FINAL REPORT

TOM WAHLIG ADVANCED SCHOLARSHIP

for research into Hereditary Spastic Paraplegia and related diseases

Title: Therapies for SPG4-HSP using a new genetic mouse expressing mutant human SPAST

Peter W. Baas, PhD
Professor, Department of Neurobiology and Anatomy
Director, Graduate Program in Neuroscience

College of Medicine
Drexel University
Department of Neurobiology and Anatomy
2900 Queen Lane
Philadelphia, PA 19129
Tel: 215.991.8298 | Fax: 215.843.9082 | Cell: 215.880.4226
Email: pbaas@drexelmed.edu

http://neurobio.drexelmed.edu/BaasWeb/

Project Summary/Abstract

Hereditary Spastic Paraplegia (HSP) is a neurodegenerative disease that results in muscle weakness and spasticity in the lower limbs due to degeneration of axons within the corticospinal tracts. The degeneration does not appear until affected neurons have made appropriate connections and have been functionally normal for years. Eventually, the affected neurons lose functional synaptic contacts and exhibit a dying-back neuropathy that begins in the distal region of the axon. The most common cause of HSP is mutations of SPAST, the gene that encodes for spastin, a microtubule-severing protein. Severing of microtubules is important because short microtubules have greater mobility than longer ones. Genetic analyses on the mutations in SPAST in HSP patients have led to the predominate view that haploinsufficiency is the molecular mechanism of the disease. In this view, axons degenerate because of insufficient microtubule severing. However, haploinsufficiency does not adequately explain why the disease is generally adult-onset or why the degeneration is confined mainly to the corticospinal tracts. Another potential explanation is that axonal degeneration results from gain-of-function toxicity of the mutated spastin proteins that accumulate in afflicted neurons. If this explanation is correct, effective therapies would be quite different from those based only on haploinsufficiency. To date, vertebrate models for the disease have catered only to the haploinsufficiency model. This grant proposal requests funds for the characterization of a new mouse model of the disease in which a human pathogenic mutated form of spastin has been introduced on an inducible expression system such that crossing the animal with

various different Cre lines can induce expression of the pathogenic mutant spastin in different tissues of the body. When crossed with a universal Cre, the resulting animals (both homozygotes and heterozygotes) display adult-onset gait deficiencies as well as trembling that is similar to spasticity, all of which are reminiscent of the human disease and none of which are observed in SPAST knockout mice. These symptoms are more severe in males than females. In addition to characterizing this animal as a powerful new model for SPAST-based HSP, the proposed studies will test whether symptoms of the disease can be alleviated with drugs based on two different mechanistic gain-of-function hypotheses that are buoyed by preliminary data from Drosophila, squid, and cultured cells.

Projektzusammenfassung / Zusammenfassung

Hereditäre Spastische Paraplegie (HSP) ist eine neurodegenerative Erkrankung, die zu Muskelschwäche und Spastizität in den unteren Gliedmaßen aufgrund der Degeneration von Axonen innerhalb der Kortikospinale führt. Die Degeneration tritt nicht auf, bis die betroffenen Neuronen angemessene Verbindungen hergestellt haben und seit Jahren funktional normal sind. Schließlich verlieren die betroffenen Neuronen funktionale synaptische Kontakte und zeigen eine zurückbleibende Neuropathie, die im distalen Bereich des Axons beginnt. Die häufigste Ursache für HSP sind Mutationen von SPAST, dem Gen, das für Spastin kodiert, ein Mikrotubuli-trennendes Protein. Das Trennen von Mikrotubuli ist wichtig, weil kurze Mikrotubuli eine größere Beweglichkeit aufweisen als längere. Genetische Analysen zu den Mutationen in SPAST bei HSP-Patienten haben zu der vorherrschenden Ansicht geführt, dass Haploinsuffizienz der molekulare Mechanismus der Erkrankung ist. In dieser Ansicht degenerieren sich Axone aufgrund unzureichender Mikrotubuliabtrennung. Allerdings erklärt die Haploinsuffizienz nicht hinreichend, warum die Erkrankung im Allgemeinen erwachsen ist oder warum die Degeneration hauptsächlich auf die kortikospinale Banden beschränkt ist. Eine weitere potenzielle Erklärung ist, dass die axonale Degeneration aus der Toxizität der Toxizität der mutierten Spastinproteine resultiert, die sich in betroffenen Neuronen ansammeln. Wenn diese Erklärung richtig ist, würden wirksame Therapien ganz anders sein als jene, die nur auf Haploinsuffizienz basieren. Bis heute haben Wirbeltiermodelle für die Krankheit nur dem Haploinsuffizienzmodell gerecht. Dieser Zuschussantrag verlangt Mittel für die Charakterisierung eines neuen Mausmodells der Erkrankung, bei dem eine menschliche pathogene mutierte Form von Spastin auf einem induzierbaren Expressionssystem eingeführt worden ist, so dass das Überqueren des Tieres mit verschiedenen verschiedenen Cre-Linien die Expression der pathogenen Mutante induzieren kann Spastin in verschiedenen Geweben des Körpers. Wenn sie mit einer universellen Cre gekreuzt werden, zeigen die daraus resultierenden Tiere (sowohl Homozygoten als auch Heterozygoten) erwachsene Köderdefizite sowie Zittern, die der Spastizität ähnlich sind, die alle an die menschliche Erkrankung erinnern und keiner von ihnen im SPAST-Knockout beobachtet wird Mäuse Diese Symptome sind bei Männern schwerer als Frauen. Neben der Charakterisierung dieses Tieres als leistungsstarkes neues Modell für SPAST-basierte HSP werden die vorgeschlagenen Studien untersuchen, ob die Symptome der Erkrankung mit Medikamenten gemildert werden können, die auf zwei verschiedenen mechanistischen Gain-of-Function-Hypothesen basieren, die durch vorläufige Daten getragen werden Drosophila, Tintenfisch und kultivierte Zellen.

PROGRESS AND DELIVERABLES – As of July 2019

1. Primary Research Article 1. This work was in progress at the time I received the award. The award was helpful in finishing up and publishing the article. The work was not on our new mouse model, but rather on cultured cells and squid axon.

Mutant spastin proteins promote deficits in axonal transport through an isoform-specific mechanism involving casein kinase 2 activation. Leo L, Weissmann C, Burns M, Kang M, Song Y, Qiang L, Brady ST, Baas PW, Morfini G. Hum Mol Genet. 2017 Jun 15;26(12):2321-2334. doi: 10.1093/hmg/ddx125.

Abstract
Mutations of various genes cause hereditary spastic paraplegia (HSP), a neurological disease involving dying-back degeneration of upper motor neurons. From these, mutations in the SPAST gene encoding the microtubule-severing protein spastin account for most HSP cases. Cumulative genetic and experimental evidence suggests that alterations in various intracellular trafficking events, including fast axonal transport (FAT), may contribute to HSP pathogenesis. However, the mechanisms linking SPAST mutations to such deficits remain largely unknown. Experiments presented here using isolated squid axoplasm reveal inhibition of FAT as a common toxic effect elicited by spastin proteins with different HSP mutations, independent of microtubule-binding or severing activity. Mutant spastin proteins produce this toxic effect only when presented as the tissue-specific M1 isoform, not when presented as the ubiquitously-expressed shorter M87 isoform. Biochemical and pharmacological experiments further indicate that the toxic effects of mutant M1 spastins on FAT involve casein kinase 2 (CK2) activation. In mammalian cells, expression of mutant M1 spastins, but not their mutant M87 counterparts, promotes abnormalities in the distribution of intracellular organelles that are correctable by pharmacological CK2 inhibition. Collectively, these results demonstrate isoform-specific toxic effects of mutant M1 spastin on FAT, and identify CK2 as a critical mediator of these effects.

2. Primary Research Article 2: This work was in progress at the time I received the award. The award was helpful in finishing up and publishing the article. The work was not on our new mouse model, but rather on cultured cells.

Truncating mutations of *SPAST* associated with hereditary spastic paraplegia indicate greater accumulation and toxicity of the M1 isoform of spastin. Solowska JM, Rao AN, Baas PW. Mol Biol Cell. 2017 Jul 1;28(13):1728-1737. doi: 10.1091/mbc.E17-01-0047. Epub 2017 May 11.

Abstract
The *SPAST* gene, which produces two isoforms (M1 and M87) of the microtubule-severing protein spastin, is the chief gene mutated in hereditary spastic paraplegia. Haploinsufficiency is a popular explanation for the disease, in part because most of the >200 pathogenic mutations of the gene are truncating and expected to produce only vanishingly small amounts of shortened proteins. Here we studied two such mutations, N184X and S245X, and our results suggest another possibility. We found that the truncated M1 proteins can accumulate to notably higher levels than their truncated M87 or wild-type counterparts. Reminiscent of our earlier studies on a pathogenic mutation that generates full-length M1 and M87 proteins, truncated M1 was notably more detrimental to neurite outgrowth than truncated M87, and this was true for both N184X and S245X. The greater toxicity and tendency to accumulate suggest that, over time, truncated M1 could damage the corticospinal tracts of human patients. Curiously, the N184X mutation triggers the reinitiation of translation at a third start codon in *SPAST*, resulting in synthesis of a novel M187 spastin isoform that is able to sever microtubules. Thus microtubule severing may not be as reduced as previously assumed in the case of that mutation.

3. **Primary Research Article 3:** The work is on our new genetic mouse model, as described in the award proposal.

Hereditary spastic paraplegia: gain-of-function mechanisms revealed by new transgenic mouse. Qiang L, Piermarini E, Muralidharan H, Yu W, Leo L, Hennessy LE, Fernandes S, Connors T, Yates PL, Swift M, Zholudeva LV, Lane MA, Morfini G, Alexander GM, Heiman-Patterson TD, Baas PW. Hum Mol Genet. 2019 Apr 1;28(7):1136-1152. doi: 10.1093/hmg/ddy419.

Abstract
Mutations of the SPAST gene, which encodes the microtubule-severing protein spastin, are the most common cause of hereditary spastic paraplegia (HSP). Haploinsufficiency is the prevalent opinion as to the mechanism of the disease, but gain-of-function toxicity of the mutant proteins is another possibility. Here, we report a new transgenic mouse (termed SPASTC448Y mouse) that is not haploinsufficient but expresses human spastin bearing the HSP pathogenic C448Y mutation. Expression of the mutant spastin was documented from fetus to adult, but gait defects reminiscent of HSP (not observed in spastin knockout mice) were adult onset, as is typical of human patients. Results of histological and tracer studies on the mouse are consistent with progressive dying back of corticospinal axons, which is characteristic of the disease. The C448Y-mutated spastin alters microtubule stability in a manner that is opposite to the expectations of haploinsufficiency. Neurons cultured from the mouse display deficits in organelle transport typical of axonal degenerative diseases, and these deficits were worsened by depletion of endogenous mouse spastin. These results on the SPASTC448Y mouse are consistent with a gain-of-function mechanism underlying HSP, with spastin haploinsufficiency exacerbating the toxicity of the mutant spastin proteins. These findings reveal the need for a different therapeutic approach than indicated by haploinsufficiency alone.

4. **Position/Review Article 1:** This article brings together the results of our research on our new genetic mouse together with observations from other mouse models to present a unifying new hypothesis for the etiology of the disease.

New hypothesis for the etiology of SPAST-based hereditary spastic paraplegia. Qiang L, Piermarini E, Baas PW. Cytoskeleton (Hoboken). 2019 Apr;76(4):289-297. doi: 10.1002/cm.21528. Epub 2019 Jul 3.

Abstract
Mutations of the SPAST gene are the chief cause of hereditary spastic paraplegia. Controversy exists in the medical community as to whether the etiology of the disease is haploinsufficiency or toxic gain-of-function properties of the mutant spastin proteins. In recognition of strong reasons that support each possible mechanism, here we present a novel perspective, based in part on new studies with mouse models and in part on the largest study to date on patients with the disease. We posit that haploinsufficiency does not cause the disease but makes the corticospinal tracts vulnerable to a second hit, which is usually the mutant spastin proteins but could also be proteins generated by mutations of other genes that may or may not cause the disease on their own.

Other Activities - Seminar Presentations

Mar 2018 "A new genetic mouse model for SPAST-based Hereditary Spastic Paraplegia reveals the importance of toxic gain-of-function mechanisms." American Society for Neurochemistry meeting. Riverside, CA.

April 2018 “A new genetic mouse model for SPAST-based Hereditary Spastic Paraplegia reveals the importance of toxic gain-of-function mechanisms.” University of Illinois at Chicago, Chicago, IL.

Mar 2019 “A new genetic mouse model for SPAST-based Hereditary Spastic Paraplegia reveals the importance of toxic gain-of-function mechanisms.” Center for Molecular Neurobiology (ZMNH), University Medical Center Hamburg-Eppendorf, Hamburg, Germany

Mar 2019 “A new genetic mouse model for SPAST-based Hereditary Spastic Paraplegia reveals the importance of toxic gain-of-function mechanisms.” Bambino Gesù Children’s Research Hospital, IRCCS, Rome, Italy.

June 2020 “Using mice to understand the cause of Hereditary Spastic Paraplegia and develop new treatments.” Spastic Paraplegia Foundation Meeting. San Antonio, Texas. USA. (Note: This meeting is mainly for patients and their families and caregivers).