Editorial

Neurodegeneration: diseases of the cytoskeleton?

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The identification of a number of genes associated with hereditary neurodegenerative disorders has revealed important clues to pathophysiology. Emerging evidence suggests that mutations contributing to disease are associated with dysfunction of cytoskeletal components that influence vesicular biogenesis, vesicle/organelle trafficking and synaptic signaling. In most familial cases of neurodegenerative disorders, dysfunction of the cytoskeleton results from mutations that alter the conformation and result in accumulation of the affected gene product. The data suggest a mechanism by which cytoskeletal disruption initiates a cascade of events including mitochondrial dysfunction and oxidative stress that, ultimately, activates the DNA damage response.

This editorial, introducing three reviews on neurodegeneration, summarizes current data that link the neurodegeneration in a number of distinct diseases to dysfunction of the cytoskeleton as an underlying cause of cell death. Defects of the cytoskeleton may be a common feature contributing to neurodegeneration.

Alzheimer's disease (AD)

The most common form of dementia occurring in mid-to-late life is Alzheimer's Disease (AD).¹ Late onset AD is influenced by the genetic risk-factor apolipoprotein E (APOE).² However, most of the early onset, familial forms of AD are caused by mutations associated with amyloid precursor protein (APP), and the presenilins (PS).^{1,2} Inherited mutations located on chromosomes 14 and 1 are associated with presenilins 1 (PS1) and 2 (PS2).^{3,4} Presenilins are membrane bound proteins that participate in the Notch-like cleavage of the APP protein within its b-amyloid region.⁵ Missense mutations in PS1 and PS2 have been shown to selectively increase the production of the Ab42 cleavage product relative to more easily degraded Ab40 product.⁵ In human cells, PS cleavage of APP occurs on the surface of recycling endosomes that originate at the trans-Golgi and fuse with the plasma membrane.⁶ Localization within transport vesicles may account for both intracellular production of Ab42 peptide and the secretion of the Ab peptides in the extracellular space. The decreased solubility and tendency to aggregate increases the lifetime

of the Ab42 peptide and results in its accumulation in extracellular plaques.^{1,2,7} The function of PS1 and PS2 as gamma-secretase-like enzymes have been established.⁸ Cleavage by PS1 and PS2 occurs by the juxtaposition of two aspartate residues in the intramembrane region of the receptor.⁹ Interestingly, the mechanism may involve cell-cell adhesion. Recently, an interacting partner of PS1 was found to be delta-catenin, an adheren junction protein involved with cell motility.¹⁰ Cell motility is associated with a massive restructuring of the actin cytoskeleton.¹¹ Therefore, it is possible that defects in PS or aggregation of Ab may stimulate structural alterations in the cytoskeleton of neurons preceding the formation of fibril-containing dystrophic neurites associated with senile plaques.¹²

A fundamental alteration of the cytoskeleton as an underlying cause for AD may, in part, explain why, despite their abundance, accumulation of APP and plaque formation cannot be definitively confirmed as a causative event in AD. Transgenic animals that overexpress the mutant APP protein form plaques but do not display neuronal death.¹³ These data suggest that plaque formation *per se* is insufficient to cause disease, and other factors must play a role in human pathophysiology. Neuropathology of AD is defined by accumulation of another form of insoluble neurofibrillary tangles (NFT). NFT are fibrillar structures comprising largely tau, a microtubule binding protein that stabilizes the microtubule tracts necessary for vesicular trafficking, endo- and exocytosis and axonal polarity. No tau mutations have yet been identified in AD families. Although the relationship between tau, NFT and APP remains to be elucidated, it is possible that defects may influence aggregation of tau protein leading to impairment or misdirection of recycling endosomes that contain the APP protein. Tau forms up to six different isoforms by alternative splicing, and all six isoforms have been found in NFTs.¹⁴ Tau in NFT is typically hyperphosphorylated and, in this state, tubulin assembly is impaired. Hyperphosphorylated forms of tau have lower binding affinities to microtubules and possibly destabilize them.¹⁵ However, hyperphosphorylated forms of tau fail to form fibrils¹⁶ *in vitro* unless sulphated aminoglycans such as heparin sulfate are present (17). Interactions or modifications of microtubules may facilitate fibril formation. As discussed below, modifications of tubulin by nitration are also associated with motor neuron loss in Amyotrophic lateral sclerosis (ALS).

In summary, known susceptibility genes in AD have a direct influence on the microtubule or actin filaments that govern neuronal shape and size and/or movement of vesicle/organelle traffic along the neurite. PS1 and PS2 may exacerbate secretory defects that depend on an intact cytoskeleton resulting in the accumulation of APP-derived peptides as a hallmark of disease.

Tauopathies

Filamentous tau protein and/or microtubule defects are also associated with a range of neurodegenerative disorders known as tauopathies.¹⁸ Tauopathies are generally characterized by the presence of NFT and the absence of neuritic plaques. As with AD, NFT comprise either mutated tau (in the case of frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP17)),¹⁹ or hyperphosphorylated tau protein.¹⁸ In addition to AD, recent evidence indicates that NFT are associated with Pick's Disease, FTDP17, cortico-basal degeneration, progressive supranuclear palsy, and amyotrophic lateral sclerosis/parkinsonism-dementia complex of Guam, among others.¹⁸ As it stands, however, the sole set of known mutations in tau are associated with FTDP17. In the C-terminal portion of tau, three imperfect repeats are present and each contains a binding domain to microtubules.²⁰ Several mutations in exon 10 alter the ratio of microtubule-binding domains from three to four repeats.²⁰ The four-repeat form of tau binds more strongly to microtubules and tends to aggregate more rapidly than the three-repeat form. NFT may result from polymerization of free tau subunits thus depleting the amount of tau available for binding. Mice null for tau shows little phenotype²¹ while mice over-expressing the four-repeat form of tau display axon abnormalities but no NFTs.²² These data indicate that severe effects on microtubule stability are unlikely to account for disease. However, subtle differences in stabilization of microtubules may play an important and common component for neuronal survival over a lifetime.

Parkinson's disease (PD)

In addition to the FTDP17 mutation in tau, Parkinson's dementia is associated with defects in two other gene products, synuclein located on chromosome 4 and parkin located on chromosome 6. Little is known about Parkin other than that it is widely expressed in rat brain and particularly associated with substantia nigra.²³ A-synuclein is a 14 kDa protein of unknown function that is located in the presynaptic terminal of the neuron.²⁴ Although the function of the gene products is unknown, a-synuclein is a prominent component of Lewy bodies and forms a similar fibrillar structure as tau in NFT.²⁵ Early onset of Parkinson has been associated with two point mutations in a-synuclein that both increase the rate of fibril formation *in vitro*.²⁵ Interestingly, a-synuclein shares sequence homology with 14-3-3 proteins that are a family of cytoplasmic chaperones.²⁶ Further, a-synuclein shares physical association with some targets of 14-3-3 proteins including PKC, BAD and ERK.²⁶ Similar to tau protein, a-synuclein is highly phosphorylated when present in fibrillar Lewy bodies. The kinases that modify synuclein are not known. However, it is possible that a-synuclein itself may function as a molecular chaperone whose mutated form can promote its own aggregation and hyper-phosphorylation. Although not detected in huntingtin-containing aggregates *in vivo*, aggregation of huntingtin can be facilitated by the presence of a-synuclein²⁷ suggesting there may be similarities in the mechanism of pathophysiology.

Amyotrophic lateral sclerosis (ALS)

Mutations in the copper-zinc superoxide dismutase (SOD1) are known to underlie 2% of familial cases of ALS.²⁸ Superoxide dismutase is an enzyme that scavenges oxygen free radicals and protects cells from oxidative damage. Initially, mutations in SOD were predicted to alter the enzymatic properties of this protein. However, SOD null mice do not develop disease²⁹ and expression of mutated SOD isoforms in some transgenic mice developed disease without change in or even elevation of SOD activity.³⁰ Instead, recent evidence indicates that SOD mutations may be directly linked to defects in both cytoskeleton components and vesicular transport motors. Aggregates containing both neurofilament and kinesin are hallmarks of ALS suggesting that these targets may be sequestered in disease. Kinesin and dynein facilitate transport of organelles along microtubules in an anterograde and retrograde direction, respectively.³¹ In ALS, there is not only selective loss of kinesin motors but there is also measurable slowing of axonal transport in motor neurons.³² The data indicate that impairment of slow axonal transport may be an early event in ALS pathophysiology.³² Slow transport is associated with movement along the axon of structural elements and cytoplasmic proteins including neurofilaments.³³ The role of SOD in this process is not entirely clear. However, recent evidence indicates there may be disruption of tubulin by nitration near the dynactin binding site in motor neurons.³⁴ Nitration occurs by the formation of peroxynitrite that can modify tyrosine residues on the aromatic ring.³⁵ Since the role of SOD is to prevent peroxynitrite and ROS formation, it has been speculated that SOD may cause nitration in motor neurons that will destabilize microtubule tracts. Nitration of tubulin alters the rate of tubulin polymerization in microtubules and has the potential to interfere with the tubulin interaction with the dynein motor. Thus, impairment of oxygen scavenging function of SOD may be a direct link between oxidative stress and transport defects along microtubules.

Polyglutamine disease

Eight progressive neurodegenerative disorders result from CAG expansion encoding a polyglutamine tract.^{36–38} The exact mechanism for disease is unknown for any of the polyglutamine diseases. However, protein-protein interactions leading to insoluble bodies called inclusions have been identified in human disease brains in all of these diseases. Both cytoplasmic and nuclear inclusions appear to mark the pathology of polyglutamine disorders but inclusion themselves are unlikely to be causative in disease.³⁹ Rather, it is the targets of aggregation that appear to be associated with toxicity. The number of important targets is unknown. However, both nuclear and cytoplasmic pathways may play a role in toxicity, depending on the normal subcellular localization of the respective protein. For example, ataxin-1, the gene product underlying spinocerebellar ataxia type I (SCAI), normally resides in the nucleus. Ataxin-1 is actively directed to the nucleus by a functional nuclear localization signal (NLS),⁴⁰ and altering the NLS abrogates nuclear transport. Transgenic animals expressing ataxin-1 with

expanded polyglutamine and functional NLS develop ataxia,⁴¹ but transgenic animals expressing expanded ataxin-1 without functional NLS develop neither pathology nor nuclear inclusions.⁴⁰ The expanded ataxin-1 (with NLS) is known to alter the subnuclear localization of leucine acidic nuclear protein expressed highly in the affected Purkinje cells of the cerebellum.^{41,42} Aggregation and sequestration of nuclear targets by the mutant protein may lead directly to improper DNA surveillance, mounting of a damage response and cell death.

However, for other polyglutamine proteins including the human Huntington's protein (htt) and spinocerebellar ataxia type 3 (SCA3), the protein normally resides in the cytoplasm and both are associated with primarily cytoplasmic inclusions in adult onset cases.^{43–47} Htt, for example, has a primarily cytoplasmic distribution in perikarya, axons, dendrites and some nerve terminals.^{48,49} Yeast two-hybrid experiments reveal that huntingtin-interacting proteins tend to be (although not exclusively) cytoplasmic. These include ubiquitin conjugating enzyme, E2-25K,⁵⁰ SH3GL3 proteins,⁵¹ a brain specific protein, HAP-1 (Huntingtin associated protein),⁵² the cytoskeletal associated protein, HIP1,⁵³ tubulin,⁵⁴ dynactin,^{55,56} WW domain proteins,⁵⁷ the cytoskeletal, *ras*-related protein Duo,⁵⁸ and *Slal*, a cytoskeletal assembly protein involved in the nucleation of actin microfilaments.⁵⁹ The latter may be associated with clathrin coated vesicles.⁶⁰ Although nuclear events are also associated with htt pathophysiology³⁹ most of the associated proteins represent components of the vesicular transport machinery or cytoplasmic tracts on which they move. Therefore, it is possible that cytoplasmic aggregation and the cytoskeleton defects may play a role in toxicity.

Among the important cargo that is transported along the cytoskeleton is mitochondria. Thus, disruption of the cytoskeleton may lead to cell death by impairing the function of MT and energy depletion in the cell. As discussed below, disruption of mitochondrial function and glucose metabolism has been proposed to mediate neuronal death in many neurodegenerative diseases. Perhaps, the most direct example of these is Friedreich's Ataxia (FRDA).³⁷ FRDA gene product encodes a protein that binds to the MT membrane and regulates the concentration of iron in MT.⁶¹ GAA expansion in the gene⁶² is thought to cause transcriptional shut-down and loss of the FRDA gene product³⁷ that results in iron accumulation in MT, MT dysfunction and cell death.⁶¹ Dysfunction of MT has also been implicated in HD. In rats, systemic administration of the mitochondrial complex II inhibitor 3-nitropropionic acid causes neurobehavioral and pathological abnormalities consistent with HD; and, in HD patients, the caudate has severe deficiencies in mitochondrial complexes II and III.^{63,64} It has been suggested that association of the htt expanded gene product with mitochondrial respiratory chain isoenzymes may account for the pathogenic effects of this polyglutamine disorder. Recent studies support this hypothesis and indicate that the membrane potential of the MT is diminished in fibroblast from HD patients.⁶⁵ It is known that striatal and cerebral glucose metabolism are decreased and precede bulk tissue loss in HD patients. Furthermore, HD protein binds

glyceraldehyde-3-phosphate dehydrogenase (GAPDH), suggesting that mutant HD protein may inactivate this enzyme.

In conclusion, neurons cannot synthesize proteins along the axon and are particularly dependent on vesicular transport to provide these. Most, if not all of the neurodegenerative disorders discussed above have some defect in the cytoskeletal tracts that either sustain neuronal shape and trafficking or a defect in the motors that provide energy for vesicle/organelle movement including MT. These events may be responsible for energy depletion in the neuron as well as provide a source for oxidative damage in the cell. Oxidative damage may lead directly to 8-OH guanine modification⁶⁶ and a DNA damage response that ultimately kills the cell. Alternatively, in the case of the nuclear polyglutamine proteins, there may be direct nuclear pathway that involves inactivation of the DNA surveillance factors themselves. Several reviews that follow in this issue not only report on additional neurodegenerative disorders but also provide ideas on a mechanism by which neurons might mount a DNA damage response. Both Morrison and Kinoshita⁶⁷ and Miller *et al.*⁶⁸ describe the involvement of p53, and its family member p73, in regulating neuronal apoptosis in both the developing and injured, mature nervous system. This includes DNA damage, ischemia and excitotoxicity, as well as neurodegenerative diseases in general. Finally, Aguzzi and Hepper⁶⁹ review the current understanding of the pathogenesis of prion diseases (spongiform encephalopathies).

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