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Extended Abstract

Molecular pathogenesis of motor neuron disease as revealed by mass spectrometry-based proteomics

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Abstract

The heterogeneous group of neurodegenerative syndromes that encapsulates motor neuron diseases is inherited or spontaneous disorders that are associated with progressive muscular atrophy. Our laboratory has initiated a proteomic profiling initiative to identify novel muscle-associated biomarkers of motor neuron disease using the wobbler mouse model of primary motor neuronopathy. We employed two complementary methods, fluorescence two-dimensional difference ingel electrophoresis and liquid chromatography in combination with label-free mass spectrometry. The proteomic analysis of disease-induced muscular atrophy has revealed highly complex alterations in the abundance or isoform expression pattern of a large number of skeletal muscle proteins involved in cellular signaling, excitation-contraction coupling, the cytoskeletal network, ion homeostasis, energy metabolism and the cellular stress response. Interestingly, the complex changes in the muscle proteome due to the progressive degeneration of individual motor neurons appears to be considerably different to the more unilateral skeletal muscle transformation observed in disuse-associated muscular atrophy or denervated muscle fibers. Hence, a subtype-specific vulnerability of neuromuscular synapses and compensatory mechanisms of fiber type shifting seem to exist in motor neuron disease as compared to other forms of muscular atrophy. The newly identifiedproteomic biomarker candidates of motor neuron disease may be useful for improving diagnostic, prognostic and therapeutic approaches.

Spinal and bulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS) are representative motor neuron diseases in which selective neuronal degeneration occurs. In this paper, some molecular aspects are discussed related to the pathogenesis of the neuronal degeneration. SBMA is a an X-linked neurodegenerative disease caused by the expansion of a CAG repeat in the first exon of the androgen receptor (AR) gene. To date, eight CAG repeat diseases have been identified, including spinal and bulbar muscular atrophy (SBMA), Huntington's disease (HD), dentatorubralpallidoluysian atrophy (DRPLA), and five spinocerebellar ataxias (SCAs 1, 2, 3, 6, 7). These disorders very likely share a common pathogenesis caused by the gain of a toxic function associated with the expanded polyglutamine tract. Several mechanisms have been postulated as a pathogenic process for neurodegeneration caused by the expanded polyglutamine tract. In SBMA, nuclear inclusions (NIs) containing mutant AR protein have been observed in regions of SBMA central nervous system susceptible to degenerations. Transcriptional factors or their cofactors, such as CREB or creb-binding protein (CBP) sequestrated in NIs, may alter the major intracellular transcriptional signal transduction and ultimately may result in neuronal degeneration. The components in the ubiquitin-proteasome pathway also colocalized in NIs and contribute to the path-ogenesis of SBMA. We generated two types of transgenic mice expressing 239Q under the control of human AR promoter and full-size AR containing 97Q. Marked neurological symptoms and extensive nuclear inclusions were observed in both transgenic lines, but there was no neuronal cell death, suggesting that major neurological phenotype was due to neuronal dysfunction instead of neuronal cell death. As for the therapeutic strategies, the overexpression of Hsp70 and Hsp40 chaperones acted together to protect a cultured neuronal cell model of SBMA from inclusion formation and cell death by mutant AR with expanded polyglutamine tract. In regard to ALS, we are screening the gene expression profiles of the motor neurons from the human ALS and SOD transgenic mouse spinal cord. Motor neurons were microdissected from the spinal cord samples by a lazer-captured microdissection system. Gene expression profiles were screened by cDNA microarray and molecular indexing. Several new molecules were cloned and characterized for their function and relation to neuronal cell dysfunction. Some molecules characterized in this procedure were briefly described.

The causative pathomechanism of sporadic amyotrophic lateral sclerosis (ALS) is not clearly understood. Using microarray technology combined with laser-captured microdissection, gene expression profiles of degenerating spinal motor neurons isolated from autopsied patients with sporadic ALS were examined. Gene expression was quantitatively assessed by real-time reverse transcription polymerase chain reaction and in situ hybridization. Spinal motor neurons showed a distinct gene expression profile from the whole spinal ventral horn. Three percent of genes examined were downregulated, and 1% were upregulated in motor neurons. Downregulated genes included those associated with cytoskeleton/axonal transport, transcription, and cell surface antigens/receptors, such as dynactin, microtubule-associated proteins, and early growth response 3 (EGR3). In contrast, cell death-associated genes were mostly upregulated. Promoters for cell death pathway, death receptor 5, cyclins A1 and C, and caspases-1, -3, and -9, were

upregulated, whereas cell death inhibitors, acetyl-CoA transporter, and NF-kappaB were also upregulated. Moreover, neuroprotective neurotrophic factors such as ciliary neurotrophic factor (CNTF), Hepatocyte growth factor (HGF), and glial cell line-derived neurotrophic factor were upregulated.

Inflammation-related genes, such as those belonging to the cytokine family, were not, however, significantly upregulated in either motor neurons or ventral horns. The motor neuron-specific gene expression profile in sporadic ALS can provide direct information on the genes leading to neurodegeneration and neuronal death and are helpful for developing new therapeutic strategies.

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