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Research article

The mouse/human cross-species heterodimer of leucine-rich repeat kinase 2: Possible significance in the transgenic model mouse of Parkinson's disease

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HIGHLIGHTS

- Mouse LRRK2 and human LRRK2 form cross-species heterodimers.
- Human I2020 T mutant LRRK2 promotes the degradation of mouse LRRK2.
- The cross-species heterodimer will affect the phenotypes of transgenic model mouse.
- We propose a new concept of cross-species dimer/oligomer in transgenic model mice.

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ABSTRACT

Leucine-rich repeat kinase (LRRK2) is the causal molecule of autosomal dominant Parkinson's disease (PD). We previously reported that intracellular degradation of wild-type (WT) LRRK2 is promoted by formation of heterodimers with the I2020T mutant LRRK2. In the present study, we investigated whether this is also the case for mouse/human cross-species heterodimers, which could be formed in transgenic mice. First, by co-transfection and immunoprecipitation, we identified the cross-species heterodimer of mouse LRRK2 and human LRRK2. Next, we found that the protein level of mouse LRRK2 decreased when co-transfected with human I2020T LRRK2, but not with human WT LRRK2. These results suggested that degradation of mouse LRRK2 was promoted by formation of a cross-species heterodimer with the mutant LRRK2. In I2020T LRRK2-transgenic mice, the lower protein level of brain LRRK2 in comparison with control mice, together with higher expression of the mRNA, suggested that endogenous LRRK2 was degraded by formation of cross-species heterodimers. Our results suggest a new concept of cross-species dimer/oligomer formation in transgenic disease-model mice.

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1. Introduction

Leucine-rich repeat kinase (LRRK2) was originally identified as the causal molecule of autosomal dominant Parkinson's disease (PD) [1–3]. LRRK2 consists of several domains: a leucine-rich repeat, ROC, the C-terminal of ROC, kinase, and WD40 [4]. LRRK2 exists as a dimer under physiological conditions [5,6]. We have reported that I2020T-mutant LRRK2 detected in the Sagamihara PD family has a higher intracellular degradation rate than the wild-type (WT) molecule [7,8]. Furthermore, degradation of WT LRRK2 is promoted by formation of WT/I2020T heterodimers, indicating a dominant-negative mechanism of neurodegeneration [9].

One of the important approaches for clarifying the etiology of PD resulting from LRRK2 mutation is analysis of transgenic mice expressing human mutant LRRK2. We previously established a transgenic mouse line expressing the I2020T LRRK2 [10]. The transgenic mice exhibited impaired locomotive ability and several cellular and biochemical abnormalities. Because mouse LRRK2 has a molecular structure very similar to that of human LRRK2,

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i.e., in terms of the domain as well as dimer structures and showing 86% amino acid homology [2,5,6], it would be important to examine whether the mouse and human LRRK2 molecules form cross-species heterodimers, and if so, whether the I2020T-mutant human LRRK2 would affect endogenous mouse LRRK2 in transgenic mice.

In the present study, we co-transfected HEK 293 cells with mouse LRRK2 and human LRRK2 cDNAs. Immunoprecipitation and Western analysis identified the cross-species heterodimer of LRRK2. Furthermore, the protein level of mouse LRRK2 became low when co-transfected with I2020T mutant LRRK2, but not with WT LRRK2. In the I2020T transgenic mice, the protein level of brain LRRK2 was low, although not to a significant degree, in comparison with control mice. Because the expression of mRNA was higher [10], it appeared that cross-species dimer formation led to degradation of endogenous mouse LRRK2.

2. Materials and methods

2.1. Transfection with LRRK2 cDNAs

The WT and I2020T-mutant human LRRK2 cDNAs bearing a C-terminal V5 tag have been reported previously [7]. The mouse LRRK2 cDNA was kindly provided by Dr Ted Dawson of Johns Hopkins University [11] and introduced into the pEF1/Myc-His mammalian expression vector (Invitrogen). The cDNA plasmids were transfected into HEK293 cells using Lipofectamine 2000 (Invitrogen) as described previously [7]. The Myc-mouse LRRK2 cDNA was also transfected into SH-SY5Y clones (WT6-A52) stably expressing V5-WT human LRRK2. Cell lysates were prepared 48 h after transfection.

2.2. Immunoprecipitation and western analysis

Mouse LRRK2 and human LRRK2 were immunoprecipitated from lysates of the transfected HEK 293 cells using Myc-Tag (9B11) Mouse mAb Sepharose Bead Conjugate™ (Cell Signaling) and Anti-V5 Agarose Affinity Gel antibody™ (Sigma), respectively. As a control, normal mouse IgG-conjugated agarose beads (Sigma) were used. The precipitates were subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) followed by Western analysis using horseradish peroxidase-conjugated antibodies against the V5 and Myc tags (Invitrogen). The LRRK2 was also detected with a rabbit monoclonal antibody, MJFF2, recognizing both mouse and human LRRK2 (Epitomics).

2.3. Transgenic mice

The transgenic mice expressing V5-tagged I2020T-mutant human LRRK2 have been reported previously [10]. Cell lysates were prepared from the whole brain of both transgenic and non-transgenic control mice aged 10 and 96 wks. The experiments performed in this study were approved by the Animal Ethics Committee of Kitasato University.

3. Results

3.1. Mouse/human cross-species heterodimer of LRRK2

Myc-tagged mouse LRRK2 and V5-tagged human WT LRRK2 cDNAs were co-transfected into HEK293 cells. Cell lysates were prepared after 48 h of transfection, and mouse LRRK2 was immunoprecipitated using the anti-Myc antibody. Western analysis of the precipitate using anti-V5 antibody revealed that the mouse LRRK2 was associated with human LRRK2 (Fig. 1A). Conversely, Western analysis using anti-Myc antibody showed that human LRRK2 immunoprecipitated with the anti-V5 antibody was associated with mouse LRRK2 (Fig. 1C). These results indicated that mouse LRRK2 and human LRRK2 formed cross-species heterodimers. The native gel electrophoresis revealed that moue LRRK2 and human LRRK2 exist as monomer, dimer, and polymer in the molecular weight region identical to each other, supporting the coexistence as cross-species heterodimers (Supplementary Fig. 1A and B). The cross-species heterodimers were also detected by transfecting Myc-mouse LRRK2 into neuroblastoma SH-SY5Y clone which permanently expressing V5-human LRRK2 [7]. Western analysis using anti-Myc antibody revealed that the mouse LRRK2 was associated with human LRRK2 immunoprecipitated with anti-V5 antibody (Supplementary Fig. 2A).

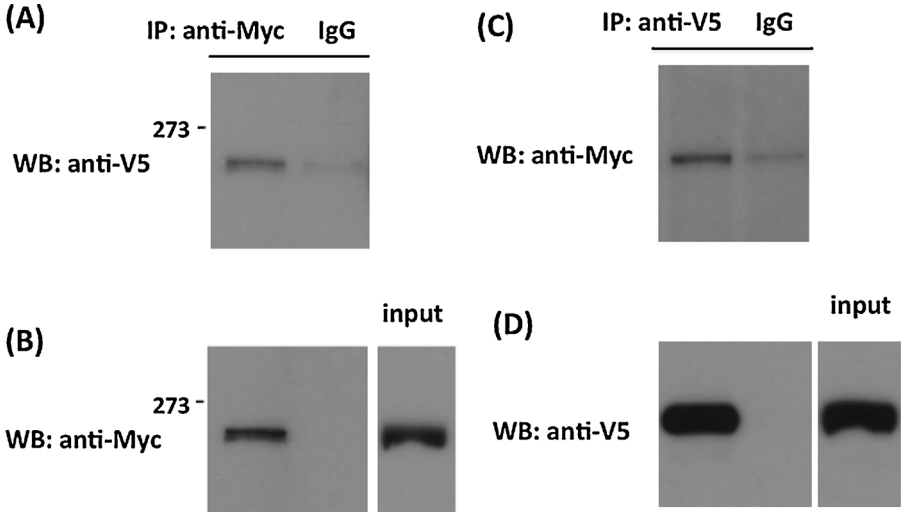

Fig. 1. Mouse LRRK2 and human LRRK2 form cross-species heterodimers. Equal amounts of Myc-tagged mouse LRRK2 and V5-tagged human wild-type LRRK2 cDNAs were co-transfected into HEK 293 cells. After 48 h of culture, cell lysates were prepared and used for immunoprecipitation with antibodies against Myc (A, B) and V5 (C, D). As a control, normal mouse IgG was used. The immunoprecipitates or cell lysates (input) were subjected to SDS-PAGE followed by Western analysis using antibodies against V5 (A, D) and Myc (B, C).

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