Advancing the pathologic phenotype of giant axonal neuropathy: early involvement of the ocular lens

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Abstract

Giant axonal neuropathy (GAN; ORPHA: 643; OMIM# 256850) is a rare, hereditary, pediatric neurodegenerative disorder associated with intracellular accumulations of intermediate filaments (IFs). GAN knockout (KO) mouse models mirror the IF dysregulation and widespread nervous system pathology seen in human GAN. Validation of therapeutic efficacy and viral vector delivery systems with these GAN KO models has provided the springboard for the development of a viral vector being delivered intrathecally in an ongoing Phase I gene therapy clinical trial for the treatment of children with GAN (https://clinicaltrials.gov/ct2/show/NCT02362438). During the course of a comprehensive pathologic characterization of the GAN KO mouse, we discovered the very early and unexpected involvement of the ocular lens. Light microscopy revealed the presence of intracytoplasmic inclusion bodies within lens epithelial cells. The inclusion bodies showed strong immunohistochemical positivity for glial fibrillary acidic protein (GFAP). We confirmed that intracytoplasmic inclusion bodies are also present within lens epithelial cells in human GAN. These IF inclusion bodies in lens epithelial cells are unique to GAN. Similar IF inclusion bodies in lens epithelial cells have not been reported previously in experimental animal models or human diseases. Since current paradigms in drug discovery and drug repurposing for IF-associated disorders are often hindered by lack of validated targets, our findings suggest that lens epithelial cells in the GAN KO mouse may provide a potential target, in vivo and in vitro, for evaluating drug efficacy and alternative therapeutic approaches in promoting the clearance of IF inclusions in GAN and other diseases characterized by intracellular IF accumulations.

Keywords: Giant axonal neuropathy (GAN), Intermediate filaments (IF), GAN KO mouse model, Gigaxonin, Human GAN, Lens epithelium, IF accumulations

Giant axonal neuropathy (GAN, OMIM# 256850) is a rare, hereditary, pediatric neurodegenerative disorder associated with intracellular accumulations of intermediate filaments (IFs) [1]. The disease affects both the peripheral nervous system (PNS) and central nervous system (CNS), and patients nearly always succumb to disease by the third decade. The pathologic signature of GAN in the PNS and CNS is giant axonal swellings filled with dense accumulations of whorled, structurally normal neurofilaments. GAN is caused by autosomal recessive loss-of-function mutations in the GAN gene that encodes the protein gigaxonin. Gigaxonin plays a pivotal role in the cytoskeletal organization and degradation of IFs. Loss of gigaxonin leads to accumulation of different types of IFs within a variety of cells, including desmin in muscle cells, vimentin in fibroblasts, neurofilaments in neurons, and glial fibrillary acidic protein (GFAP) in astrocytes [2]. Most GAN patients also have characteristically tightly curled hair due to alterations of keratin IFs [3].

Three mouse models of GAN have been developed by knocking out part of the endogenous GAN gene [4–6]. All three mouse models mirror the IF dysregulation and widespread nervous system pathology seen in human GAN [7]. Validation of therapeutic efficacy and viral vector delivery...
systems with these GAN KO models [8] has provided the springboard for the development of a viral vector to be delivered intrathecally in a Phase I gene therapy clinical trial for the treatment of children with GAN [9].

During the course of a comprehensive study of the pathologic findings in the GAN KO mouse, we encountered the unexpected and very early involvement of the ocular lens (Fig. 1). Here, described for the first time, we document the early appearance of abundant IF accumulations in lens epithelial cells of the GAN KO mouse. Lens epithelial cells potentially provide an easily accessible target for accelerating complementary drug discovery and drug repurposing strategies for human GAN.

GAN KO mice with a deletion of GAN exons 3–5 (GAN/Y) [4] or a deletion of GAN exon 1 (GAN/J) [6] were maintained at the University of North Carolina at Chapel Hill (UNC–CH) as previously described [8]. Heterozygous GAN mice are phenotypically normal [4, 6] and were used as controls. Mixed sex and age-matched litters from both GAN KO models were used in these studies (4-month-old cohort: 4 KO, 2 heterozygotes; 24-month-old cohort: 10 KO, 15 heterozygotes).

In 4-month-old GAN KO mice, light microscopic examination of H&E-stained sections revealed oval, intracytoplasmic eosinophilic inclusion bodies within lens epithelial cells (Fig. 2a). Histologically identical inclusion bodies were found in 24-month-old GAN KO mice (Fig. 2b). In both 4-month-old and 24-month-old cohorts, inclusion bodies were present in almost every epithelial cell. A panel of immunohistochemical stains for lens IF proteins (GFAP, vimentin, keratin 8/18, CP49 and filensin) [10] showed strong immunoreactivity of inclusion bodies for GFAP (Fig. 2c). The epithelial cell inclusion bodies were present in both GAN/J and GAN/Y KO mice. Age-matched control mice had no inclusion bodies (Fig. 2d). The inclusion bodies were not present in lens fiber cells in the GAN KO mice or age-matched controls. No lens fiber cell degeneration was identified histologically in 4-month-old GAN KO mice or age-matched controls. Lens fiber cell degeneration, morphologically consistent with age-related degeneration [11], was present to a similar degree in both 24-month-old GAN KO mice and age-matched controls.

The neuropathological phenotype of the GAN KO mouse model shares many morphological features with the human disease [7]. Here, described for the first time in the GAN KO mouse, we document the presence of intracytoplasmic IF inclusion bodies in lens epithelial cells. The inclusion bodies were present in the young 4-month-old KO mice and served as a reliable, easily identifiable, early marker of GAN.

These IF inclusion bodies in lens epithelial cells appear to be unique to GAN, as similar IF inclusion bodies have not been reported previously in experimental animal...
models or human diseases. Although lens abnormalities have not been reported in clinical or postmortem studies of human GAN [12–16], we confirmed in a specimen obtained at autopsy that similar appearing, intracytoplasmic inclusion bodies are also present in lens epithelial cells in human GAN (Fig. 2e).

The presence of GFAP-positive inclusion bodies in lens epithelial cells and their absence in lens fiber cells is intriguing. One difference between lens epithelial cells and lens fiber cells is the large concentration of the chaperone protein complex alpha-crystallin in lens fiber cells [17]. The chaperone activity of alpha-crystallin modulates the assembly of IFs, including GFAP, and assists IFs in recovery from stress by preventing inappropriate filament-filament interactions that would otherwise lead to aggregation [18].

Current paradigms in drug discovery and drug repurposing for IF-associated disorders are often hindered by lack of validated targets [19]. One strategy to circumvent this constraint is to screen against a disease phenotype in cell culture or animal model that recapitulates the pathologic phenotype of the human disease [19, 20]. Our findings suggest that lens epithelial cells in the GAN KO mouse may provide a potential target cell, in vivo, for evaluating the efficacy of drugs and other therapeutic approaches in promoting clearance of IF inclusions. Additionally, lens epithelial cells can be grown on their native basement membrane or as dissociated cells [21] and serve as a simple in vitro model system of target cells.

Intracytoplasmic accumulations of IFs are a distinctive pathological feature shared by common neurodegenerative diseases of adulthood, such as Alzheimer’s disease and Parkinson’s disease, as well as rare neurodegenerative diseases of childhood, such as Alexander disease and GAN [2]. It is possible that lens epithelial cells from the GAN KO mouse, if used as a drug repurposing screen, could be extended to address multiple diseases that share an IF accumulation pathologic phenotype [20, 22].

In summary, the GAN KO mouse exhibits great fidelity to the characteristic pathologic features and selected functional deficits of human GAN [7]. Here, we present...
the novel finding of GAN pathology in both mouse and human lens epithelial cells. We suggest that lens epithelium may serve as a target tissue in which to study the effects of pharmacological interventions on GAN and potentially other disorders characterized by intracytoplasmic IF accumulations.

Additional files

Additional file 1: Details regarding experimental procedures and immunohistochemistry (IHC). (DOCX 15 kb)

Additional file 2: Details regarding immunoreactivity (IR). (DOCX 16 kb)

Abbreviations

CNS: Central nervous system; GAN: Giant axonal neuropathy; GFAP: Glial fibrillary acidic protein; IF: Intermediate filament; KO: Knockout; PNS: Peripheral nervous system

Acknowledgements

We thank Kimberlee A. Burns, Research Specialist at the Marsico Lung Institute at UNC-CH, for her anatomical and histological expertise. We acknowledge Yuhui Hu and Mary Keener, UNC-CH, and Jowaly Schneider, the Johns Hopkins University School of Medicine, for their histological technical skills and support. The UNC Histology Research Core Facility in the Department of Cell Biology and Physiology provided some histology services for this project. We acknowledge the Microscopy Services Laboratory, Pathology and Laboratory Medicine, UNC-CH. We are especially thankful for the Johns Hopkins Autopsy Service. Finally, we are grateful for C. Bönemann, MD and his team at NIH/NINDS, for their collaboration and the initiation of the Phase I GAN clinical trial.

Funding

This study was supported by Hannah’s Hope Fund, the NIH National Institute of Neurological Disorders and Stroke (NS087175, S.J.G; NS095315, R.M.B.), and the NIH National Institute of Child Health and Human Development (HD040127, R.M.B.; US4HD079124). Indirect administrative support for S.J.G. was provided by the Research to Prevent Blindness to the UNC-CH Department of Ophthalmology.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its Additional files 1 and 2].

Authors’ contributions

DA, TWB, RMB, and S.J.G. contributed to study concept and design. DA, TWB, RMB, JEH, DXB and SIG contributed to data acquisition and analysis. DA, TWB, RMB, JEH, DXB and SIG contributed to drafting and reviewing the text. DA, TWB, RMB, and SIG contributed to composing the figures. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals [DHHS Publication No. (NIH) 85–23] and approved by the UNC–CH Institutional Animal Care and Use Committee. Human specimens were obtained through a validated autopsy consent from the legal next of kin, which explicitly stated that tissues could be used for research purposes. The consent and diagnostic autopsy report were filed in the deceased patient’s medical record.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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Received: 17 August 2018 Accepted: 18 November 2018

Published online: 01 February 2019

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