Biomedicine and Diseases: Review

Neurofilament proteins in neurodegenerative diseases


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Abstract. The function of neurofilaments, the major component in large myelinated neurons, is not well understood even though they were discovered as structures over 100 years ago. Recent studies have suggested that neurofilaments are closely related to many neurodegenerative diseases, such as amyotrophic lateral sclerosis, Parkinson disease Alzheimer disease, and diabetes. Using in vitro assays, cultures and transgenic mice, these studies provided new insights into neurofilament function. The function of each subunit, the relationship of neurofilaments with other cytoskeletal elements and their clinical significance are topics of increasing attention.

Key words. Alzheimer disease; amyotrophic lateral sclerosis; cytoskeleton; glycosylation; neurofilament proteins; phosphorylation; transgenic mice; Parkinson disease.

Introduction

Neurofilaments (NFs) are intermediate filaments of neurons that are considered to add rigidity, tensile strength and possibly intracellular transport guidance to axons and dendrites [1]. Exclusively expressed in neurons, NFs are members of the cytoskeleton proteins that act together to form and maintain cell shape and facilitate the transport of particles and organelles within the cytoplasm. Based on differences in diameter and protein components, cytoskeletal polymers are classified into three groups: microtubules (MTs) (~24 nm), microfilaments (MFs) (~6–8 nm) and intermediate filaments (IFs) (~10 nm). MTs, which are predominantly composed of tubulin, are responsible for maintaining cell shape, organelle and vesicle movement, and the formation of spindle fibers during mitosis. MFs, which are composed predominantly of actin, are responsible for cellular movement, muscle contraction, mechanical strength and cytokinesis. IFs are composed of different proteins and are prominent in cells that withstand mechanical stress. Moreover, IFs are the most insoluble components of the cells. In contrast to the similarity in polymer composition of MTs and MFs in all tissues, IF polymers differ in different tissues and different cells. Based on molecular structural homology, five types of IFs have been identified, and NFs belongs to type IV IFs (table 1).

In this review, current knowledge about NF history, general properties, expression, assembly, transport, post-translational modification, degradation, functions, transgenic mouse models, related diseases and clinical implications will be summarized.

History

As early as the 1830s, neuronal networks had already been described [2, 3]. The discovery of the silver staining method in the late 19th century resulted in a clear vision
of NFs [4, 5], which also led to the characterization of neurofibrillary tangles and senile plaques by Alois Alzheimer [6]. With the development of electron microscopy after 1931 (Max Knoll and Ernst Ruska in Germany), the molecular structures of NFs were further defined as filaments ~10 nm in diameter and present exclusively in neuronal cells. For a long time, it was known that NFs are involved in several neuronal diseases, and in the past decade, NFs have been linked to more human diseases, which will be discussed here. With the development of specific antibodies, transgenic animal models and molecular genetic methodologies, studies of NFs advanced to the molecular level.

**General properties**

Together with peripherin, α-internexin and nestin, NFs belong to type IV IFs and share common sequence structures (table 1). A central α-helical rod domain of about 310 amino acids is flanked by a globular N-terminal region and non-α-helical carboxy-terminal side-arm domains. The central rod domains, including regions 1a, 1b, and 2, contain highly conserved motifs and every seventh residue is hydrophobic, which facilitates the formation of α-helical coiled-coil parallel homodimers or heterodimers. A linker region aligns the hydrophobic residues. These properties are characteristic of the IFs and are essential for their proper assembly (fig. 1).

NFs are composed of three subunits, and these subunits are defined by their molecular weight: NF-L (light), NF-M (medium) and NF-H (heavy), which are 60 kDa, 100 kDa and 110 kDa, respectively, as predicted from the DNA sequences [7]. These subunits exhibit higher molecular weights on SDS-polyacrylamide gel electrophoresis (PAGE): 68 kDa, 160 kDa and 205 kDa, respectively, because of the enriched negatively charged amino acids (glutamic acid) in their sequences and post-translational modifications such as phosphorylation and glycosylation [7, 8]. NFs constitute the most abundant struc-

### Table 1. Types of IFs. IFs include five defined types and other undefined types. Their molecular weights are varied, and they are found in different cell types. Neurofilament subunits belong to type IV IFs.

<table>
<thead>
<tr>
<th>Type</th>
<th>Name</th>
<th>Molecular weight</th>
<th>Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>acid keratins</td>
<td>40–60 kDa</td>
<td>epithelial</td>
</tr>
<tr>
<td>II</td>
<td>basic keratins</td>
<td>50–70 kDa</td>
<td>epithelial</td>
</tr>
<tr>
<td>III</td>
<td>desmin, GFAP, peripherin/vimentin</td>
<td>50–70 kDa</td>
<td>muscle, astroglia, mesenchymal, tissue cultured cells</td>
</tr>
<tr>
<td>IV</td>
<td>NF-L, NF-M, NF-H, α-internexin, nestin</td>
<td>50–200 kDa</td>
<td>neuronal, immature neuronal, CNS stem cells</td>
</tr>
<tr>
<td>V</td>
<td>nuclear lamins</td>
<td>60–70 kDa</td>
<td>most other undefined types</td>
</tr>
</tbody>
</table>

![Figure 1. Comparison of the structures of NF subunits and other type IV filaments. The NF subunits and other type IV IFs all include α-helical rod domain and are varied in N- and C-termini. A unique character of NF-M and NF-H is that their C-termini have multiple KSP repeats which are heavily phosphorylated. Also shown here, are posttranslational modifications, including phosphorylation and glycosylation on NF subunits (summarized from [7, 8, 11]).](image-url)