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Hsp27 decreases inclusion body formation from mutated GTP-cyclohydrolase I protein

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Abstract

GTP cyclohydrolase I (GCH), an oligomeric protein composed of 10 identical subunits, is required for the synthesis of neurotransmitters; mutations in GCH are associated with dopa-responsive dystonia (DRD) and hyperphenylalaninemia. Mutated GCH proteins are unstable and prone to dominant-negative effect. We show herein that expression of the GCH mutant GCH-201E or the splicing variant GCH-II caused intracellular inclusion bodies. When Hsp27 was expressed together with the GCH mutants, Hsp27 expression decreased the formation of inclusion bodies by GCH (as assessed by immunofluorescence) and decreased the amount of insoluble GCH mutant proteins (as assessed by Western blot). Transfection of pcDNA-Hsp27-S3D, a phosphorylation-mimicry Hsp27 mutant, was more effective at the mutated GCH proteins than transfection with pcDNA-Hsp27, but okadaic acid, a phosphatase inhibitor, enhanced the effect of pcDNA-Hsp27. Hsp27-S3D also abolished the dominant-negative action of GCH-II. The mutated GCH proteins interacted with the wild-type GCH protein; the inclusion bodies were positive for lysosomal marker LAMP1, soluble in 2% SDS, and were not ubiquitinated. Phophorlyated Hsp27 also decreased the inclusion body formation by the huntingtin polyglutamines. Therefore, diseases involving mutated oligomeric proteins would be manageable by chaperone therapies.

Keywords: GTP-cyclohydrolase I; Dopa-responsive dystonia; Inclusion body formation; Chaperone; Heat shock protein 27; Phosphorylation

1. Introduction

GTP cyclohydrolase I (GCH; EC 3.5.4.16) [1] is a homo-decameric protein [2,3], and mutations of GCH gene are associated with a wide range of clinical conditions, ranging from benign dopa-responsive dystonia (DRD) to malignant hyperphenylalaninemia [4–7]. The oligomeric quaternary structure of GCH seems to amplify its molecular defects and cause phenotype variabilities. Previously we have shown that mutated GCH proteins are unstable and prone to dominant-negative effect [4,5]. In this study, we show that expression of GCH-201E (a mutation causing DRD) or splicing variant GCH-II (which encodes a shorter peptide) in baby hamster kidney (BHK) cells causes the formation of prominent punctate cytoplasmic inclusion bodies in immunofluorescence staining.

Protein aggregates are involved in neurodegenerative diseases including Alzheimer disease, Parkinson disease, Huntington disease, and prion diseases [8]. The aggregates consist of fragments of mutated proteins like the polyglutamines in Huntington disease, or the aberrantly cleaved amyloid beta protein in Alzheimer disease [8]. These polypeptides have a highly abnormal conformation, which prevents normal folding, and therefore form aggregates through polymerization. For example, polyglutamines form pleated sheets of beta-strands held together by hydrogen bonds between their amides, and associate irreversibly into oligomers firmly interlocked by either strand- or domain-swapping [9] or covalent bonds [10]. The aggregates themselves, or early steps in the cascade, cause toxicity and subsequent death of the cells.

Abbreviations: Hsp, heat shock protein; GCH, GTP-cyclohydrolase I; BHK cells, baby hamster ovary cells; DRD, dopa-responsive dystonia

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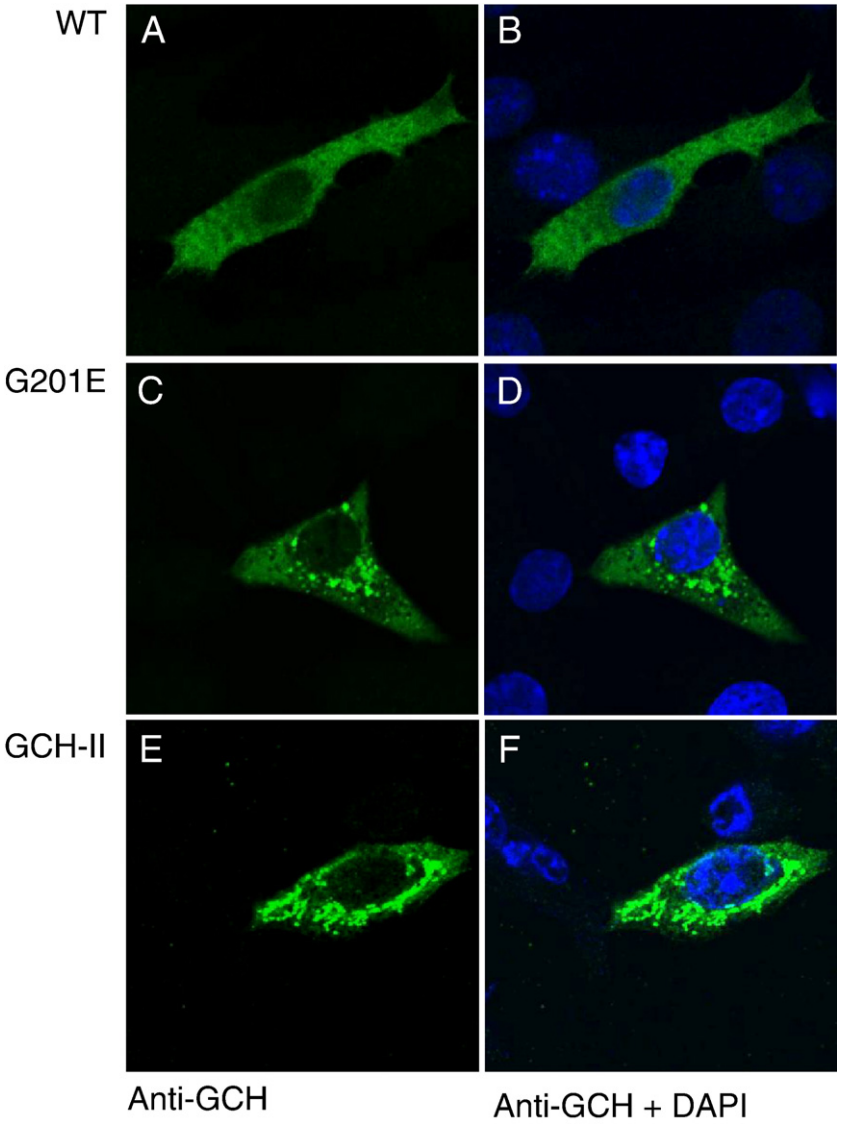

Fig. 1. GCH-II and GCH-201E form cytoplasmic inclusion bodies. Immunofluorescence staining of BHK cells expressing pCMV-A16-GCH-WT (WT), pCMV-GCH-201E (G201E), or pCMV-GCH-II (GCH-II). The left panel shows GCH staining (green) and the right panel shows both GCH and 4′,6-diamidino-2-phenylindole (DAPI) staining (blue). DAPI stains the nuclei. Both GCH-G201E and GCH-II form small punctate cytoplasmic inclusion bodies.

Molecular chaperones, or heat shock proteins, are initially known as cellular machineries against the accumulation of damaged proteins during stress. Hsp70 and other chaperones also protect partially synthesized peptides when they emerge from the ribosomes [11,12]. Overexpression of chaperones has been evaluated as a therapeutic strategy to promote proper folding or degradation of misfolded proteins [13,14]. It has been shown that chaperones HDJ-2/HSDJ [13], Hsp40 and Hsp70 [15], and MRJ [16] modulate polyglutamine pathogenesis. Chaperonin TRiC promotes the assembly of polyglutamine expansion proteins into nontoxic oligomers [17]. Actually, upregulation of Hsp70 may be an intrinsic cellular response against the neuronal degeneration mediated by the huntingtin mutant [18].

In this study, we tested the effect of small heat shock protein Hsp27 [19] on GCH inclusion body formation. We demonstrated that Hsp27, in its phosphorylated form, effectively prevented GCH-201E- and GCH-II-mediated formation of inclusion bodies, so chaperone therapy could be helpful in these conditions.

2. Materials and methods

2.1. Vectors and chemicals

Expression vectors pCMV-A16-GCH-WT (GCH-WT), pCMV-GCH-II (GCH-II), and pCMV-GCH-201E (GCH-201E) have been described previously [4,5]. N-terminal AGP/EBP A16 epitope was added to the GCH proteins for the convenience of detection, and the A16-tagged GCH proteins have molecular weights higher than the HA-tagged or untagged GCH proteins [20]. A rabbit antiserum (anti-N20) against the A16 epitope was developed in house. pcDNA3-Hsp27 (Hsp27) and pcDNA3-Hsp27-S3D (Hsp27-S3D) were gifts from Dr. Gaestel [21]. Replacing serine 15, 78, and 82 by aspartate, Hsp27-S3D mimics Hsp27 phosphorylation [21]. Okadaic acid was obtained from Sigma Chemical Company (St Louis, Mo, USA). A polyglutamine tract with alternating CAG/CAA repeats, encoding 71 glutamines (71Q-GFP), was inserted into the context of the