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Transglutaminase 6 interacts with polyQ proteins and promotes the formation of polyQ aggregates

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ABSTRACT

A common feature of polyglutamine (polyQ) diseases is the presence of aggregates in neuronal cells caused by expanded polyglutamine tracts. PolyQ proteins are the substrates of transglutaminase 2, and the increased activity of transglutaminase in polyQ diseases suggests that transglutaminase may be directly involved in the formation of the aggregates. We previously identified the transglutaminase 6 gene to be causative of spinocerebellar ataxia type 35 (SCA35), and we found that SCA35-associated mutants exhibited reduced transglutaminase activity. Here we report that transglutaminase 6 interacts and co-localizes with both normal and expanded polyQ proteins in HEK293 cells. Moreover, the overexpression of transglutaminase 6 promotes the formation of polyQ aggregates and the conversion of soluble polyQ into insoluble polyQ aggregates. However, SCA35-associated mutants do not affect their interactions with polyQ proteins. These data suggest that transglutaminase 6 could be involved in polyQ diseases and there may exist a common pathological link between polyQ associated SCA and SCA35.

1. Introduction

Polyglutamine (polyQ) diseases are neurodegenerative disorders caused by expansion in specific genes with a trinucleotide repeat, cytosine–adenine–guanine (CAG), which encodes glutamine (Q). The first gene encoding polyQ proteins is that of an androgen receptor, whose mutations result in spinal and bulbar muscular atrophy [1]. Subsequently, eight other polyQ-associated neurodegenerative diseases were identified, including Huntington's disease (HD), dentatorubral-pallidoluysian atrophy and six types of spinocerebellar ataxia (SCA). PolyQ diseases share several features, such as the phenomenon of genetic anticipation, and a correlation between the mutant polyQ length, the severity of the diseases and the age of onset [2]. It seems that the presence of expanded polyQ in a protein confers a toxic gain-of-function mechanism, ultimately leading to neuronal dysfunction and cell death. Proteins with expanded polyQ repeats tend to take on an abnormal configuration, resulting in the formation and deposition of aggregates in neurons, the typical pathological hallmarks in polyQ diseases [3,4].

Transglutaminases (TGs) are Ca^{2+}-dependent enzymes that catalyze the formation of isopeptide bonds of glutaminyl residues and various other amine-bearing compounds. As an enzyme responsible for the majority of TG activity in the brain, the activity and expression of transglutaminase 2 (TG2) and TG-catalyzed products are increased in a variety of neurodegenerative diseases [5,6]. In 1988, Green et al. demonstrated that an increase in the number of glutamine residues beyond a certain threshold may result in a protein becoming a TG substrate [7]. Later, Green et al. demonstrated that peptides containing small polyQ domains (where $n \leqslant 18$) are excellent substrates for TG2-catalyzed attachment to glycine ethyl ester [8]. Further studies have confirmed that mutant huntingtin is an excellent TG substrate [9–11]. Besides huntingtin, TG2 also crosslinks the SCA1 gene product ataxin-1 into aggregates [12]. These data demonstrated that TG may be involved in the pathogenesis of polyQ diseases.

Previously, we identified the transglutaminase 6 (*TGM6*) gene to be causative of SCA35 [13]. Recently, we further demonstrated that TG6, the protein encoded by *TGM6*, was mainly localized in cytoplasm, and the SCA35-associated mutants exhibited reduced transglutaminase activity [14]. As TG2 could increase the formation of intracellular polyQ aggregates, this study demonstrated that TG6 also interacted with polyQ proteins and promoted polyQ proteins to form aggregates.

2. Materials and methods

2.1. Plasmid constructs

The truncated N-terminal fragment of huntingtin, which contained 150 CAG repeats, and full length ataxin-3 constructs, which

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contained 20 or 70 CAG repeats in pEGFP-N1, were kindly provided by Dr. Guanghui Wang (College of Pharmaceutical Sciences, Soochow University, Suzhou, China) and were described previously [15,16]. Wild-type (WT) or mutant pcDNA3.1-Myc-his(-)B-*TGM6* constructs were described previously [14]. *TGM6* cDNA was obtained by PCR from pcDNA3.1-Myc-his(-)B-*TGM6* and inserted into pGEX-3X at BamHI/EcoRI sites. *TGM6* mutations (D327G/L517W) were introduced using the QuikChange site-directed mutagenesis protocol (Stratagene). All constructs were verified by Sanger direct sequencing.

2.2. Cell culture and transfections

HEK293 cells were grown at 37 °C under a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U/ml of penicillin/streptomycin. All transfections were performed using Lipofectamine 2000 (Invitrogen). For experiments assessing polyQ aggregates, 1 μg of plasmid encoding the polyQ proteins was co-transfected with 2 μg of TG6 or pcDNA3.1-Myc-his(-)B as an empty vector control.

2.3. Immunofluorescence assays

HEK293 cells were grown on poly-D-lysine-coated coverslips and transiently co-transfected with WT or mutant Myc-tagged TG6 along with EGFP-tagged ataxin-3-20Q, ataxin-3-70Q or Htt-150Q, respectively. At 48 h after transfection, the cells were fixed in 4% paraformaldehyde. Anti-c-Myc primary antibody (Cell Signaling) was added, and the coverslips were incubated for 2 h at room temperature. Fluorescence-labeled secondary antibodies (Invitrogen) were then added, and the cells were incubated for 1 h in a dark room. After incubation with 4, 6-diamino-2-phenylindole (DAPI, Invitrogen) for 3 min, the cells were mounted in Fluoromount medium (Sigma) and examined with a laser scanning confocal system installed on a Carl Zeiss microscope. The images were analyzed using the Metaphor software package.

2.4. Glutathione S-transferase (GST) pull-down assay

The expression of GST fusion proteins was induced by 100 mM IPTG for 3 h at 37 °C. Recombinant GST fusion proteins were purified by incubating the *Escherichia coli* extracts with a 30 μl slurry of glutathione-Sepharose beads (GE healthcare) in buffer A (PBS, 1% NP-40 with 1 mM EDTA) supplemented with 1 mM PMSF and pro-

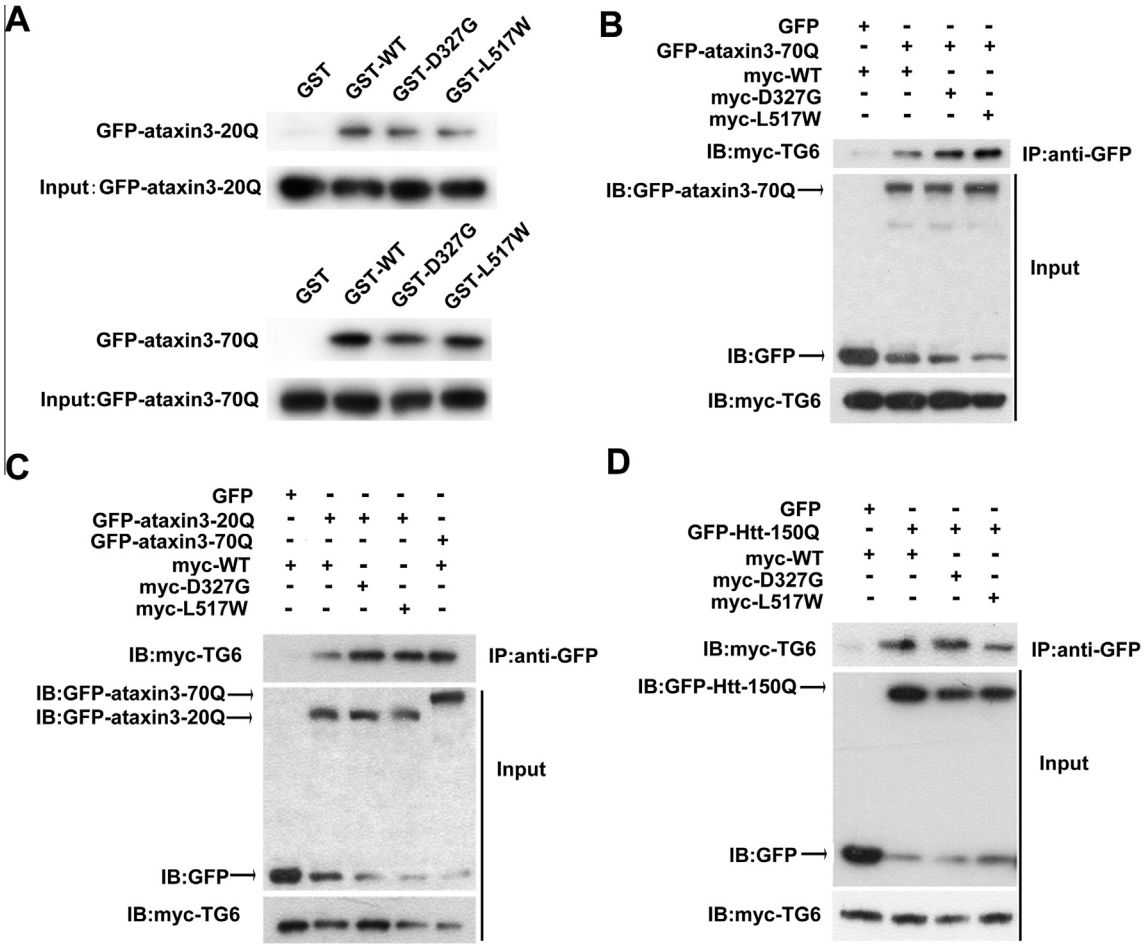

Fig. 1. TG6 interacts with polyQ proteins as demonstrated by GST pull-down and co-immunoprecipitation. (A) GST pull-down assay with HEK293 cells that ectopically expressed EGFP-ataxin-3-20Q or EGFP-ataxin-3-70Q. HEK293 cells were transiently transfected with plasmids that expressed EGFP-ataxin-3-20Q or EGFP-ataxin-3-70Q, respectively. Cell extracts were subjected to GST pull-down assay using glutathione-sepharose beads followed by immunoblotting with anti-GFP antibody. (B–D) Immunoprecipitation (IP) of TG6 and polyQ proteins. HEK293 cells were co-transfected with plasmids that expressed EGFP-tagged polyQ and Myc-tagged TG6, respectively. As a control, HEK293 cells co-transfected with plasmids that expressed GFP and WT TG6 were used. Forty-eight hours after transfection, cells were collected for IP with anti-GFP antibody and immunoblotted with anti-GFP antibody or anti-c-Myc antibody.

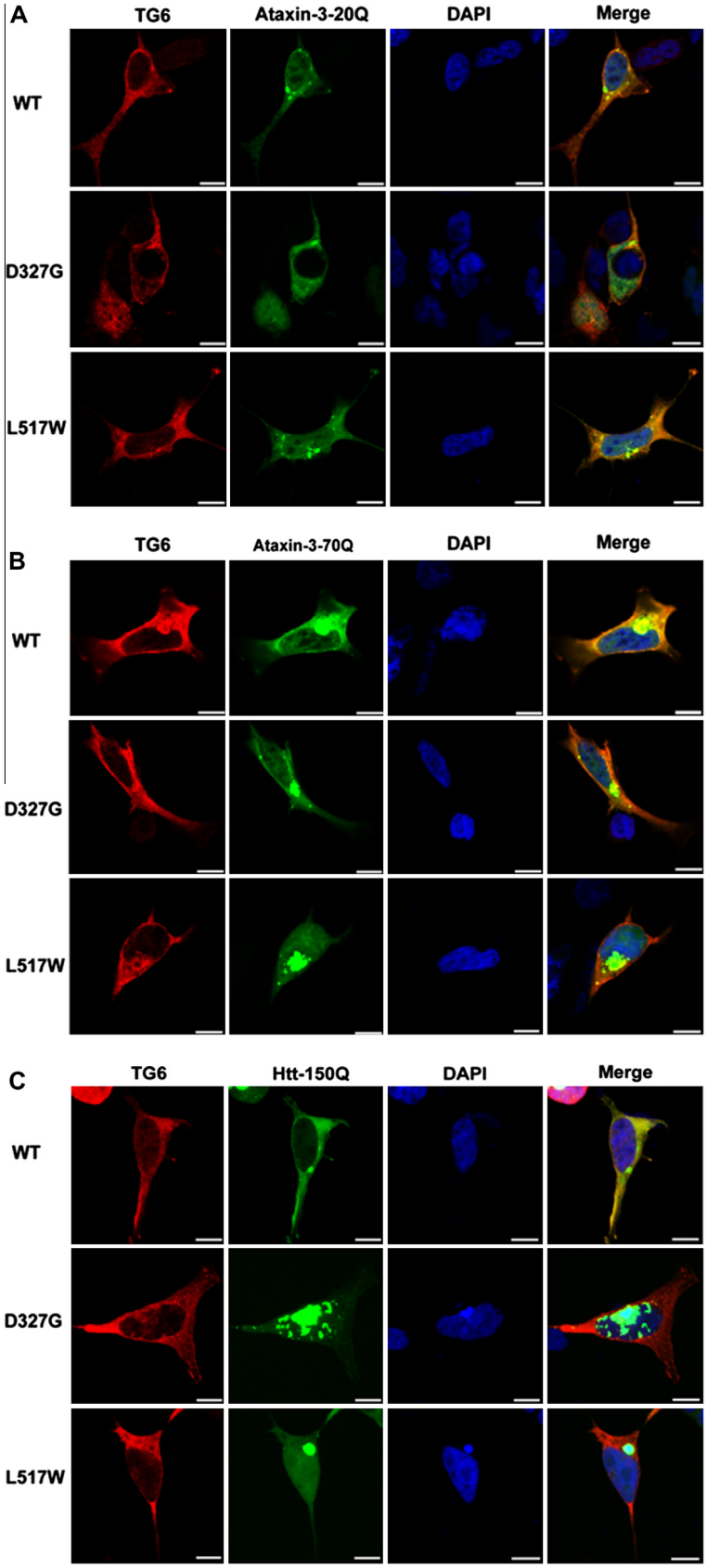

Fig. 2. TG6 co-localizes with polyQ aggregates. (A–C) HEK293 cells were co-transfected with plasmids that expressed Myc-tagged TG6 and EGFP-tagged polyQ proteins. An anti-c-Myc antibody was used to detect exogenous TG6. Co-localization was analyzed by confocal laser scanning microscopy. TG6 is shown in red, DAPI-stained nuclei are blue, polyQ proteins are shown in green, and merged images are shown in the third row. Scale bar = 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

tease inhibitor cocktail (Sigma) for 3 h at 4 °C with constant agitation. After washing with buffer A, the beads were resuspended in 1 ml of buffer A containing lysates from cells that transiently expressed EGFP-tagged ataxin-3-20Q or ataxin-3-70Q and incubated at 4 °C overnight with constant agitation. The glutathione beads were collected by centrifugation and washed extensively in buffer A. The bound proteins were eluted in 2 × SDS loading buffer and subjected to immunoblotting.

2.5. Co-immunoprecipitation and immunoblot analysis

HEK293 cells were transfected with plasmids expressing Myc-tagged WT or mutant TG6 and EGFP-tagged ataxin-3-20Q, ataxin-3-70Q or Htt-150Q, respectively. At 48 h after transfection, cells were harvested with the RIPA lysis buffer (150 mM NaCl, 1% NP-40, 2 mM EDTA, 50 mM Tris, pH 8.0, 1 mM PMSF, and protease inhibitor cocktail) at 48 h after transfection. The total protein content of the lysates was measured with the BCA protein assay (Pierce). Approximately 600 μg of whole cell lysates were incubated with 1 μg of anti-GFP antibody (Sigma) with constant agitation overnight at 4 °C. Then, 30 μl of a protein G agarose bead slurry (Sigma) was added to pull down the immunocomplexes. The beads were collected by centrifugation, washed extensively with PBS, and boiled with 2 × SDS loading buffer, the protein samples separated by 10% SDS–PAGE were transferred to a PVDF membrane (Millipore), probed with the appropriate antibody (1:500–1000 dilution), and then visualized using an ECL plus Western blotting detection system (GE Healthcare). Bands were scanned and quantified by densitometric analysis with Image J software and normalized with β-actin as a loading control.

2.6. Fractionation of cell lysates

HEK293 cells were transfected with plasmids that expressed Myc-tagged WT or mutant TG6 and EGFP-tagged ataxin-3-20Q, ataxin-3-70Q or Htt-150Q, respectively. To analyze the distribution of polyQ proteins in the soluble and insoluble fractions, at 48 h after transfection, we lysed HEK293 cells in RIPA lysis buffer, clarified by centrifugation at 13,000 rpm for 30 min. The supernatants were collected and referred to as the soluble fraction. The pellets (referred to as the insoluble fraction) were washed three times with RIPA lysis buffer and re-suspended with 2 × SDS lysis buffer, then were sonicated 3–4 times to disperse the aggregated materials. Equal amounts of proteins in each fraction were analyzed by immunoblotting.

3. Results

3.1. Interaction between TG6 and polyQ proteins

We first analyzed the interaction between TG6 and SCA3-associated polyQ protein ataxin-3 (20Q/70Q) by GST pull-down assay and co-immunoprecipitation. As shown in Fig. 1A, GST-fused WT TG6 protein, but not GST, was able to pull down EGFP-tagged ataxin-3 (20Q/70Q). These data suggest that both normal and expanded ataxin-3 interact with WT TG6 in vitro. To further examine the possible interaction between ataxin-3 and TG6, we co-transfected HEK293 cells with Myc-tagged TG6 and EGFP or EGFP-tagged ataxin-3 (20Q/70Q) expression plasmids, respectively. We found that the antibody that specifically recognized EGFP-tagged ataxin-3 (20Q/70Q) was able to pull down TG6 when ataxin-3 (20Q/70Q) and Myc-tagged TG6 were co-expressed, but not when TG6 was co-expressed with an EGFP control vector. (Fig. 1B and C). We thus demonstrated specific binding between TG6 and ataxin-3 in vivo. Further, we also examined the interactions between TG6 and another polyQ protein HD-associated Htt-150Q. Similar to ataxin-3, Htt-150Q also interacted with TG6 as demonstrated by co-immunoprecipitation (Fig. 1D). Both SCA35-associated mutants retained the ability to interact with polyQ proteins comparable to the WT TG6. Thus, we speculate that TG6 may interact with any glutamine-containing proteins when the polyQ length exceeds a certain threshold.

3.2. TG6 co-localizes with polyQ aggregates

Because aggregate formation is a pathological hallmark of polyQ diseases, we wondered whether the interaction between TG6 and polyQ proteins is involved in this pathological process. To address

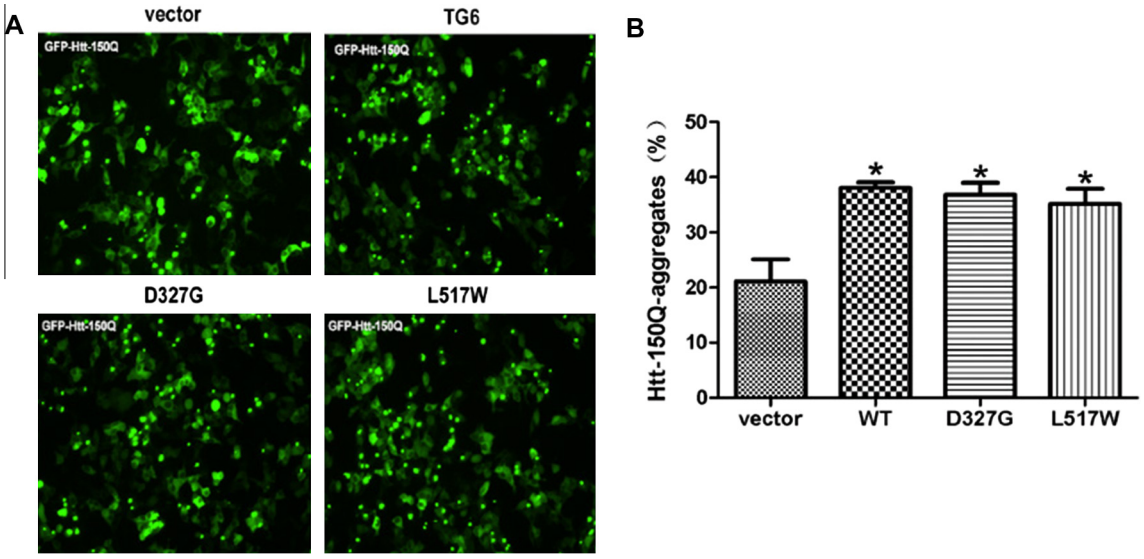

Fig. 3. Overexpression of TG6 increases the formation of polyQ aggregates. (A) HEK293 cells were co-transfected with plasmids that expressed EGFP-tagged polyQ and Myc-tagged TG6, respectively. HEK293 cells co-transfected with plasmids that expressed EGFP-tagged polyQ and empty vector were used as a control. After 48 h, the transfected cells were observed using an inverted system microscope IX71 (Olympus). (B) The percentages of aggregate-positive cells among EGFP-positive cells. Data are means ± SD values of triplicate assays. Asterisks indicate statistically significant differences compared with empty vector controls. $^{*}P < 0.05$ by one-way ANOVA with Dunnett's multiple comparison tests.

this possibility, we co-transfected HEK293 cells with plasmids that expressed WT or mutant TG6 and plasmids that expressed EGFP-tagged ataxin-3 (20Q/70Q) or EGFP-tagged Htt-150Q. Immunofluorescence analysis revealed that WT and mutant TG6 were normally localized in the cytosolic compartment of HEK293 cells. Ataxin-3-20Q was distributed throughout the cytoplasm and the nucleus, and co-localized with WT or mutant TG6 in the cytoplasm, but almost did not form aggregates (Fig. 2A). As expected, the overexpression of ataxin-3 70Q or Htt-150Q caused aggregate formation in the nucleus or perinucleus regions, with a small portion co-localized with WT and mutant TG6 (Fig. 2B and C).

3.3. Overexpression of TG6 promotes the formation of polyQ protein aggregates

To further explore the role of TG6 in polyQ aggregate formation, we counted the aggregates in the transfected cells. Fig. 3A showed that the EGFP-Htt-150Q-transfected cells presented a diffuse green fluorescence that was uniformly distributed throughout the cytoplasm and the nucleus. Twenty-four hours after transfection, the Htt-150Q fusion protein began to form large aggregate bodies, which were mainly localized in the nucleus. The co-expression of WT or mutant TG6 significantly increased aggregate formation in the GFP-positive cells (20% vs. 40%). This phenomenon was recapitulated when co-expressing TG6 and EGFP-ataxin-3-70Q aggregates (data not shown).

3.4. TG6 promotes the formation of insoluble polyQ aggregates

It has been reported that polyQ expansion is a gain-of-function mutation, causing polypeptides with expanded polyQ to acquire an unusual conformation, which leads to aggregation and eventually cell toxicity [17,18]. Because the co-expression of TG6 and Htt-150Q or ataxin-70Q increased polyQ aggregate formation in

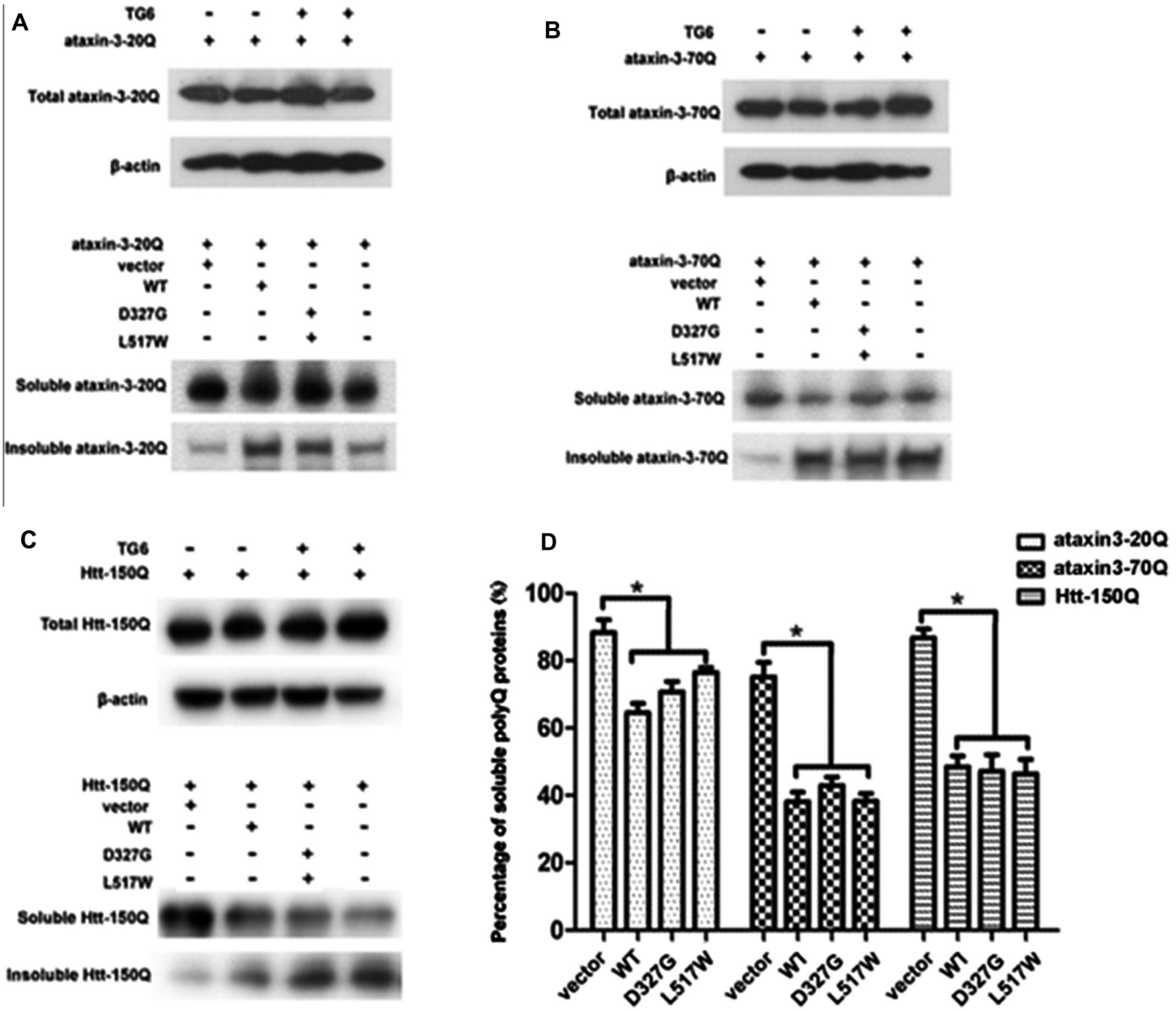

Fig. 4. Analysis of polyQ proteins in soluble and insoluble aggregates. (A–C) HEK293 cells were co-transfected with plasmids that expressed EGFP-tagged polyQ and Myc-tagged TG6, respectively. HEK293 cells co-transfected with plasmids that expressed EGFP-tagged polyQ and empty vector were used as a control. After 48 h, the total proteins were extracted by 2 × SDS lysis buffer. Soluble proteins were lysed in RIPA lysis buffer and clarified by centrifugation, and the pellets (referred as the insoluble fraction) were re-suspended with 2 × SDS lysis buffer and sonicated. Equal amounts of proteins in each fraction were analyzed by immunoblotting with anti-GFP antibody. (D) The percentage of soluble fraction was recorded as means ± SD values of triplicate assays. Asterisks indicate statistically significant differences compared with empty vector controls. $^{*}P < 0.05$ by one-way ANOVA with Dunnett's multiple comparison tests.

cells, we speculated that TG6 may facilitate the formation of insoluble aggregates. We co-transfected HEK293 cells with EGFP-tagged ataxin-3 (20Q/70Q) or Htt-150Q and WT or mutant TG6 or a vector control, respectively. First, we found that both WT and mutant TG6 have no effect on the total expression level of polyQ proteins (Fig. 4A–C). However, WT TG6 promoted the formation of polyQ aggregates by converting soluble polyQ into insoluble polyQ aggregates (Fig. 4A–C). Similar results were observed in TG6 mutants (Fig. 4A–D), indicating that TG6 may contribute to the insolubility of the expanded polyQ proteins andthat SCA35-associated TG6 mutants do not alter their impact on polyQ proteins.

4. Discussion

The aggregation of proteins that contain an expanded polyQ tract into insoluble inclusions is a key event in the pathogenesis of several neurodegenerative diseases; however, the mechanism related to neurotoxicity remains poorly understood [19–21]. Recent studies have revealed that TG2 promotes the formation of polyQ aggregates [22], and co-transfection of TG2 and mutant Htt exhibits increased cross-linked Htt in the insoluble fraction of cell lysates [10]. Several lines of evidence also indicate that TG2 involves the formation of mutant ataxin-1 aggregates [12,23]. In the present study, we first showed that TG6 interacts and co-localizes with both normal and expanded polyQ proteins in HEK293 cells. The overexpression of TG6 increased the formation of polyQ aggregates and promoted the conversion of soluble polyQ into insoluble polyQ aggregates, further supporting that TG6-catalyzed modification may be involved in the process of abnormal protein aggregation.

An increase in total TG enzymatic activity and TG-catalyzed substrates in several neurodegenerative diseases demonstrates that TG activity may be directly involved in the mechanisms of neurodegenerative diseases [24,25]. The aggregation of polyQ depends on TG activity. For example, the aggregation of Htt was inhibited by using a monoclonal antibody against TG that is known to block its activity [11]. Cystamine, a TG inhibitor, could decrease the formation of Htt aggregates and increase the viability of cells that have been transfected with truncated Htt-148Q and either TG2 or vector [10]. TG6 is the causative gene of SCA35, and two missense mutations (D327G and L517W) of TG6 were reported in our previous study. And we also found that mutant TG6 lost a part of its enzyme activities and sensitized cells to apoptosis. Here, we found that SCA35-associated TG6 mutants do not alter their interactions with polyQ proteins. This may be due to mutant TG6 still retaining partial enzyme activities that are able to catalyze the proteins that contain polyQ residues, ultimately leading to the increased formation of insoluble aggregates.

PolyQ inclusion bodies in mammalian cells are complex structures that contain many proteins, including molecular chaperones, components of the ubiquitin-proteasome system, centrosomal material, and cytoskeletal proteins [26–29]. Previous studies have shown that TG2 may play a major role in the insolubility of the aggregates. TG2 was postulated to facilitate the assembly of polyQ domains through polar-zipper formation and intermolecular cross-linking [8,30,31]. TG2 was supposed to translocate to the nucleus in response to the nuclear accumulation of mutant ataxin-1 and contribute to the formation of insoluble aggregates [12]. Similarly, we found that TG6 also translocated to the nucleus and co-localized with polyQ aggregates. Interestingly, in SCA35 pedigrees, we did not detect any mutations encoding the pathologic polyQ protein. Whether there exists a common pathological link between polyQ associated SCA and SCA35 still requires further investigation.

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References

[1] A.R. La Spada, E.M. Wilson, D.B. Lubahn, A.E. Harding, K.H. Fischbeck, Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy, Nature 352 (1991) 77–79.
[2] H.Y. Zoghbi, H.T. Orr, Glutamine repeats and neurodegeneration, Annu. Rev. Neurosci. 23 (2000) 217–247.
[3] A. Michalik, C. Van Broeckhoven, Pathogenesis of polyglutamine disorders: aggregation revisited, Hum. Mol. Genet. 12 (Spec. No. 2) (2003) R173–R186.
[4] C.A. Ross, M.A. Poirier, Protein aggregation and neurodegenerative disease, Nat. Med. 10 (Suppl.) (2004) S10–S17.
[5] T.M. Jeitner, J.T. Pinto, B.F. Krasnikov, M. Horswill, A.J. Cooper, Transglutaminases and neurodegeneration, J. Neurochem. 109 (Suppl. 1) (2009) 160–166.
[6] T.M. Jeitner, N.A. Muma, K.P. Battaile, A.J. Cooper, Transglutaminase activation in neurodegenerative diseases, Future Neurol. 4 (2009) 449–467.
[7] M. Simon, H. Green, The glutamine residues reactive in transglutaminase-catalyzed cross-linking of involucrin, J. Biol. Chem. 263 (1988) 18093–18098.
[8] P. Kahlem, C. Terre, H. Green, P. Djian, Peptides containing glutamine repeats as substrates for transglutaminase-catalyzed cross-linking: relevance to diseases of the nervous system, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 14580–14585.
[9] P. Kahlem, H. Green, P. Djian, Transglutaminase action imitates Huntington's disease: selective polymerization of Huntingtin containing expanded polyglutamine, Mol. Cell 1 (1998) 595–601.
[10] G.M. Zainelli, N.L. Dudek, C.A. Ross, S.Y. Kim, N.A. Muma, Mutant huntingtin protein: a substrate for transglutaminase 1, 2, and 3, J. Neuropathol. Exp. Neurol. 64 (2005) 58–65.
[11] M.V. Karpuj, H. Garren, H. Slunt, D.L. Price, J. Gusella, M.W. Becher, L. Steinman, Transglutaminase aggregates huntingtin into nonamyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 7388–7393.
[12] D.R. D'Souza, J. Wei, Q. Shao, M.D. Hebert, S.H. Subramony, P.J. Vig, Tissue transglutaminase crosslinks ataxin-1: possible role in SCA1 pathogenesis, Neurosci. Lett. 409 (2006) 5–9.
[13] J.L. Wang, X. Yang, K. Xia, Z.M. Hu, L. Weng, X. Jin, H. Jiang, P. Zhang, L. Shen, J.F. Guo, N. Li, Y.R. Li, L.F. Lei, J. Zhou, J. Du, Y.F. Zhou, Q. Pan, J. Wang, J. Wang, R.Q. Li, B.S. Tang, TGM6 identified as a novel causative gene of spinocerebellar ataxias using exome sequencing, Brain 133 (2010) 3510–3518.
[14] W.J. Guan, J.L. Wang, Y.T. Liu, Y.T. Ma, Y. Zhou, H. Jiang, L. Shen, J.F. Guo, K. Xia, J.D. Li, B.S. Tang, Spinocerebellar ataxia type 35 (SCA35)-associated transglutaminase 6 mutants sensitize cells to apoptosis, Biochem. Biophys. Res. Commun. 430 (2013) 780–786.
[15] E. Fei, N. Jia, T. Zhang, X. Ma, H. Wang, C. Liu, W. Zhang, L. Ding, N. Nukina, G. Wang, Phosphorylation of ataxin-3 by glycogen synthase kinase 3beta at serine 256 regulates the aggregation of ataxin-3, Biochem. Biophys. Res. Commun. 357 (2007) 487–492.
[16] G.H. Wang, K. Mitsui, S. Kotliarova, A. Yamashita, Y. Nagao, S. Tokuhiro, T. Iwatsubo, I. Kanazawa, N. Nukina, Caspase activation during apoptotic cell death induced by expanded polyglutamine in N2a cells, Neuroreport 10 (1999) 2435–2438.
[17] E. Bossy-Wetzel, R. Schwarzenbacher, S.A. Lipton, Molecular pathways to neurodegeneration, Nat. Med. 10 (Suppl.) (2004) S2–S9.
[18] H.L. Paulson, N.M. Bonini, K.A. Roth, Polyglutamine disease and neuronal cell death, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 12957–12958.
[19] L. Lorand, Neurodegenerative diseases and transglutaminase, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 14310–14313.
[20] A.J. Tobin, E.R. Signer, Huntington's disease: the challenge for cell biologists, Trends Cell Biol. 10 (2000) 531–536.
[21] G. Hoffner, P. Djian, Protein aggregation in Huntington's disease, Biochimie 84 (2002) 273–278.
[22] V. Gentile, C. Sepe, M. Calvani, M.A. Melone, R. Cotrufo, A.J. Cooper, J.P. Blass, G. Peluso, Tissue transglutaminase-catalyzed formation of high-molecular-weight aggregates in vitro is favored with long polyglutamine domains: a possible mechanism contributing to CAG-triplet diseases, Arch. Biochem. Biophys. 352 (1998) 314–321.

[23] P.J. Vig, J. Wei, Q. Shao, M.D. Hebert, S.H. Subramony, L.T. Sutton, Role of tissue transglutaminase type 2 in calbindin-D28k interaction with ataxin-1, Neurosci. Lett. 420 (2007) 53–57.
[24] N.A. Muma, Transglutaminase is linked to neurodegenerative diseases, J. Neuropathol. Exp. Neurol. 66 (2007) 258–263.
[25] A. Martin, G. De Vivo, V. Gentile, Possible role of the transglutaminases in the pathogenesis of Alzheimer's disease and other neurodegenerative diseases, Int. J. Alzheimers Dis. 2011 (2011) 865432.
[26] C.A. Vidair, R.N. Huang, S.J. Doxsey, Heat shock causes protein aggregation and reduced protein solubility at the centrosome and other cytoplasmic locations, Int. J. Hyperthermia 12 (1996) 681–695.
[27] C. Wojcik, D. Schroeter, S. Wilk, J. Lamprecht, N. Paweletz, Ubiquitin-mediated proteolysis centers in HeLa cells: indication from studies of an inhibitor of the chymotrypsin-like activity of the proteasome, Eur. J. Cell Biol. 71 (1996) 311–318.
[28] W.C. Wigley, R.P. Fabunmi, M.G. Lee, C.R. Marino, S. Muallem, G.N. DeMartino, P.J. Thomas, Dynamic association of proteasomal machinery with the centrosome, J. Cell Biol. 145 (1999) 481–490.
[29] R.P. Fabunmi, W.C. Wigley, P.J. Thomas, G.N. DeMartino, Activity and regulation of the centrosome-associated proteasome, J. Biol. Chem. 275 (2000) 409–413.
[30] P. Kahlem, H. Green, P. Djian, Transglutaminase as the agent of neurodegenerative diseases due to polyglutamine expansion, Pathol. Biol. (Paris) 46 (1998) 681–682.
[31] M.F. Perutz, B.J. Pope, D. Owen, E.E. Wanker, E. Scherzinger, Aggregation of proteins with expanded glutamine and alanine repeats of the glutamine-rich and asparagine-rich domains of Sup35 and of the amyloid beta-peptide of amyloid plaques, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 5596–5600.