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Neuronal Intranuclear Inclusions in Polyglutamine Diseases: Nuclear Weapons or Nuclear Fallout?

Minireview

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An increasing number of progressive neurodegenerative diseases have been found to be caused by expanded CAG triplet repeats coding for variable length polyglutamine stretches. All of the known polyglutamine repeat diseases, including Huntington's disease (HD) and several forms of the spinocerebellar ataxias, are characterized by the loss of select populations of neurons. Excitotoxicity, impaired energy metabolism, oxidative stress, and apoptosis (programmed cell death) have all been postulated as general mechanisms of cell death operating in HD and other polyglutamine neurodegenerative diseases. Increasing length of the glutamine repeat in affected individuals strongly correlates with lower ages of onset (Gusella et al., 1997). In the past several years, identification of the causative genes coding for specific target proteins has provided new opportunities for exploring the molecular mechanisms underlying the pathogenesis of polyglutamine repeat neurodegenerative diseases, including huntingtin in HD, ataxin-1 in spinocerebellar ataxia type 1 (SCA1), ataxin-2 in SCA2, ataxin-3 in SCA3 (also known as Machado-Joseph disease), ataxin-7 in SCA7, atrophin-1 in dentatorubral-pallidoluysian atrophy (DRPLA), and the androgen receptor in spinobulbar muscular atrophy (SBMA) (reviewed by Gusella et al., 1997; Lunkes and Mendel, 1997; Ross, 1997; Davies et al., 1998).

Abnormal protein aggregation has been postulated to explain the molecular basis for many neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease, and prion diseases. Prior to the discovery of polyglutamine aggregates in vivo, in vitro studies have suggested that expanded polyglutamine-containing protein fragments form aggregates more readily than fragments containing nonexpanded glutamine residues. This has been proposed to occur either by the mechanism of transglutaminase-catalyzed cross-linking (Kahlem et al., 1996) or by polar-zipper hydrogen bonding (Perutz et al., 1994). Proteolytic cleavage of huntingtin specifically containing the pathogenic polyglutamine expansion has been shown to lead to the formation of insoluble aggregates, with tinctorial staining properties similar to those of the potentially neurotoxic β-amyloid-like fibrils observed in AD (Scherzinger et al., 1997). This has raised the question of whether conformational changes imparted by the expanded polyglutamine tract lead to the formation of insoluble aggregates with β-sheet structure, which like β-amyloid might be toxic to selective neurons. Several recent studies have provided valuable new clues regarding the potentially neurotoxic roles of the polyglutamine repeat proteins and their aggregates in neurodegenerative disorders.

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Neuronal Intranuclear Inclusions: Nuclear Beasts or Molecular Roadkill?

A pathological hallmark of polyglutamine diseases is the presence of ubiquitin-positive intranuclear inclusions in affected neurons of the CNS. Neuronal intranuclear inclusions (NIIs) were initially observed in mouse models of HD and SCA1, in which animals develop neurologic symptoms similar to those associated with their respective human disease (reviewed by Lunkes and Mendel, 1997; Ross, 1997; Davies et al., 1998). NIIs have subsequently been found in the brains of patients affected with HD, DRPLA, SCA1, and SCA3 (reviewed by Lunkes and Mendel, 1997; Ross, 1997; Davies et al., 1998). In cases of HD, the increasing length of the polyglutamine tract appears to correlate with the increasing numbers of NIIs (DiFiglia et al., 1997). While these aggregates have been detected predominantly in the nucleus in SCA1 or in juvenile HD (DiFiglia et al., 1997; Skinner et al., 1997), they have also been observed in dystrophic neurites and the cytoplasm, as well as the nucleus, in the cortex and striatum of patients with adult-onset HD (DiFiglia et al., 1997). Cells transfected with mutant forms of huntingtin, ataxin-1, or atrophin-1 also contain aggregates, although their properties appear to be somewhat different from those found in the animal models or brains of affected individuals. For instance, while ubiquitin-positive aggregates were found to be almost exclusively intranuclear in brains of patients with DRPLA and SCA3, in cells transfected with atrophin-1 or ataxin-3 both perinuclear and intranuclear aggregate bodies were observed. In addition, while the aggregates found in the dentate nucleus of the cerebellum in DRPLA patients were composed mainly of fine granular structures and only occasional filamentous ones, atrophin-1-transfected cells consisted primarily of fibrous filaments (Paulson et al., 1997; Igarashi et al., 1998). It is also noteworthy that while in patients NIIs were only detected in the subpopulation of neurons that were affected in each of these diseases, they could be found experimentally in many types of cultured cells. Thus, it appears that multiple factors, including cell type and level of expression, may regulate a cell's susceptibility to form aggregates, as well as the form and subcellular localization of these aggregates.

The most pressing question regarding the presence of the polyglutamine-containing aggregates in the CAG repeat disorders is whether they, and particularly NIIs, are pathogenic agents or simply markers of the cell's demise. In a recent paper in *Cell*, Saudou et al. (1998) have shown that apoptotic neuronal cell death of cultured striatal neurons transfected with mutant huntingtin can be prevented by coexpression of the antiapoptotic gene *bcl-XL*, by treatment with the caspase-3 inhibitor Ac-DEVD-CHO, or by supplementing with the neurotrophins brain derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF). Interestingly, while Ac-DEVD-CHO prevented apoptosis, it failed to suppress the formation of NIIs, evidence that the formation

of detectable NIIs may not be a sufficient event for neuronal cell death mediated by mutant huntingtin.

In an elegant study in a recent issue of *Cell*, Klement et al. (1998) assessed the role of ataxin aggregates in the SCA1 disease process. Transgenic mice expressing an ataxin-1 construct in which a previously described self-association region was deleted were shown to develop ataxia and Purkinje cell pathology in the absence of NIIs, again suggesting that NIIs are not a necessary prerequisite for pathogenesis. Together, the data from Klement et al. (1998) and Saudou et al. (1998) suggest that detectable NIIs are more akin to nuclear "fallout" as opposed to constituting a nuclear "threat."

NIIs found in all polyglutamine diseases have been demonstrated to be ubiquitin positive (Lunkes and Mandel, 1997; Ross, 1997; Davies et al., 1998). A recent study showed that the proteasome and folding chaperone HDJ-2/HSDJ colocalized with ataxin-1 aggregates in affected neurons of SCA1 patients and transgenic mice, and that ataxin-1 aggregates were reduced by the overexpression of HDJ-2/HSDJ in HeLa cells (Cummings et al., 1998). These data suggest that formation of ubiquitin-positive NIIs may be associated with an ataxin-1-mediated perturbation in proteasome function and/or misfolding and subsequent aggregation of ataxin-1-containing expanded polyglutamine tracts (Cummings et al., 1998). A potential link between dysfunctional proteasome-mediated catabolism and mutant huntingtin has been previously implicated by the finding that huntingtin interacts with HIP2, a 25 kDa E2 ubiquitin-conjugating enzyme (Kalchman et al., 1996). Thus, both huntingtin and ataxin-1 have been linked to perturbations in proteasome function, raising the question of whether this leads to NII formation and/or enhanced cell death. In the recent report by Saudou et al. (1998), cotransfection of plasmids encoding mutant huntingtin and a dominant negative form of the ubiquitin-conjugating enzyme led to enhanced cell death but markedly decreased the occurrence of the NIIs. Surprisingly, this indicates oppositional effects of proteasomal inhibition on NII formation and neuronal survival. These observations support the notion that NIIs do not directly compromise neuronal viability.

Nuclear Entry: the Road to Death?

It is puzzling why huntingtin, a cytoplasmic protein, accumulates in the nucleus. Both Saudou et al. (1998) and Klement et al. (1998) have data linking nuclear localization to cell death. Saudou, Greenberg, and colleagues examined whether the entry of huntingtin into the nucleus is a necessary step for initiating neuronal apoptosis in neuronal cells expressing mutant huntingtin. The expression of mutant huntingtin containing a nuclear export signal (NES) in striatal neurons inhibited the formation of NIIs; this could be reversed by treatment with the nuclear export inhibitor leptomycin B. When formation of NIIs was inhibited by expression of mutant huntingtin containing an NES, neuronal apoptosis was also blocked completely.

Similar results were found in a mouse model of SCA1. In contrast to huntingtin, ataxin-1 is normally localized to both the nucleus and cytoplasm, and it contains a nuclear localization sequence (NLS) (Klement et al., 1998). Klement, Orr, and colleagues created mice expressing ataxin-1 with a mutated NLS. As a result, neither significant ataxia nor severe Purkinje cell pathology were observed in these mice, suggesting that nuclear localization of ataxin-1 is required for Purkinje cell neuropathogenesis, the onset of neurologic symptoms, and NII formation in the SCA transgenic mice. Thus, nuclear translocation, but not the formation of detectable nuclear inclusions, is required to induce neuronal apoptosis by mutant huntingtin in cultured striatal neurons (Saudou et al., 1998) or to develop neuropathogenesis by mutant ataxin-1 in SCA transgenic animals (Klement et al., 1998). Although translocation of the mutant protein to the nucleus is a critical step for pathogenesis, it is questionable as to whether inhibition of nuclear transport will ultimately be useful for providing a potential means for therapeutic intervention, as it may interfere with the normal function of these and other proteins, like ataxin-1, that are normally targeted to enter the nucleus.

Caspase-Mediated Proteolysis in Polyglutamine Disorders: First Strike or Aftermath?

Although the actual mechanism of cell death induced by expanded polyglutamine repeats remains unresolved, the nuclear presence of the expanded protein is clearly instrumental in pathogenicity. Because nuclear fractions isolated from brains of HD patients contain only truncated polyglutamine-containing fragments of the N-terminal region of huntingtin (DiFiglia et al., 1997), two possibilities present themselves: (1) only proteolytically processed fragments containing the pathogenic expanded glutamine tracts are transported to the nucleus, and (2) the full-length mutant protein is first translocated into the nucleus and then subjected to proteolytic processing or ubiquitin-dependent degradation, although the expanded polyglutamine-containing N-terminal region remains resistant to degradation. In any case, it remains important to determine whether nuclear localization and/or its consequences require proteolytic cleavage as an initial step and, if so, to identify which proteases are involved.

Several gene products associated with polyglutamine disorders, including huntingtin (Goldberg et al., 1996), atrophin-1 (Miyashita et al., 1997), ataxin-3, and the androgen receptor (Wellington et al., 1998) have been shown to be cleaved by caspases, cysteine proteases that are activated during apoptosis. Caspase-mediated cleavage of these proteins generate N-terminal truncated proteins containing expanded polyglutamine tracts that might then be prone to form aggregates and/or contribute to cell death. Along these lines, caspases might be activated by mutant polyglutamine proteins themselves or by their cellular decompartmentalization (e.g., translocation to the nucleus), and the activated caspases would then cleave these proteins during the "effector" or "execution" stage of apoptotic cell death. Alternatively, caspase cleavage of these proteins may merely be a secondary consequence of the apoptotic cell death process. In either case, it is important to determine whether caspase-cleaved products (e.g., truncated huntingtin) more readily form aggregates (e.g., NIIs) and whether the cleaved products influence the rate of cell death. The new data from Saudou et al. (1998) show that the the formation of NIIs does not appear to require cleavage by an Ac-DEVD-CHO-sensitive caspase, at least for huntingtin (see above). Nevertheless, it remains important for future studies to delineate the temporal order of

caspase activation, aggregate formation, and cell death. Along similar lines, it will also be critical to determine whether caspase cleavage is required for nuclear translocation.

Interacting Proteins: Dangerous Liaisons?

Despite the widespread expression of the polyglutamine disease–associated proteins, only select neurons are affected while others are spared in their respective disorders. Many interacting proteins for huntingtin and other polyglutamine disease proteins have now been identified (reviewed by Gusella and MacDonald, 1998), although their cellular distributions do not strictly correlate with the pathological profiles of these diseases. The aberrant interaction of mutant proteins with specific partners in selective neuronal populations has been proposed to play a role in determining cell-specific vulnerability in the polyglutamine diseases (Gusella and MacDonald, 1998). Although a number of interactors for polyglutamine disease proteins have been identified, only a few suggest involvement in selective neuropathogenesis based on cellular distribution. In the case of SCA, ataxin-1 was found to interact with leucine-rich acidic nuclear protein (LANP) (reviewed by Gusella and MacDonald, 1998). The cellular distribution of LANP closely correlates with the primary affected neuronal sites in SCA1 (Skinner et al., 1997).

In a recent issue of *Molecular Cell*, Sittler et al. (1998) identified a new class of huntingtin-binding protein, termed SH3GL3. The C-terminal SH3 domain in SH3GL3 binds to the proline-rich domain in the N-terminal portion of huntingtin encoded by exon 1. The binding of SH3GL3 with the huntingtin domain containing an expanded glutamine tract appears to promote the formation of aggregates (Sittler et al., 1998). The proline-rich region of huntingtin also serves as the interacting domain for other sets of proteins containing WW domains, termed HYPA, HYPB, and HYPC (Gusella and McDonald, 1998). Determining the cellular distribution of these interactors and defining the role of the interaction mediated by the proline-rich domain in HD-associated phenotypes (e.g., NII formation and apoptosis) will be important in the future for further evaluating the contribution of these newly identified interactors to HD neuropathogenesis.

Identification of additional cellular components in NIIs by immunohistochemical or biochemical approaches should also greatly facilitate our understanding of the potential role, if any, of NIIs in the pathogenesis of polyglutamine diseases.

Conclusion

Common pathogenic events are clearly beginning to emerge not only among the polyglutamine repeat neurodegenerative disorders but also among other CNS diseases such as AD, prion diseases, and amyotrophic lateral sclerosis. All of these disorders involve abnormal processing and aggregation of their respective target proteins and the questionable role of these aggregates in the etiology and pathogenesis of these diseases. The involvement of apoptosis and enhanced cleavage of mutant proteins by caspases has also been implicated in both HD and AD. The new data discussed in this review suggest that the disease-associated aggregates are not necessary for cell death, while aberrant cellular decompartmentalization (e.g., translocation into the nucleus) appears to be a more critical pathogenic event. However, it should be noted that a potentially neuropathogenic role for smaller submicroscopic aggregates has not been ruled out in these recent studies. Although β-amyloid is considered to be a neurotoxic agent in AD, it is also conceivable that cellular decompartmentalization of its precursor, APP, may be more detrimental to cell function than are Aβ aggregates. Finally, the issue of whether caspase cleavage of these proteins is a primary or secondary event in the neurodegenerative process remains to be resolved. Future experimentation elucidating the relationships between expanded polyglutamine repeats and potential gain-of-function effects on protein trafficking, processing (e.g., caspase cleavage), and aggregation should greatly benefit our understanding of the molecular basis of these and other related neurodegenerative disorders.

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