Identification of 17 highly expressed genes within mouse lumbar spinal cord anterior horn region from an *in-situ* hybridization atlas of 3430 genes: implications for motor neuron disease

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Abstract

In an effort to find possible new gene candidates involved in the causation of amyotrophic lateral sclerosis (ALS), a prior version of the on-line brain gene expression atlas GENSAT was extensively searched for selectively intense expression within spinal motor neurons. Using autoradiographic data of *in-situ* hybridization from 3430 genes, a search for selectively intense activity was made for the anterior horn region of murine lumbar spinal cord sectioned in the axial plane. Of 3430 genes, a group of 17 genes was found to be highly expressed within the anterior horn suggesting localization to its primary cellular constituent, the alpha spinal motor neuron. For some genes, an inter-relationship to ALS was already known, such as for heavy, medium, and light neurofilaments, and peripherin. Other genes identified include: Gamma Synuclein, GDNF, SEMA3A, Extended Synaptotagmin-like protein 1, LYNX1, HSPA12a, Cadherin 22, PRKACA, TPPP3 as well as Choline Acetyltransferase, Janus Kinase 1, and the Motor Neuron and Pancreas Homeobox 1. Based on this study, Fibroblast Growth Factor 1 was found to have a particularly selective and intense localization pattern to the ventral horn and may be a good target for development of motor neuron disease therapies; further research is needed.

Materials and Methods

As part of the Gene Expression Nervous System Atlas (GENSAT) project, the St. Jude Brain Gene Expression Map (BGEM) was the main resource for this on-line atlas search. Details on the technical aspects of the GENSAT project for tissue processing, generation of template DNA, RNA isolation, generation of the riboprobe for *in-situ* hybridization (ISH), actual hybridization method as well as autoradiographic details are found at: http://www.stjude-bgem.org/.

Using the original GENSAT on-line data base, ISH autoradiograms of axial sections of mouse lumbar spinal cord at post-natal day 7 (P7) were individually reviewed for a total of 3430 genes; attention was focused on identifying genes that were selectively and intensely expressed within the anterior horn regions of the spinal cord.

Originally researched as part of the GENSAT data base, many but not all of the results are currently archived at different location, and found within the Mouse Genome Informatics website under: http://www.informatics.jax.org/gxdll?reference=1:162220.

With this link, adult (A) gene expression patterns can be found; the limitation however is that post-natal day 7 (P7) expression is separately archived and incompletely stored and found only by individual searches by gene name. Furthermore, there is no ability to magnify the autoradiograms from the Mouse Genome Informatics website, whereas this was readily accomplished with GENSAT, which was the original information source for this study. For an illustration of the current limitations in accessing the original GENSAT data, the reader is referred to the current web archive for Fibroblast Growth Factor as an example: http://www.informatics.jax.org/assay/MGI:4945600#g01133_P7_id.

Results

Selective and intense localization within the anterior horn region of the ventral aspects of the lumbar spinal cord was noted for Neurofilament genes (light and heavy forms, as well as medium type) as shown in Figure 1; the selectivity for the ventral horn was most apparent for the light form and least evident for the medium neurofilament gene where activity was also seen within the dorsal horn gray matter. Other genes with a pattern highly suggestive for selective localization to spinal motor neurons include Peripherin, and Fibroblast Growth Factor, with little no activity found outside the ventral horn area (Figure 1).

Of the remaining 12 genes within the identified group of 17 that had patterns of interest with selective but less intense ventral horn localization included Tubulin polymerization promoting protein family member 3 (TPPP3), as well as gamma synuclein, and Extended Synaptotagmin-like protein 1 (Figure 1).

Discussion

Peripherin (PRPH) is an intermediate filament protein found mainly within peripheral nerve but highly expressed within motor neurons after axonal injury. In consideration of the extensive research that links peripherin to ALS, including studies that reveal PRPH frame shift mutations in ALS patients, it is not surprising that this search of selectively intense expression within the ventral horn identified it as a spinal motor neuron gene (Figure 1). Although likely related to functional disruption of key inter-related neurofilaments within spinal motor neurons, peripherin gene mutations overall appear to be linked to relatively small number of ALS cases.
In consideration that motor neurons have the longest axons of all, extending up to one meter, and that >99% of motor neuron cell volume is the axon itself, it is not surprising that any disruption of key axonal transport related proteins such as neurofilaments can result in major cellular dysfunction and be linked to motor neuron disease. Likewise, in consideration of the fact that extensive research has tied neurofilament mutations to certain familial cases of ALS, it is also not surprising that gene expression for the light, medium, and heavy forms of neurofilaments show prominently for gene expression within the ventral horn of the spinal cord (Figure 1). With regards to the latter, it has been found that heavy neurofilament (NF-H) gene deletions can occur for the subunit tail region in some ALS patients, producing cytoskeletal disruption as well as impaired axonal transport.

Tubulin polymerization promoting protein family member 3 (TPPP3), also known as p20, also demonstrated fairly intense and selective localization to the ventral horn as shown in Figure 1. Although TPPP3 is known to bind to microtubules, its exact natural physiological function remains unknown. However, knockdown of endogenous TPPP3 by RNA interference (RNAi) inhibited cell proliferation and caused HeLa cell cycle arrest; the same study also showed that TPPP3 depletion also caused multipolar mitotic spindles and chromosome segregation errors, leading to HeLa cells apoptosis.

The gene of greatest interest in terms of localization selectivity to the ventral horn, and in relation to what is already known about functional aspects to the expressed protein, is Fibroblast Growth Factor 1 (FGF-1). As shown in Figure 1, FGF-1 is selectively expressed within the anterior horn gray matter in a fairly intense manner, suggesting a key role in alpha motor neuron physiology. Also known as acidic Fibroblast growth factor, FGF-1 is one of many related polypeptide growth factors that use tyrosine kinase-linked FGF receptors.
Gamma Synuclein is another gene which was found to be selectively expressed within the ventral horn (Figure 1) and is of special interest as the protein is closely related to the well known alpha synuclein that undergoes disease-related aggregative event in Parkinson’s Disease and related disorders. Gamma Synuclein shares this ability to self-aggregate, and has been found in experimental studies with transgenic overproduction of synuclein in mice to be highly patho-physiologic and reproduces clinical and pathologic aspects to motor neuron disease;21 further research on the role of gamma synuclein is clearly needed. With regards to glial cell line-derived neurotrophic factor (GDNF) as an identified gene of mild to moderately high expression within the ventral horn (Figure 1), there have been extensive studies published on this protein in relation to ALS, with most of the recent studies exploring GDNF as a potential treatment for experimental animal models of ALS.22-24 A recent study genetically fused GDNF to the C-fragment of tetanus toxin (TTT) to create a peptide that could deliver trophic molecules in a specific targeted fashion to motor neurons with the results showing significant delay in onset of symptoms and functional deficits with longer lifespan for the SODG93A mouse model of ALS.23

As shown in Figure 1, Semaphorin 3a, otherwise known as Sema3A [sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A] displayed a fairly discrete and moderately intense localization to the ventral horn extending up to the margins of the dorsal horn. One study has shown that Semaphorin 3A is differentially expressed in terminal Schwann cells (TSCs) on different populations of muscle fibers;25 this study found that in regenerative conditions after injury, increased expression of Sema3A is selectively seen in fast-fatigable muscle fibers at terminal Schwann cells and may explain why motor nerve terminals that activate slow muscle fibers sprout extremely well after synaptic blockade, yet those nerves to fast-fatigable muscle fibers do not sprout at all when synaptic activity is blocked. With regards to a mouse model for amyotrophic lateral sclerosis (ALS), Sema3A was noted to be expressed at fast-fatigable muscle fiber neuromuscular junctions. The authors suggest that Semaphorin 3a may not only suppresses nerve terminal plasticity at specific neuromuscular synapses, but may also contribute to their early and selective loss in the motor neuron disease ALS. With regards to Extended Synaptotagmin-like protein 1, also known as E-syt1, a moderately intense and selective pattern was found for the ventral horn (Figure 1). Only seven articles in published medical literature mention anything at all about this relatively obscure protein. However, recent studies show E-syt1 to be of potentially great importance, as one study from 2012 discovered that the Non Small Cell Lung Carcinoma derived fusion kinase CD74-ROS,26 which for NSCLC comprises 30% of all ROS fusion kinases, is an active and oncogenic tyrosine kinase that when expressed results in phosphorylation of extended synaptotagmin-like protein (E-Syt1); the investigators found that elimination of E-Syt1 expression drastically reduced tumor invasiveness. A subsequent study published in 2013 emphasizes a key role for E-syt1 in the dynamics of endoplasmic reticulum (ER)-plasma membrane (PM) junctional interactions.27 Elevation of cytosolic Ca2+ triggers trans-location of E-Syt1 to ER-PM junctions to enhance the connection between the two. Although this represents an important new concept in cell physiology that has implication as well for oncology, it remains currently unclear how E-syt1 may influence motor neuron disease; further research is clearly needed. As shown in Figure 1, Lty/Neurotoxin 1 (otherwise known as LNX1) displays moderately intense and selective ventral horn localization. As noted by Fu et al.28 LNX1 is highly expressed in the brain and is a member of the ly-6 family of proteins, also known as three-finger toxins or proteins, which is a large family of proteins that have exactly spaced cysteine motifs and display binding to the nicotinic Acetylcholine Receptor (nAChR). Based on its homology with the nicotinic antagonist α-bungarotoxin (α-BGT), it is targeted for Elapid snake venom neurotoxin and acts as an endogenous nicotinic type negative modulator of cholinergic neuronal signaling; binding of LNX1 diminishes cholinergic neurotransmission. Although one study showed LNX1 resides are important for interaction with muscle-type and/or neuronal nicotinic receptors,29 there is no data on its role in spinal motor neurons; further research is needed. With regards to moderately intense and selective ventral horn expression of heat shock protein 12a (HSPA12a) as revealed in Figure 1, there is very little information about this protein in the medical literature to interpret its functional significance with respect for spinal motor neurons. However, one study has shown that HSPA12A (but not HSPA12B) is highly expressed in the human brain and shows strongest expression in the frontal and occipital cortical regions;30 significant reductions in HSPA12A messenger ribonucleic acid was found in the prefrontal cortex of subjects that had been affected with schizophrenia. As there are no reports on HSPA12a with respect to motor neurons in health or disease, basic research in this area is needed.

Moderately intense and somewhat selective localization of activity was noted for Cadherin 22, also known as Cdh22 (Figure 1). As noted by Saarimäki-Vire et al.,31 cadherins act as key cell adhesion molecules to specify and separate the developing brain into separate compartments. The authors noted that although cadherin-22 (Cdh22) was strongly expressed at the midbrain-hindbrain boundary during early development, no brain nuclei or compartmental defects could be found in experimental Cdh22 mutants, likely due to functional redundancy between other related type 2 cadherins. Although Zhou et al.32 noted that Cdh22 over-expression is linked to colorectal cancer invasion and metastasis at both protein and mRNA levels, there is no research on Cdh22 in motor neuron disease; further research is needed. Moderately selective and intense expression of protein kinase, cAMP dependent, catalytic alpha (PRKACA, previously known as PKA) was also noted for the ventral horn (Figure 1). As noted by Banday et al.33 the PRKACA gene encodes a CAMP dependent protein kinase catalytic alpha subunit in mice; alternatively spliced two transcript variants are known, which encode for two isoforms of PKA C-subunits: Calpha1 and Calpha2. Limited data exists with regards to ALS, where on study showed a 43% increase in PKA expression for ALS patients relative to controls, with PKCalpha showing a 100% increase.34

Conclusions

As very little is known about some of the genes identified in this search, further research is also clearly needed on motor neuron expression of Janus kinase 1 as well as other relatively obscure genes such as Extended Synaptotagmin-like protein 1, Cadherin 22, and L6/neurotoxin 1. The selectively intense expression of fibroblast growth factor 1 in normal motor neurons and its marked deficiency in ALS raises interest in exploring the possibility of using this trophic factor for disease treatment. Finally, alternative consideration needs to be given to research on treatment of motor neuron disease that may involve a combination of factors identified here.
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