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Dystonia-4 (DYT4)-associated TUBB4A mutants exhibit disorganized microtubule networks and inhibit neuronal process growth

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ABSTRACT

Dystonia-1 (DYT1) is an autosomal dominant early-onset torsion form of dystonia, a neurological disease affecting movement. DYT1 is the prototypic hereditary dystonia and is caused by the mutation of the *tor1a* gene. The gene product has chaperone functions important for the control of protein folding and stability. Dystonia-4 (DYT4) is another autosomal dominant dystonia that is characterized by onset in the second to third decade of progressive laryngeal dysphonia. DYT4 is associated with the mutation of the *tubb4a* gene, although it remains to be understood how disease-associated mutation affects biochemical as well as cell biological properties of the gene product as the microtubule component (a tubulin beta subunit). Herein we demonstrate that DYT4-associated TUBB4A missense mutants (Arg2-to-Gly or Ala271-to-Thr) form disorganized tubulin networks in cells. Transfected mutants are indeed expressed in cytoplasmic regions, as observed in wild-type transfectants. However, mutant proteins do not exhibit typical radial tubulin networks. Rather, they have diminished ability to interact with tubulin alpha subunits. Processes do not form in sufficient amounts in cells of the N1E-115 neuronal cell line expressing each of these mutants as compared to parental cells. Together, DYT4-associated TUBB4A mutants themselves form aberrant tubulin networks and inhibit neuronal process growth, possibly explaining progress through the pathological states at cellular levels.

1. Introduction

Dystonia is a range of neurological movement disorders characterized by involuntary movements and extended muscle contractions [1–4]. The dystonia patient often exhibits twisting body movements. Dystonia presents with abnormal muscle contractions leading to repetitive movements or fixed postures. In some patients, the whole body may be involved in these unusual movements. In others, only certain parts of the body are affected. Dystonia may affect as many as 10 to 20 out of 100,000 people. The disease is caused by genetic or other factors such as injuries affecting the nervous system [1–4]. Dystonia of genetic origin involves more than 25 potentially responsible genes (see the OMIM website, URL: http://omim.org/phenotypicSeries/PS128100).

One form of early-onset torsion dystonia is known as dystonia-1 (DYT1), which is the prototypic hereditary dystonia [5]. DYT1 is an autosomal dominant condition caused by the mutation of the *tor1a* gene [5]. The gene product has chaperone functions that are important for the control of protein folding, whole or partial protein refolding, and protein stability. Dystonia-4 (DYT4) is another autosomal dominant dystonia that is characterized by onset in the second to third decade of progressive laryngeal dysphonia [6,7]. DYT4 is known to be associated with two missense mutations (Arg2-to-Gly [R2G] and Ala271-to-Thr [A271T]) of the *tubb4a* gene [6,7]. The gene product is one of beta-type tubulins [8,9]. Tubulin beta and alpha (TUBB and TUBA) have many beta and alpha molecular types, which constitute microtubules [8,9].

Despite the identification of gene mutations in various

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responsible genes associated with hereditary dystonia following recent advances in sequencing techniques, the biochemical or cell biological properties of their mutated gene products remain to be characterized. In the present study, we have investigated whether and how DYT4-associated mutations affect the properties of TUBB4A. DYT4-associated mutants result in disorganized tubulin networks and less interaction with TUBAs in Cos-7 cells. Furthermore, cells expressing each of the respective mutations do not grow sufficient numbers of processes in N1E-115 cells compared to the neuronal cell model. These results may explain some of the cellular phenotypes seen during progress through the disease states.

2. Material and methods

2.1. Primary antibodies

The following antibodies were purchased: mouse monoclonal anti-actin beta type (Cat. No. M177-3; IB, 1/5000), mouse monoclonal anti-GFP (Cat. No. M048-3; IB, 1/1000), and mouse monoclonal anti-tubulin alpha type (Cat. No. M175-3; IB, 1/5000) from

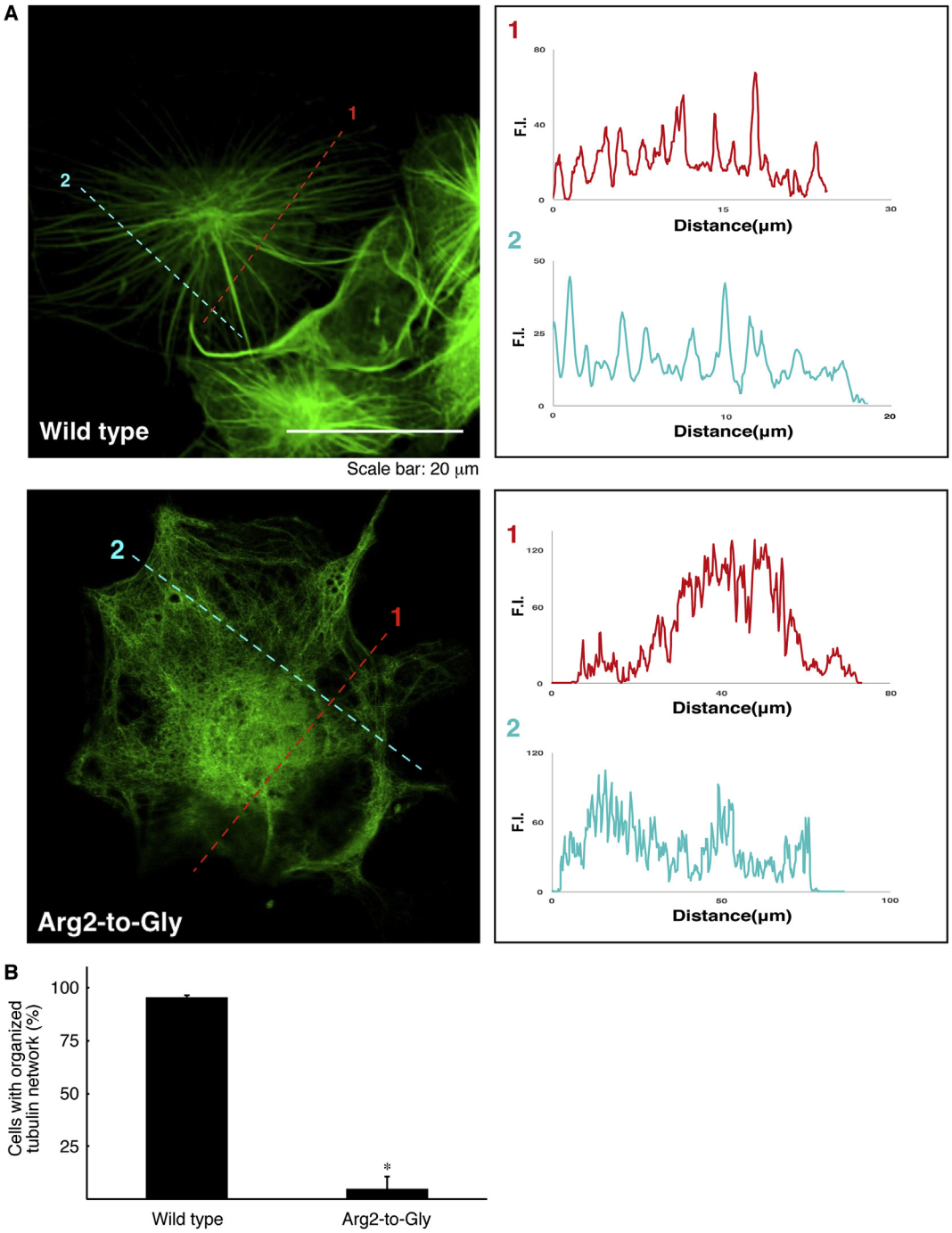

Fig. 1. TUBB4A missense (Arg2-to-Gly) mutation leads to disorganized tubulin networks in cells. (A) Cos-7 cells were transfected with the plasmid encoding the wild type or the Arg2-to-Gly (R2G) mutant of GFP-tagged TUBB4A. Representative GFP-fluorescence images (left panels) are shown. Scan plots in lines 1 and 2 (top to down direction) were performed and graphs showing fluorescent intensities (F.I.) are shown (right panels). Line profiles distinguish normal radial tubulin networks from disorganized ones. (B) Fluorescent cells without organized radial tubulin structures are counted and statistically shown (*, $p < 0.01$ of Student's t-test; $n = 3$ fields).

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