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Human cytomegalovirus UL97 kinase prevents the deposition of mutant protein aggregates in cellular models of Huntington's disease and Ataxia

Cristy Tower [a], Lianwu Fu [a], Rachel Gill [b], Mark Prichard [b], Mathieu Lesort [a,c], Elizabeth Sztul [a,*]

[a] Department of Cell Biology, University of Alabama at Birmingham, Birmingham, AL, USA
[b] Department of Pediatrics, University of Alabama at Birmingham, Birmingham, AL, USA
[c] Department of Psychiatry, University of Alabama at Birmingham, Birmingham, AL, USA

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ABSTRACT

The presence of aggregates of abnormally expanded polyglutamine (polyQ)-containing proteins are a pathological hallmark of a number of neurodegenerative diseases including Huntington's disease (HD) and spinocerebellar ataxia-3 (SCA3). Previous studies in cellular, *Drosophila*, and mouse models of HD and SCA have shown that neurodegeneration can be prevented by manipulations that inhibit polyQ aggregation. We have shown that the UL97 kinase of the human cytomegalovirus (HCMV) prevents aggregation of the pp71 and pp65 viral tegument proteins. To explore whether UL97 may act as a general antiaggregation factor, we examined whether UL97 prevents aggregation of cellular non-polyQ and polyQ proteins. We report that UL97 prevents the deposition of aggregates of two non-polyQ proteins: a protein chimera (GFP170*) composed of the green fluorescent protein and a fragment of the Golgi Complex protein (GCP-170) and a chimera composed of the red fluorescent protein (RFP) fused to the Werner syndrome protein (WRN), a RecQ helicase and exonuclease involved in DNA repair. Furthermore, we show that UL97 inhibits aggregate deposition in cellular models of HD and SCA3. UL97 prevents the deposition of aggregates of the mutant huntingtin exon 1 containing 82 glutamine repeats (HttExon1-Q82) or full length ataxin-3 containing a 72 polyQ track (AT3-72Q). The kinase activity of UL97 appears critical, as the kinase-dead UL97 mutant (K335M) fails to prevent aggregate formation. We further show that UL97 disrupts nuclear PML bodies and decreases p53-mediated transcription. The universality of the antiaggregation effect of UL97 suggests that UL97 targets a key cellular factor that regulates cellular aggregation mechanisms. Our results identify UL97 as a novel means to modulate polyQ aggregation and suggest that UL97 can serve as a novel tool to probe the cellular mechanisms that contribute to the formation of aggregates in polyglutamine disorders.

Introduction

The expansion of trinucleotide CAG repeats encoding glutamines within specific cellular proteins is the cause of inherited neurodegenerative diseases termed polyglutamine disorders. Huntington's disease, spinobulbar muscular atrophy (SBMA), dentatorubral–pallidoluysian atrophy (DRPLA), and spinocerebellar ataxias (SCA) 1, 2, 3, 6, 7, and 17 are all caused by an abnormally expanded polyQ domain (reviewed in Cummings and Zoghbi, 2000; McCampbell et al., 2001; Orr and Zoghbi, 2007). A striking neuropathological hallmark of these polyQ diseases is the presence of neuronal insoluble cytoplasmic aggregates and nuclear insoluble inclusions (NIIs) formed by mutant polyQ proteins (reviewed in Ross, 2002). The role NIIs and cytoplasmic aggregates in the pathological processes of polyQ diseases remains contentious. While some consider the NIIs/cytoplasmic aggregates as direct toxic intermediates, some have proposed that NIIs/cytoplasmic aggregates are a point of sequestration of a toxic product and thereby beneficial to a neuron (Arrasate et al., 2004; Ross et al., 1999).

Multiple lines of evidence support the view that protein aggregation is a complex process that is initiated by the accumulation of misfolded polyQ-containing proteins into a variety of higher-order intermediate conformational assemblies that ultimately form insoluble inclusion bodies. Conformational rearrangements of these mutated proteins likely change their biological activities and contribute to their toxicities. Importantly, recent reports suggest that the toxicity of mutant polyQ-containing proteins might be not related to the insoluble inclusion bodies but rather to soluble oligomeric or other intermediate conformations (Kayed et al., 2003, 2004). In support of this idea, polyQ-induced neurodegeneration can be prevented by pharmacological and molecular manipulations that target processes leading to oligomerization and aggregation. For instance, inhibition of polyglutamine oligomerization in a transgenic mouse model of HD has a marked protective effect on survival, weight loss, and motor function (Sanchez et al., 2003), supporting the idea that oligomerization of expanded

* Corresponding author. Department of Cell Biology, University of Alabama at Birmingham, 1918 University Blvd, McCallum Health Science Building, Room 657, Birmingham, AL 35294, USA. Fax: +1 205 934 0950.
E-mail address: esztul@uab.edu (E. Sztul).
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polyglutamine may play a pivotal role in the protein's toxicity. Similarly, the green tea polyphenol epi-gallocatechin-3-gallate (EGCG) inhibits aggregation of polyQ Httex1 *in vitro* and reduces polyQ-induced cytotoxicity in yeast cells and *Drosophila* models of the disease (Ehrnhoefer et al., 2006).

In addition to pharmacological approaches, modulations of cellular pathways that prevent aggregation also reverse neurotoxicity. Molecular chaperones are the first line of defense against protein aggregation, and numerous studies have shown that overexpression of chaperones prevents polyQ aggregation and reduces the pathology of neurodegenerative diseases (Muchowski and Wacker, 2005). In cultured cells, the chaperones heat shock protein 40 kDa (Hsp40) and 70 kDa (Hsp70) have been found to prevent the formation of spherical and annular oligomeric structures by a polyQ Httex1 (Wacker et al., 2004). Significantly, overexpression of Hsp70 in SCA1 transgenic mice decreases neurodegeneration and improves motor coordination (Cummings et al., 2001). Similarly, inhibiting polyQ aggregation by overexpressing Hsp70 (Warrick et al., 1999, 2005) and Hsp40 (Chan et al., 2000) rescues neurodegeneration in *Drosophila* models of SCA3. Altogether, these findings strongly suggest that specific polyQ conformational structures confer the cellular toxicity of the mutated proteins and that the identification of novel pharmacological, molecular, or genetic means to prevent polyQ aggregation is likely to have direct impact on developing therapeutic strategies for neurodegenerative diseases such as HD and SCA3.

We have recently documented that the UL97 kinase of HCMV prevents the aggregation of viral components during infection (Prichard et al., 2008). UL97 is a ~80-kDa tegument protein composed of 707 amino acids that is expressed during HCMV infection (Michel et al., 1998). UL97 contains a nuclear localization signal within its N-terminal domain and is targeted to the nucleus in infected or transfected cells (Prichard et al., 2005). UL97 is a kinase and shows homology to cellular serine/threonine kinases within conserved subdomains involved in substrate and ATP binding (Michel et al., 1998). The kinase activity of UL97 requires the invariant lysine at position 355, and the K355M mutant is inactive (Marschall et al., 2001). UL97 is important for viral replication, and a recombinant virus with a large deletion in UL97 replicates poorly and contains abnormal aggregates of viral proteins within the nuclei (Prichard et al., 1999, 2005). One of the aggregated proteins is the pp65 viral tegument protein, which also forms nuclear aggregates when expressed in transfected mammalian cells. Importantly, UL97, but not the catalytically inactive UL97 K355M, prevents pp65 aggregation (Prichard et al., 2005), suggesting that UL97 has antiaggregation activity and that the antiaggregation effect of UL97 is dependent on its kinase activity.

In addition to viral proteins, UL97 also prevents aggregation of GFP170*, a protein chimera formed by fusing an internal segment (amino acids 566–1375) of the Golgi protein GCP-170 to the C-terminus of GFP (Misumi et al., 2001; Hicks and Machamer, 2002; Prichard et al., 2008). We have shown previously that GFP170* forms nuclear aggregates similar in structure to those formed by the viral pp65 and also deposits in large ribbon-like aggregates within the cytoplasm (Fu et al., 2005a). UL97 prevents the formation of both the nuclear and the cytoplasmic GFP170* aggregates (Prichard et al., 2008). As with pp65, the catalytically inactive UL97/K355M mutant is unable to prevent GFP170* aggregation. Thus, UL97 prevents the aggregation of both a viral and a cellular protein. Herein, we examined the possibility that UL97 may possess a general antiaggregation activity and may serve as a tool for understanding and inhibiting the mechanisms that contribute to aggregation in polyQ diseases.

We report that UL97 has a strong antiaggregation effect on non-polyQ proteins as well as polyQ-expanded proteins associated with HD and SCA3. We show that UL97 prevents the deposition of aggregates of the non-polyQ Werner protein (WRN) that causes the premature aging disease Werner syndrome. We also show that UL97 prevents aggregation of a pathogenic construct that encodes the full-length ataxin-3 containing a 72-glutamine expansion (AT3-72Q), and of a pathogenic N-terminal huntingtin domain corresponding to the exon1 of this protein and containing an expanded track of 82 glutamine residues (HttExon1-82Q). In all cases, the catalytically inactive UL97/K355M mutant does not prevent aggregation. The similarity of the UL97 effect on the viral pp65 protein, the non-polyQ GFP170* and WRN proteins, and the polyQ AT3-72Q and HttExon1-82Q proteins suggests that UL97 has general antiaggregation effect. This similarity is also consistent with the hypothesis that aggregation of diverse proteins may occur through a common mechanism that is targeted by the UL97 kinase. In agreement, we show that UL97 disperses nuclear PML bodies and causes a decrease in p53-mediated transcription.

Our results identify UL97 as a novel means to inhibit the aggregation of polyQ proteins. They also designate UL97 as a new molecular tool to further examine the cellular mechanisms that lead to polyQ aggregation and neurodegeneration in HD and SCA3.

Materials and methods

Antibodies and reagents

Monoclonal antibody to the V5-epitope was purchased from Invitrogen (catalogue no. R960-25; Carlsbad, CA). Polyclonal anti-GFP antibody was purchased from Abcam (catalogue no. ab290-50; Cambridge, MA). Anti-myc monoclonal antibody was purchased from Covance (catalogue no. PRB-150B; Denver, PA). Anti-myc (A-14) (catalogue no. sc-789) polyclonal antibody, anti-PML (PG-M3) (catalogue no. sc-966) monoclonal antibody, and anti-PML (H-238) (sc-5621) polyclonal antibody were from Santa Cruz (Santa Cruz, CA). Fugene 6 transfection reagent was purchased from Roche (catalogue no. 11814443001; Indianapolis, IN), and was used in luciferase experiments, Mirus IT-LTI transfection reagent (catalogue no. MIR2300; Madison, WI) was used for transfection of cells for immunoflourescence microscopy. BCA protein assay kit was purchased from Thermo Scientific (catalogue no. 23225; Rockford, IO). Alexa Fluor 594-labeled goat anti-rabbit, Alexa Fluor 488-labeled goat anti-mouse, and Hoechst 33258 were from Invitrogen Molecular Probes, Inc (Eugene, OR).

DNA constructs

The construction of the GFP-GCP170* chimera has been previously described (Fu et al., 2005b). UL97 and K355M V5-epitope tagged plasmids have been previously described (Prichard et al., 2005). Plasmids encoding pGL2-p21A luciferase have been previously described (Chinery et al., 1997) and were a generous gift from Dr. Xinbin Chen (UC Davis School of Veterinary Medicine). HttExon1-8299Q-GFP plasmid has been described in Chun et al. (2001). The AT3-72Q plasmid was generously provided by Dr. Randall Pittman (University of Pennsylvania School of Medicine). The mRFP-Werner construct has been described previously (Vaitiekunaite et al., 2007) and was a gift from Dr. Marek Rusin (Maria Sklodowska-Curie Memorial Institute, Gliwice, Poland).

Cell culture and transfections

HeLa cells were purchased from ATCC (Manassas, VA). HeLa cells were cultured in minimum essential medium (MEM) supplemented with glucose, and glutamine (Mediatech, Comprehensive Cancer Center of the University of Alabama at Birmingham, AL). Media were supplemented with 10% fetal bovine serum (FBS, Life Technologies, Grand Island, NY), 100 U/ml of penicillin and streptomycin (Invitrogen Corporation, Grand Island, NY), nonessential amino acids, and 1 mM sodium pyruvate. HT1080 cells were a gift from Dr. Susan Nozell (University of Alabama at Birmingham). HT1080 cells were maintained in MEM plus L-glutamine and 10% fetal bovine serum. These cells express endogenous wild-type p53 and were used for luciferase assays. All cells were grown in incubator with 95% air in the atmosphere and 5% carbon dioxide (CO_2) at 37 °C.