NUCLEUS
2016, VOL. 7, NO. 3, 275–283
http://dx.doi.org/10.1080/19491034.2016.1183859

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EXTRA VIEW

Alzheimer's disease: An acquired neurodegenerative laminopathy

Bess Frost

Barshop Institute for Longevity and Aging Studies, Department of Cellular and Structural Biology, University of Texas Health Science Center San Antonio, San Antonio, Texas, USA

ABSTRACT

The nucleus is typically depicted as a sphere encircled by a smooth surface of nuclear envelope. For most cell types, this depiction is accurate. In other cell types and in some pathological conditions, however, the smooth nuclear exterior is interrupted by tubular invaginations of the nuclear envelope, often referred to as a "nucleoplasmic reticulum," into the deep nuclear interior. We have recently reported a significant expansion of the nucleoplasmic reticulum in postmortem human Alzheimer's disease brain tissue. We found that dysfunction of the nucleoskeleton, a lamin-rich meshwork that coats the inner nuclear membrane and associated invaginations, is causal for Alzheimer's disease-related neurodegeneration *in vivo*. Additionally, we demonstrated that proper function of the nucleoskeleton is required for survival of adult neurons and maintaining genomic architecture. Here, we elaborate on the significance of these findings in regard to pathological states and physiological aging, and discuss cellular causes and consequences of nuclear envelope invagination.

ARTICLE HISTORY
Received 18 March 2016
Revised 20 April 2016
Accepted 25 April 2016

KEYWORDS
Alzheimer's disease; aging; heterochromatin; laminopathy; lamin; nucleoplasmic reticulum; progeria; tauopathy

Nuclear architecture

The nuclear envelope is a lipid bilayer that encases the genome and provides a physical boundary between the cytoplasm and nucleoplasm. On its external surface, the nuclear envelope anchors to the cytoskeleton via the giant Nesprins, proteins that embed in the outer nuclear membrane and bind directly to cytoplasmic actin, intermediate filaments, and microtubules.[1,2] On its internal surface, the nuclear envelope anchors to the lamin nucleoskeleton via SUN proteins, which reside on the inner nuclear membrane and bind directly to lamin proteins.[3] Together, Nesprins and SUN proteins partner to form the LINC complex (LInker of Nucleoskeleton and Cytoskeleton),[4] a bridge that physically connects the cytoskeleton to the nucleoskeleton. The lamin nucleoskeleton provides a scaffold for the anchoring of highly condensed heterochromatic DNA.[5] Proper regulation of nuclear and genomic architecture thus requires harmony between the cytoskeleton, the LINC complex, the nucleoskeleton, and heterochromatin (Summarized in Fig. 1).

Laminopathies

Consequences of nuclear architecture disruption can be gleaned from the laminopathies, most of which are caused by mutations in the gene encoding A-type lamins, *LMNA*.[6] Over 300 disease-causing mutations have been identified in the *LMNA* gene, with phenotypes including muscular dystrophy, lipodystrophy, cardiomyopathy, and progeriod or "premature aging" syndromes such as Hutchinson-Gilford Progeria Syndrome (HGPS). While children affected by HGPS have no disease-associated phenotype at birth, they develop aging-related phenotypes within the first few years of life, including hair loss, sclerotic skin, low subcutaneous fat, osteoarthritis, low bone density, hearing loss and vascular abnormalities, which generally lead to death via cardiac disease or stroke around the age of 13.[7,8] Instead of producing prelamin A, cells of patients with HGPS produce "progerin," a version of prelamin A that lacks amino acids 607–656 within its C-terminus. Unlike prelamin A, progerin cannot be processed into mature Lamin A, and thus constitutively associates

CONTACT Bess Frost BFrost@uthscsa.edu Barshop Institute for Longevity and Aging Studies, Department of Cellular and Structural Biology, University of Texas Health Science Center San Antonio, 15355 Lambda Drive, STCBM 3.100.02, San Antonio, Texas 78245, USA.
Extra view to: Frost B., Bardai F.H., Feany M.B. 2016. Lamin disfunction mediates neurodegeneration in taupathies. Current Biology 26(1):129–136. http://dx.doi.org/10.1016/j.cub.2015.11.039

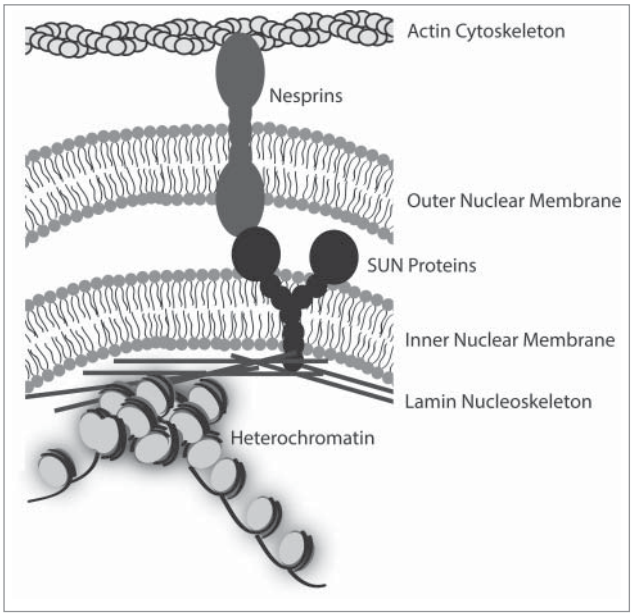

Figure 1. Schematic representation of nuclear anchoring. Cytoplasmic filamentous actin (shown here), microtubules, and microfilaments bind giant Nesprins. Nesprins binds to SUN proteins in the perinuclear space. Together, Nesprins and SUN proteins make up the LINC complex. SUN proteins bind directly to the lamin nucleoskeleton, which anchors heterochromatin to the internal nuclear periphery.

with the inner nuclear membrane.[7] Progerin induces irregularities in nuclear morphology, including invagination and evagination of the nuclear envelope.[9,10] Progerin-associated deletion of amino acids 607-656 reduces its ability to bind heterochromatin-associated histone modifications, which causes relaxation of peripheral heterochromatin.[11,12] Progerin has been detected at low levels in healthy individuals, and increases with age in human skin and liver,[13,14] indicating that progerin may play a role in physiological aging. Similar to HGPS-associated progerin, age-associated progerin accumulates at the inner nuclear membrane and is associated with changes in nuclear morphology and relaxation of peripheral heterochromatin.[13]

HGPS is a segmental aging disorder, meaning that patients manifest some typical features of aging, but not all (e.g. neurodegeneration). Since age is the greatest risk factor for most neurodegenerative disorders, the lack of neurodegeneration in HGPS has been an anomaly in aging research. Why are many tissues affected by A-type lamin dysfunction while the brain is spared? Evidence supports 2 non-mutually exclusive hypotheses. First, lamin A and progerin protein levels are very low in the brain due to a brain-specific microRNA, mir-9, that targets the destruction of prelamin A and progerin transcripts.[15,16] B-type lamins are thus more highly expressed in the brain compared to lamin A. Second, while transgenic expression of progerin in mouse brain distorts the morphology of neuronal nuclei in the hippocampus, no significant effects on behavior, neurogenesis, or gene expression are detected.[17] Thus, the lack of neuropathy in HGPS may be due to the relative lack of progerin in the brains of affected individuals, and/or the relative insensitivity of the brain to progerin protein.

B-type lamins, on the other hand, are expressed widely in all stages of development and in most tissues. At the cellular level, B-type lamins are important for maintaining heterochromatin organization,[18-20] DNA replication,[21] mitotic spindle organization,[22] positioning of chromosomes during interphase,[23] gene transcription,[23-25] maintaining functional plasticity of nucleoli,[26] and managing oxidative stress.[27] At the organismal level, B-type lamins are a critical determinant of neuronal development. The *Drosophila* B-type lamin controls migration of photoreceptor neuronal nuclei during eye formation.[2] In mice, lamin B1 and B2 are required for development-associated neuronal migration and layering of neurons, and neuronal survival.[28-30] Mice lacking lamin B1 or lamin B2 die shortly after birth.[28,30,31]

To date, 3 mutations in B-type lamins are associated with human disease. Duplication of *LMNB1* causes autosomal dominant adult-onset leukodystrophy, which involves progressive loss of myelin, the fatty substance surrounding neuronal axons that aids with neuron firing.[32] A heterozygous mutation of *LMNB2* is associated with increased risk of acquired partial lipodystrophy,[33] which begins in childhood and involves the loss of adipose tissue. A second missense mutation in *LMNB2* was recently identified in 2 sisters with progressive myoclonic epilepsy-9 with early ataxia.[34]

Until recently,[35] it was unknown if lamin B dysfunction affects mature, adult neurons. We demonstrated that dysfunction of B-type lamin drives heterochromatin relaxation, cell cycle activation, and apoptosis of adult *Drosophila* neurons *in vivo*.[35] Furthermore, we identified a role for acquired B-type lamin dysfunction in mediating neuronal death in Alzheimer's disease and related tauopathies.[35] Our study is the first to connect laminopathy with an age-related neurodegenerative disorder.

Identification of a neurodegenerative laminopathy

Tauopathies are age-related progressive neurodegenerative disorders, including Alzheimer's disease, which are pathologically characterized by aggregates of tau protein in the brain.[36] Dominant mutations in the tau gene demonstrate that tau dysfunction is sufficient to cause neurodegeneration in humans.[37] We have previously identified widespread relaxation of constitutive heterochromatin as a mechanism of tau-induced neurodegeneration in tau transgenic *Drosophila*, mice, and postmortem tissue from human Alzheimer's disease brains.[38] Our studies suggest that heterochromatin relaxation is a causal factor in disease progression, since reversing heterochromatin relaxation significantly suppresses tau neurotoxicity, while promoting heterochromatin relaxation significantly enhances tau neurotoxicity in *Drosophila*.[38]

Due to the association between lamins, heterochromatin and aging, we became interested in a potential role for lamin in mediating tau-induced heterochromatin relaxation. Starting with a *Drosophila* model of tauopathy,[39] we found an overall reduction of B-type lamin protein (but not A-type lamin protein) in adult neurons in the context of transgenic human tau. Direct visualization of the nuclear lamina in neurons revealed invaginations of the nuclear envelope in tau transgenic *Drosophila*, similar to what had been previously described in patients with laminopathies. Comparative analyses in postmortem tissue from human brains affected by Alzheimer's disease revealed reduced levels of lamin B1 in neurons, alongside significant invaginations of the nuclear envelope based on staining with lamin B1, the lamin B receptor, and nuclear pores. Genetic reduction of B-type lamin levels in tau transgenic *Drosophila* enhanced tau neurotoxicity, suggesting that lamin dysfunction drives neuronal death in tauopathy.[35] We were unable to detect changes in total B-type lamin levels or alterations in nuclear morphology in a *Drosophila* model of polyglutamine-induced neurotoxicity. Furthermore, genetic reduction of lamin B did not affect polyglutamine mediated neuronal loss, suggesting that lamin dysfunction is not a general feature of neurodegeneration in *Drosophila*.[35]

Consequences of B-type lamin dysfunction in adult neurons

Data from tau transgenic *Drosophila*, mice, and postmortem human brain suggest that pathological tau activates a toxic cascade in which tau-induced heterochromatin relaxation and aberrant expression of genes that are normally silenced by heterochromatin activate the cell cycle in postmitotic neurons, which causes neuronal death.[36] Since lamin dysfunction causes relaxation of peripheral heterochromatin in other tissues, we hypothesized that disruption of the lamin nucleoskeleton is the upstream cause of heterochromatin relaxation in tauopathy. While lamin is clearly important for maintaining chromatin structure and regulating neuronal development, as discussed above, the downstream consequences of lamin dysfunction in adult neurons had not been investigated. We utilized a strong loss-of-function allele, lam^{A25}, of the *Drosophila* B-type lamin to investigate chromatin structure, neuronal cell cycle activation, and neuronal death in neurons of adult flies. Lam^{A25} lacks the domain responsible for targeting lamin to the nuclear envelope,[2] and was used in our studies as a homozygote.

In neurons of lam^{A25} mutant adult flies, we documented significantly reduced levels of heterochromatin protein 1 and dimethylated histone 3 of lysine 9, a histone modification associated with constitutive heterochromatin,[35] suggesting that B-type lamin is required for maintaining heterochromatin structure in fully differentiated neurons. We next investigated neuronal cell cycle activation in the brains of adult lam^{A25} *Drosophila*. Exogenous activation of the cell cycle in postmitotic neurons induces cell death,[40] and the coincidence of cell cycle markers with tau pathology is a well-described feature of tauopathies.[36] Tau-induced cell cycle activation is known to be a causal event in tau-induced neurodegeneration. Brains of adult lam^{A25} mutant *Drosophila* stained positively for proliferating cell nuclear antigen, which detects DNA synthesis, and phosphorylated histone 3, which detects the G2/M transition, suggesting that B-type lamin dysfunction activates the cell cycle in neurons. We also detected significant TUNEL staining in brains of adult lam^{A25} *Drosophila*, which detects DNA fragmentation associated with apoptotic cell death,[35] indicating that proper B-type lamin function is important for neuronal survival. Together, these experiments clearly

illustrate that dysfunction of B-type lamins is of significant consequence to fully differentiated, adult neurons, and suggest that B-type lamin dysfunction is upstream of heterochromatin relaxation, neuronal cell cycle re-entry, and apoptosis in tauopathy.

Other groups have reported a decline in lamin B1 protein levels as fibroblasts cells enter cellular senescence,[41-43] a state in which cells lose replicative ability and secrete pro-inflammatory factors. In senescent cells, lamin B1 depletion causes global reorganization of chromatin and subsequent changes in gene expression.[44] Despite the fact that neurons are postmitotic, a role for cellular senescence in the context of neurodegeneration has been proposed. The theory of proteinopathy-induced neuronal senescence posits that aggregation-prone proteins such as tau are recognized as non-self and stimulate an immune reaction that induces neuronal senescence, causing a pro-inflammatory secretory response in the absence of decreased proliferative potential.[45] The possibility that reduced lamin B1 protein levels cause cellular senescence is a matter of debate,[41-43] and it is currently unknown if tau-associated reduction of B-type lamins affects cellular senescence.

Mechanism of lamin dysfunction in tauopathy

We next determined the mechanism whereby pathological tau reduces lamin levels and induces morphological changes in the nuclear envelope. Since pathological tau induces over-stabilization and bundling of filamentous actin,[36,46] we hypothesized that the actin cytoskeleton acts through the LINC complex to disrupt the lamin nucleoskeleton in tauopathy. While the LINC complex is distributed fairly evenly across the nuclear envelope in neurons of adult control flies, transgenic tau or genetic stabilization of filamentous actin caused clustering of the LINC complex along the nuclear envelope. Like pathological tau, genetically stabilizing filamentous actin also reduced total B-type lamin protein levels and caused the nuclear envelope to invaginate in neuronal nuclei of adult *Drosophila*. Reducing the interaction between filamentous actin and the LINC complex rescued B-type lamin loss in tau transgenic *Drosophila* brains, and significantly reduced tau-induced neurotoxicity.[35] Nuclear envelope invaginations were filled with hyperphosphorylated, disease-associated tau and filamentous actin in neurons from human Alzheimer's disease brains, suggesting that filamentous actin may exert a physical force on the nuclear envelope, which causes it to invaginate.[35] Taken together, our data suggest that pathological tau-induced stabilization of filamentous actin disrupts cytoskeletal-nucleoskeletal coupling, which leads to heterochromatin relaxation and subsequent neuronal death.

Nucleoplasmic reticulum expansion in pathological and physiological settings

We observed that 60% of neuronal nuclei from postmortem human Alzheimer's disease brains harbored nuclear envelope invaginations, which is a 3-fold increase over age-matched control brains.[35] In addition to Alzheimer's disease and laminopathy, expansion of a so-called "nucleoplasmic reticulum"[47] is associated with several pathological states, including cancer, viral infection, and host-cell colonization (for a review see ref.[48]). An increase in nuclear envelope invagination is also associated with physiological aging. Nuclei from frontal cortex and hippocampus of aged marmosets contain a marked increase in nuclear envelope invaginations compared to young marmosets,[49] as do neurons of the dorsal lateral geniculate nucleus,[50,51] and suprachiasmatic nucleus[52] in rats, pyramidal neurons of the motor cortex in hamsters,[53] and cortical neurons in humans.[54] However, despite being present at high levels during development, nuclear envelope invaginations decrease to low incidence with age in facial neurons of hamsters.[55] Similarly, nuclear envelope invaginations do not increase with age in neurons of *C. elegans*, despite obvious age-related changes in nuclei of most non-neuronal tissues.[56,57] Increased incidence of nuclear envelope invagination inversely correlates with the degree of cellular de-differentiation in cultured cells, i.e., cells that are more differentiated contain less invaginations.[58] Interestingly, expression of tau in neuroblastoma cells induces nuclear lobulation, but this phenomenon is not associated with reduced A- or B-type lamin protein, changes in the cell cycle, or cell death.[59] Presence of a nucleoplasmic reticulum may thus differ based on differentiation status, age, species and neuronal type. Significant advances in microscopy have occurred in the decades since many of these studies were first published, and could facilitate more rigorous studies of how neuronal nuclei change with age.

Functional consequences of nucleoplasmic reticulum expansion

The nuclear envelope is at the crossroads of communication between the cytoplasm and the nucleus. In addition to its role in nuclear anchoring and maintaining genome architecture, the nuclear envelope regulates many cellular processes, including nuclear calcium signaling and macromolecular trafficking of RNAs and proteins. The nucleoplasmic reticulum is thought to bring functions of the peripheral nuclear envelope into the deep nuclear interior (Fig. 2).

The shared lumen of the endoplasmic reticulum, the nuclear envelope, and the nucleoplasmic reticulum is rich in calcium, which is a critical regulator of nuclear function. (for a review see ref.[60]). Alongside high calcium concentrations, nuclear envelope invaginations also contain inositol triphosphate receptors[47] and ryanodine receptors,[61] which provide a mechanism whereby calcium can be released from the nucleoplasmic reticulum into the nucleus (Fig. 2). In neurons, synaptic activity can induce formation of a nucleoplasmic reticulum, which increases the rate at which calcium signals are relayed from the synapse to the nuclear interior.[62] It is currently unknown if nucleoplasmic reticulum expansion in Alzheimer's disease and related tauopathies affects nuclear calcium signaling.

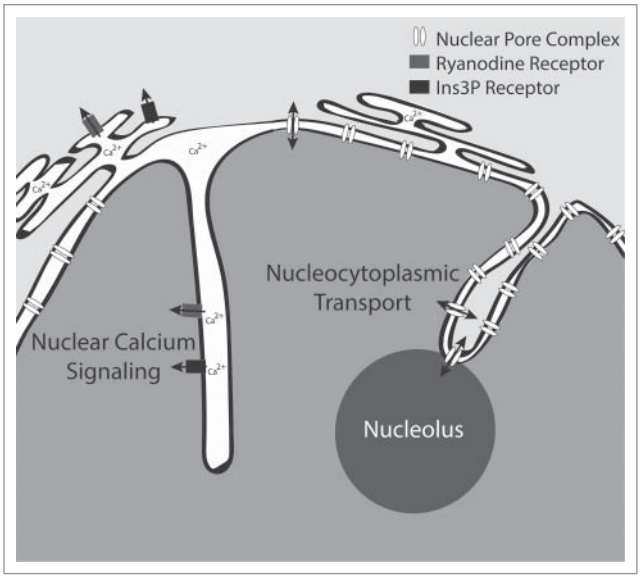

Figure 2. Schematic representation of potential consequences of nuclear envelope invaginations. Type I nuclear invaginations (left) are composed of the internal nuclear membrane, whereas type II nuclear invaginations (right) involve the inner and outer nuclear membranes. The perinuclear space is contiguous with the endoplasmic reticulum, and both are enriched in calcium. Ryanodine receptors and Ins3 receptors are present in the endoplasmic reticulum and in nuclear envelope invaginations, providing a mechanism whereby calcium can be deposited into the nucleus. Type II nuclear invaginations are lined with nuclear pores, are filled with cytoplasm, often associate with nucleoli, and may facilitate transport of macromolecules between the nucleus and cytoplasm.

Type II nuclear envelope invaginations involve both the inner and outer nuclear membranes, are lined with nuclear pores, and contain a cytoplasmic core. Type II invaginations often associate with nucleoli,[63,64] which are sites of high rRNA synthesis. While the coupling of a pore lined, cytoplasm-filled nuclear envelope invagination to a transcriptionally active nuclear compartment could facilitate the nuclear export of RNAs (Fig. 2), the functional significance of nuclear envelope invaginations in regard to nucleocytoplasmic transport is currently unknown. ABC50, a protein involved in translation initiation, has been detected inside nuclear invaginations in cultured cells, suggesting that translation may occur within the nucleoplasmic reticulum itself.[65]

Finally, type I nuclear envelope invaginations, which involve only the inner nuclear envelope, were recently shown to contain lipid droplets.[66] While lipid droplets are known to store lipid esters and participate in lipid metabolism, protein storage, and protein degradation,[67] the significance of lipid droplet enrichment in nuclear envelope invaginations is not known.

Concluding remarks

Neurons of tau transgenic *Drosophila* and of postmortem human Alzheimer's disease brains harbor significant invaginations of the nuclear envelope and have reduced levels of B-type lamin protein compared to controls. Dysfunction of B-type lamins has functional consequences in adult neurons in regard to heterochromatin formation, cell cycle activation, and neuronal survival.[35] Taken together, our results suggest that pathological tau-induced stabilization of filamentous actin disrupts the LINC complex, which reduces lamin protein levels and causes the nuclear envelope to invaginate. Lamin reduction or dysfunction, in turn, causes constitutive heterochromatin to relax, allowing expression of genes that are normally silenced by heterochromatin and activating the cell cycle in postmitotic neurons, which causes their death.

Our findings suggest that Alzheimer's disease and associated tauopathies are, in fact, acquired neurodegenerative laminopathies. We demonstrate that loss of

lamin function can lead directly to age-related neurodegeneration, indicating that basic mechanisms of aging are conserved between neurons and other somatic tissues.[35] The lamin nucleoskeleton is thus a plausible molecular link between aging, the single most important risk factor for developing common neurodegenerative diseases, including Alzheimer's disease, and basic mechanisms of cellular senescence.

Functional consequences of nucleoplasmic reticulum expansion in physiological aging and pathological conditions including cancer and Alzheimer's disease remain to be determined. Investigating a potential role for increased nuclear calcium signaling and nucleocytoplasmic transport in Alzheimer's disease and related tauopathies is of particular interest to our group. It will also be of great value to apply recent advances in microscopy to many of the intriguing electron microscopy-based observations that were made in the late 1900s regarding the nucleoplasmic reticulum and aging.[18,49,51,53-55,64]

Abbreviations

HGPS Hutchinson-Gilford progeria syndrome
LINC linker of nucleoskeleton and cytoskeleton

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

B.F. is supported by R00NS088429 (NINDS).

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