ABSTRACT

INVESTIGATION OF LIX1 AND ITS ROLE IN FELINE SPINAL MUSCULAR ATROPHY PATHOGENESIS

By

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The spinal muscular atrophies (SMA) are a group of inherited disorders distinguished by proximal muscle weakness and atrophy due to spinal cord lower motor neuron degeneration. Autosomal recessive SMA is the leading genetic cause of infant mortality and divided into four subtypes based on severity, age of onset and survival time. In humans, at least 97% of autosomal recessive SMA is due to a mutation in the survival of motor neuron gene. Despite intensive research, the molecular mechanism by which SMN depletion results in motor neuron death remains poorly defined. Feline SMA is an autosomal recessive, juvenile onset lower motor neuron disease that resembles Type 3 human SMA. Molecular analysis identified a 140 kb deletion on feline chromosome A1q that eliminated exons 4-6 of limb expression 1 (LIX1) and nearly all of leucyl/cystinyl aminopeptidase (LNPEP). Little is currently known about LIX1, except that it is highly expressed in the spinal cord. LNPEP is an ubiquitously expressed aminopeptidase and a Lnpep knockout mouse has been generated and did not demonstrate any overt neuromuscular phenotype. The aim of my research was to characterize feline SMA onset and progression and to identify molecular functions of LIX1.

Motor neurons and muscles of SMA affected cats developed normally until 8 weeks. At this age, motor axons in affected cats failed to undergo radial expansion to generate the bimodal distribution of axon diameters found in normal animals. Significant ventral root axon loss and atrophic muscle fibers were observed at 12 weeks. No cell body loss was detected at

21 weeks, although chromatolytic cells and acentric nuclei were observed. Thus the first pathological change in feline SMA detected in this study was a failure of axon radial outgrowth. In order to confirm that LIX1 is the feline SMA disease gene and facilitate future investigation of LIX1 function, a Lix1 knockout mouse was ordered from Lexicon Genetics, Inc. Homozygous Lix1 knockout mice did not demonstrate any neuromuscular phenotype even at two years of age, as assessed by motor function tests and spinal cord and muscle histology. However, expression of a Lix1 alternative transcript (Lix1alt) with an independent promoter was maintained in Lix1-/- mice and may compensate for the loss of Lix1.

A yeast two-hybrid screen of a human fetal brain cDNA library was conducted to identify putative LIX1 interacting partners. This screen of 2 x 107

clones identified twelve unique preys,

some of which were identified multiple times. Confirmation of the protein interactions was accomplished by in vitro co-immunoprecipitation and band shift assays. The cytoplasmic domain of DACHSOUS1 (DCHS1) was identified 20 times in the yeast two-hybrid screen and was the only prey that had a confirmed interaction with LIX1 in vitro. GFP tagged Lix1 and Lix1alt co-localized with DsRed tagged Dchs1 in cultured Cos7 and NSC34 cells. Although the co-localization in cultured cells supports an in vivo interaction between Lix1 or Lix1alt and Dchs1, no such interaction could be detected by in vivo co-immunoprecipitation.

Computational analysis of LIX1 predicted amino acids 22-99 to fold into a double

stranded RNA binding domain. To test this prediction I conducted gel mobility shift assays with single and double stranded RNA and purified, recombinant LIX1. No interaction with RNA was observed in vitro. Furthermore, GFP-tagged Lix1 did not co-localize with Poly A Binding Protein

1 (Pabp1) a marker of ribonucleoprotein complexes in cultured NSC34 cells. Therefore, this does not support the predicted RNA binding activity of LIX1. More work is necessary to confirm that LIX1 is the feline SMA disease gene and whether the interaction of LIX1 and DCHS1 is directly involved or completely unrelated to feline SMA pathogenesis