6.2.2B: Graphical representation showing average number of puncta/field in HD150Q cells after different concentrations of Kinetin with/without Ponasterone A for 24 hours

Figure 6.2.3A: 40X Fluorescence microscopic images showing GFP tagged mHTT aggregates in HD150Q cells after different concentrations of Kinetin with/without Ponasterone A for 48 hours.

Figure 6.2.3B: Graphical representation showing average number of puncta/field in HD150Q cells after different concentrations of Kinetin with/without Ponasterone A for 48 hours.

Figure 6.2.4: (A) Western Blot image and **(B)** Graphical representation for densitometric analysis showing mHTT aggregates in HD150Q cells after different concentrations of Kinetin with/without Ponasterone A for 24 hours.

Figure 6.2.4: (C) Western Blot image and **(D)** Graphical representation for densitometric analysis showing mHTT aggregates in HD150Q cells after different concentrations of Kinetin with/without Ponasterone A for 48 hours.

Figure 6.2.5A: 40X Fluorescence microscopic images showing GFP tagged mHTT aggregates in HD150Q cells after Ponasterone A with/without different concentrations of BMS 345541 and Bay 11-7082 for 48 hours.

Figure 6.2.5B: Graphical representation showing Average number of puncta/field in HD150Q cells after Ponasterone A with/without different concentrations of BMS 345541 and Bay 11-7082 for 48 hours.

Figure 6.2.6: (A) Western Blot image and **(B)** Graphical representation for densitometric analysis showing mHTT aggregates and soluble HTT expression in HD150Q cells after Ponasterone A with/without different concentrations of BMS 345541 for 48 hours.

Figure 6.2.6: (C) Western Blot image and **(D)** Graphical representation for densitometric analysis showing mHTT aggregates and soluble HTT expression in HD150Q cells after Ponasterone A with/without different concentrations of Bay 11-7082 for 48 hours.

Figure 6.3.1: Graphical representation of normalized ATP levels in HD150Q cells after different concentrations of Kinetin with/without Ponasterone A obtained through ATP determination kit.

Figure 6.3.2: Expression profile of some of the key mitochondrial genes (*Pgc1 α* and *Nrf1*) and *Bdnf* obtained through RT-qPCR.

Figure 6.3.3A: 40X Fluorescence microscopic images showing mitochondrial ROS levels using MitoSOX Red in HD150Q cells after Ponasterone A treatment for 24, 48, 72 and 96 hours, respectively.

Figure 6.3.3B: Graphical representation showing % fluorescence intensity of MitoSOX Red in HD150Q cells after Ponasterone A treatment for 24, 48, 72 and 96 hours, respectively.

Figure 6.3.3C: 40X Fluorescence microscopic images showing mitochondrial ROS levels using MitoSOX Red in HD150Q cells after different concentrations of Kinetin with/without Ponasterone A for 24 and 48 hours.

Figure 6.3.3D: Graphical representation showing % fluorescence intensity of MitoSOX Red in HD150Q cells after treatment of Kinetin with/without Ponasterone A for 24 and 48 hours.

Figure 6.3.4: Graphical representation of normalized ATP levels in HD150Q cells after Ponasterone A with/without different concentrations of (A) BMS 345541 and (B) Bay 11-7082 for 24 hours.

Figure 6.3.5: Expression profile of some of the key mitochondrial genes (*Pgc1 α* and *Nrf1*) and *Bdnf* after Ponasterone A with/without BMS 345541 and Bay 11-7082 treated HD150Q cells for 24 hours obtained through RT-qPCR.

Figure 6.3.6A: 40X Fluorescence microscopic images showing mitochondrial ROS levels using MitoSOX Red in HD150Q cells after Ponasterone A with/without different concentrations of BMS 345541 and Bay 11-7082 for 48 hours.

Figure 6.3.6B: Graphical representation showing % fluorescence intensity of MitoSOX Red in HD150Q cells after Ponasterone A with/without different concentrations of BMS 345541 and Bay 11-7082 for 48 hours.

Figure 6.4.1: Expression profile of some of the crucial genes involved in ER stress in HD150Q cells after treatment of Kinetin with/without Ponasterone A obtained through RT-qPCR.

Figure 6.4.2: Western Blot image showing some of the key proteins involved in ER stress in HD150Q cells after different concentrations of Kinetin with/without Ponasterone A treatment for 24 and 48 hours.

Figure 6.4.3: Graphical representations for densitometric analysis of some of the key proteins involved in ER stress in HD150Q cells after different concentrations of Kinetin with/without Ponasterone A for 24 and 48hours.

Figure 6.4.4: Expression profile of some of the crucial genes involved in ER stress after Ponasterone A with/without BMS 345541 and Bay 11-7082 treated HD150Q cells for 24 hours obtained through RT-qPCR.

Figure 6.4.5A: Western Blot image showing some of the key proteins involved in ER stress after Ponasterone A with/without different concentrations of BMS 345541 and Bay 11-7082 treated HD150Q cells for 48 hours.

Figure 6.4.5B: Graphical representations for densitometric analysis of some of the key proteins involved in ER stress after Ponasterone A with/without different concentrations of BMS 345541 and Bay 11-7082 treated HD150Q cells for 48 hours.

Figure 6.5.1A: Graphical representation of Ponasterone A and/or Kinetin treated HD150Q cells for cell survivability by MTT assay.

Figure 6.5.1B: Graphical representation of Ponasterone A and/or Kinetin treated HD150Q cells for cell survivability by MTT assay for 24, 48, 72 and 96 hours, respectively.

Figure 6.5.1C: Graphical representation of Ponasterone A with/without different concentrations of BMS 345541 and Bay 11-7082 treated HD150Q cells for cell survivability by MTT assay.

Figure 6.5.1D: Graphical representation of Ponasterone A with/without different concentrations of BMS 345541 and Bay 11-7082 treated HD150Q cells for cell survivability by MTT assay for 24, 48, 72 and 96 hours, respectively.

CHAPTER 7

Figure 7.1.1: Heatmap showing the "switch" behavior of 127 proteins, downregulated upon Pon A treatment and upregulated with Kinetin, BMS 345541, and Bay 11-7082, and vice versa.

Figure 7.1.2: The Venn diagram illustrates the overlap in protein expression between different experimental groups.

Figure 7.1.3: Venn diagram illustrating the overlap of upregulated proteins among Kinetin, BMS 345541, and Bay 11-7082 treatment groups compared to Pon A alone (disease condition).

Figure 7.1.4: The Venn diagram shows the overlap in downregulated proteins among Kinetin, BMS 345541, and Bay 11-7082 treatment groups compared to Pon A alone (disease condition).

Figure 7.2.1: Downregulated Pathways in Ponasterone A alone (HD Condition).

Figure 7.2.2: Upregulated Pathways Post-Treatments with Ponasterone A and different concentrations of Kinetin, BMS 345541 and Bay 11-7082.

Figure 7.2.3: Upregulated Pathways in Ponasterone A alone (HD Condition).

Figure 7.2.4: Downregulated Pathways Post-Treatments with Ponasterone A and different concentrations of Kinetin, BMS 345541 and Bay 11-7082.

Figure 7.3.1: Downregulated Biological Processes in Ponasterone A alone (HD Condition).

Figure 7.3.2: Upregulated Biological Processes Post-Treatments with Ponasterone A and different concentrations of Kinetin, BMS 345541 and Bay 11-7082.

Figure 7.3.3: Upregulated Biological Processes in Ponasterone A alone (HD Condition).

Figure 7.3.4: Downregulated Biological Processes Post-Treatments with Ponasterone A and different concentrations of Kinetin, BMS 345541 and Bay 11-7082.

Figure 7.4.1: Downregulated Cellular Components in Ponasterone A alone (HD Condition).

Figure 7.4.2: Upregulated Cellular Components Post-Treatments with Ponasterone A and different concentrations of Kinetin, BMS 345541 and Bay 11-7082.

Figure 7.4.3: Upregulated Cellular Components in Ponasterone A alone (HD Condition).

Figure 7.4.4: Downregulated Cellular Components Post-Treatments with Ponasterone A and different concentrations of Kinetin, BMS 345541 and Bay 11-7082.

CHAPTER 8

Figure 8.1.1: Representative microscopy images showing the growth of B95-8 cells.

Figure 8.1.2: A schematic representation illustrating Epstein Barr Virus production.

Figure 8.1.3: A diagram depicting the *in-vitro* infection process of purified mononuclear cells with Epstein-Barr virus.

Figure 8.1.4: 2% Agarose gel showing PCR confirmation of marker genes expression of healthy control and HD patient LCLs.

Figure 8.1.5: Representative microscopy images depicting cell growth post-infection with Epstein-Barr Virus (EBV).

Figure 8.1.6: 2% Agarose gel showing PCR confirmation for *HTT* gene using *HTT* specific primers.

Figure 8.1.7: Western Blot image showing huntingtin expression from control and HD LCLs.

Figure 8.2.1: Gene expression profiles of key (A) pro-inflammatory and (B) anti-inflammatory genes in Kinetin, BMS 345541, and Bay 11-7082 treated LCLs, as determined by RT-qPCR.

Figure 8.3.1: Expression profile of key pro-inflammatory and anti-inflammatory genes in PBMCs and THP-1 cells, following treatment with HD150Q cell secretome, as determined by RT-qPCR.

Figure 8.3.2: Graphical representation of normalized ATP levels in *STHdh*^{Q7/Q7} cells after different treatments of HD150Q secretome for (A) 24 hour and (B) 48 hours obtained through ATP determination kit.

CHAPTER 9

Figure 9.1: Schematic representation of the current study.