Neurodegenerative Protein Misfolding Disorders and the Skin
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Abstract
Recent evidence has demonstrated that many neurodegenerative disorders, such as Alzheimer’s disease and Parkinson’s disease are in fact proteinopathies in which misfolded proteins propagate in a manner not unlike the prions, the infectious protein pathogens associated with a group of transmissible prion diseases. Hereditary, lifestyle, or other environmental factors may trigger the formation of insoluble protein aggregates, termed amyloids, which accumulate to disrupt normal cell function and may ultimately contribute to progressive neuron death. Clinical progress in the treatment and diagnosis of neurodegenerative proteinopathies, however, has remained challenging in spite of the advances in understanding the events leading to neurotoxicity. No effective therapies are currently available and definitive diagnosis relies on brain biopsy or autopsy. Methods for less-invasive and especially earlier diagnosis of these devastating diseases are an active area of research. The skin is the most accessible organ of the human body, and is not only highly innervated, but also shares a common embryonic origin with the brain. With cutaneous symptoms having been reported with neurodegenerative diseases, the skin has become an attractive target in searching for potential biomarkers and exploring pathogenesis for neurodegenerative diseases.

INTRODUCTION
Neurodegenerative disorders can be broadly classified as a type of local amyloidosis, diseases which involve the accumulation of abnormally folded proteins in the central nervous system (CNS). A diverse variety of proteins and peptides has been shown to be amyloidogenic-capable of obtaining abnormal, β-sheet rich confirmations, which polymerize into fibrillar structures in a concentration dependent manner. Prion diseases are a prototype of neurodegenerative amyloidosis. The normal soluble cellular prion protein (PrPsc) is highly conserved among mammals and can be converted into an insoluble misfolded conformer (PrPSc) by genetic mutations, exogenous infection, or other as-of-yet undescribed factors [1]. This abnormal conformer is pathogenic and famously transmissible between individuals and across species due to its ability to self-propagate in a template-directed manner. Recently, several lines of studies have demonstrated that this self-assisted propagation phenomenon is not unique to the prion protein [2-4]. Indeed, amyloid-beta (Ab) and tau of Alzheimer’s disease (AD), and α-synuclein of Parkinson’s disease (PD) have been demonstrated to spread throughout not only within the CNS, but also from the peripheral tissues to the CNS in a prion-like manner [2,5]. The latter diseases, however, in contrast to the prion diseases, have not been observed to be transmissible on an individual-to-individual basis, resulting in a new term, ‘prionoid’-self-replicating conformers without infectivity-to refer to and distinguish them as different from prions [3].

Although there is ample evidence supporting the association of amyloid deposits with neurodegenerative diseases, the precise mechanisms of toxicity, as well as the events initiating protein aggregation remain unclear. Evidence for direct toxicity of amyloid has been inconsistent. It is unknown to what degree neuronal death is mediated by a loss of function or a toxic gain of function from the related proteins [6]. Poor understanding of the downstream events following amyloidosis has been a major hindrance to the development of therapeutics. The effects of protein aggregation seem to occur largely during middle to late age, even in the case of disease-associated mutations. Neurodegenerative diseases typically have a long dormancy period. Symptoms may not become apparent until years, even decades, after the onset of aggregation, thereby obscuring an effective therapeutic window.

As existing therapeutics remain largely supportive, the development of advanced and sensitive pre-clinical diagnostic tools could be critical for developing effective treatment. Current definitive diagnosis of neurodegenerative diseases is primarily dependent on the examination of brain tissues obtained at biopsy or autopsy [7]. Cerebrospinal fluid (CSF) analysis of 14-3-3 and

Keywords
- Amyloidosis
- Cerebrospinal fluid
- Frontotemporal dementia
- Prion disease
- Parkinson’s disease
- Beta-amyloid deposits

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Submitted: 11 October 2017
Accepted: 06 November 2017
Published: 10 November 2017
ISSN: 2476-2032
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β-sheet rich PrP on the misfolded normal prion protein (PrPSc) is the agent of scrapie [13,14]. The normal prion protein PrP C is a membrane bound protein highly expressed in neuronal tissue, normally rich in α-helices. Through a yet to be fully understood mechanism, the α-helical rich PrP C can be converted into the β-sheet rich PrPSc. PrPSc acquires resistance to proteolysis during this process, and more importantly, can recruit other normal PrP molecules to also adopt the PrPSc conformation [1-4]. This self-directed propagation results in the accumulation of PrPSc, which ultimately induces neuronal death [1,13,15].

Self-directed propagation is not a property exclusive to the prion protein. Accumulated evidence suggests strongly that the prion model of progression describes a generic mode of replication of misfolded proteins common to many neurodegenerative proteinopathies [2,5,6,16,17] where misfolded, partially folded, or otherwise abnormal conformers of a disease-associated protein interact to form a beta-rich nucleus (Figure 1). This nucleus acts as a seed, inducing alteration in the secondary structure of other proteins of the same species to assemble into fibrillary aggregates. Mature large fibrils subsequently fragment to release additional small seeds, which act as templates themselves-establishing the self-propagating ability of these neurodegenerative diseases. Transmission between individuals has so far only been documented in prion diseases. Other protein misfolding diseases, pending future evidence, should be contained in the category of non-infectious prionoids.

It is important to note, however, that the model of cyclical amplification does not fully explain the toxicity of protein aggregates. Despite shared β-sheet rich ultrastructure in aggregates associated with neurodegenerative diseases, it is unclear if such fibrils possess inherent toxicity. Oligomeric species and fibrils of PrP assembled in vitro, for example, exhibit only mixed toxicity and transmissibility [18], and the identification of variably protease sensitive infectious species of PrPSc indicate that detergent insolubility and protease resistance are not obligatory requirements for transmission [19,20]. Additionally, the existence of a class of ‘functional amyloids’ suggests that prion and prionoid replication and neurotoxicity are separate phenomena. Functional amyloids are proteins which exist physiologically in an amyloid-like or prion-like conformation but are not pathological, and some of which even exert important biological functions [21,22]. It has been shown, for example, that mammalian peptide hormones are stored in secretory granules in natural β-sheet rich conformations [23] and most fungal prions have roles in gene expression or RNA binding [24]. Furthermore, the identification of ‘strains’ [13]-distinct conformers with different biochemical and physicochemical signatures-in prion and prionoid replication demonstrates that there is still much to be known about the underlying processes of these diseases. Nonetheless, disruption of the misfolding cycle is the primary therapeutic target [3,25,26].

**THE SKIN AND THE BRAIN**

Neuronal and dermal tissues share a common developmental origin, both arising from the ectoderm germ layer formed during embryogenesis. Differential commitment to nervous or...
The role of the skin in neurodegenerative diseases, due to the non-as a target for therapeutics. biomarkers for these diseases, but also the possibility of the skin the possibility of not only the cutaneous presence of diagnostic cascades, at least in a subset of patients. This hypothesis raises suggest that it may be the site of origin of the neurodegenerative an extensively innervated organ [32], it is not unreasonable to mutagenic insults, UV light being most prominent. As the skin is against the environment, is subjected daily to a variety of nature of neurons [37]. The skin, as a first line physical defense 

**PARKINSON’S DISEASE (PD)**

PD is a neurodegenerative disorder commonly manifesting as progressive motor dysfunction to the point of immobility. The triad hallmark of symptoms of PD includes rigidity, resting tremor, and bradykinesia, caused by the loss of dopaminergic neurons in the substantia nigra region of the midbrain. The mechanisms responsible for the death of nigrostriatal neurons are not totally clear, but in part involve abnormal accumulation of the protein α-synuclein. A major histological finding in PD patients is the presence of Lewy bodies-round cytoplasmic inclusions which are eosinophilic and with a dense core surrounded by a lighter halo. Immunohistochemistry confirms the strong presence of α-synuclein in the Lewy body core. The association of α-synuclein with PD is further supported by analysis of rare genetic forms of PD. Missense mutations in the SNCA gene coding for α-synuclein have identified in familial PD. Additionally, mutations in the protein kinase LRRK2 have been associated with PD presenting with Lewy bodies containing tau in addition to α-synuclein. LRRK2 is involved in autophagic pathways [38].

An *in vivo* propagation mechanism of PD was first suggested with the demonstrated development of Lewy bodies within neuronal stem cells transplanted into PD patients [39,40]. This phenomenon was replicated in mice and the seeding behavior of α-synuclein confirmed in a cell culture model [41]. Inoculation with brain homogenate from PD mice was also found to accelerate the course of disease in an A53T PD mouse model [42]. Though still considered a prionoid, some evidence suggests the capacity for α-synuclein to behave in a prion-like manner. Recent investigation has demonstrated that the disease could be induced in A53T mice not only by PD brain homogenate, but remarkably also by recombinant α-synuclein aggregated *in vitro* [43]. Brain homogenates from human multiple system atrophy (MSA) patients, another α-synucleinopathy, have been shown to induce PD-like neurodegeneration in A53T mice [44]. These results, though landmark, require further investigation before α-synuclein can be declared a prion. Individual-to-individual transmission has not yet been demonstrated in α-synucleinopathies [3].

Although motor symptoms are the most prominent feature, PD patients present with several other important non-motor clinical manifestations. Autonomic disturbances, difficulties with sleep and olfaction are other symptoms associated with PD, and are often seen in advance of motor dysfunction. Dementia, typically less severe, but featuring hallucinations, may be present in the later stages of the disease. These extra-motor dysfunctions are indeed associated with the presence of Lewy body lesions beyond the basal ganglia [38,44].
Interestingly, some have suggested an association between PD and melanoma. Not only do PD patients seem to be at greater risk of developing melanoma, melanoma patients seem to have a higher incidence of PD [45,46]. Melanin and dopamine are functionally distinct compounds, but share a synthetic precursor in the amino acid L-DOPA. A form of melanin called neuromelanin is abundant in the substantia nigra and the locus coeruleus of the brain and is in fact responsible for the characteristically dark appearance of the two structures. Melanocytes and dopaminergic neurons therefore share a set of genes involved in the production of melanin and dopamine, and it is conceivable that perturbations in these pathways may have a role in the etiology of PD [47].

Excessive sweating, or conversely, dry skin, is commonly reported among PD patients. As previously mentioned, Lewy bodies have been detected within autonomic structures, and PD patients have been found to have reduced innervation of sweat glands and erector pili muscles, though some have suggested that these symptoms are a side effect of standard PD therapy. Peripheral dermal neuropathology has been demonstrated to be an intrinsic feature of PD, and phosphor-α-synuclein (p-α-synuclein) could be reliably detected in a group of patients. The association of p-α-synuclein with PD was highly specific [48]. A follow up study by the same group detected dermal deposits of p-α-synuclein in PD patients, and additionally, detected p-α-synuclein in patients with REM sleep behavior disorder (RBD), a group considered to be at high risk for developing PD [49]. Taken together, these findings not only generate great interest in the potential for a skin based histological biomarker for PD, but also stimulate conversation regarding a possible dermal origin of PD pathology.

ALZHEIMER’S DISEASE (AD)

AD is the most common cause of dementia in the elderly population and is characterized by progressive degeneration of the limbic and cortical structures, leading to deterioration in memory, higher executive functions, and alterations in mood and behavior. Patients are profoundly disabled during the end stages of the disease, becoming mute and immobile in a catatonic-like state [50]. The social costs of AD are high as the gradual progression of the disease places complicated burdens on health care institutions and families. The World Health Organization estimates that the global prevalence of AD is expected to triple by the year 2050 [51], highlighting the need for improved diagnostics and management.

AD is associated with the accumulation of extracellular amyloid-beta (Aβ) plaques and intracellular tau neurofibrillary tangles in the brain. Aβ is an insoluble fragment of the normal amyloid precursor protein (APP) and tau is a microtubule-associated protein. A causal role for either protein in AD is supported by development of disease in mouse models overexpressing APP. Additionally, individuals with Down’s syndrome, which inherit an extra copy of the APP gene, almost universally develop symptoms of AD. Mutations in APP, enzymes responsible for processing of APP, and tau are associated with inherited forms of the disease [52,53].

Like PD, application of exogenous Aβ has been shown to accelerate disease in AD mouse models. Inoculation of AD mice with brain homogenate from human AD patients or aged AD mice, both of which contain high levels of Aβ, have been shown to hasten the progression of disease [54]. This phenomenon is
not limited to Aβ. Inoculation of transgenic mice expressing human tau with brain homogenate containing tau aggregates has been demonstrated to induce host aggregation [55]. Unlike α-synuclein and PrP, recombinant Aβ has not been shown to induce aggregation or trigger neuronal death [56]. Aβ has been found, however, to possess strain-like characteristics, which may influence the rate of propagation [57,58]. This phenomenon must be better understood prior to concluding that AD harbors no infective potential.

Beta-amyloid deposits have been detected in non-neuronal tissues in Alzheimer’s disease, in structures such as the vascular endothelium, GI tissues, and the skin, suggesting the circulating ability of precursors to Aβ aggregation [59]. Indeed, Aβ in the skin has been found to primarily exist as the more toxic soluble form rather than in mature fibrils [60]. It is perhaps due to this discrepancy of form that immunodetection of beta-amyloid in skin has not been supported as a reliable biomarker for AD [61]. Interest in a cutaneous biomarker for AD has instead focused on alterations in skin physiology. Presenilin-1, a component of the gamma secretase complex critical for the generation of Aβ from APP, has been implicated in skin cancer [62]. Presenilin-1 has also been found involved in the regulation skin turnover and epidermal growth factor receptor (EGFR) and its loss has been associated with the development of inflammatory skin conditions. Additionally, patients with AD have been reported to exhibit changes in cutaneous vascular function [63,64].

Fibroblasts from AD patients have demonstrated altered responses to stimuli, raising the possibility of a culture-based assay for early AD detection. Oxidative stress responses have been shown to be reduced in AD fibroblasts compared to non-AD patients [65]. Cultured fibroblasts from AD patients and APOE4 carriers have also demonstrated altered expression of the metabolic enzyme transketolase [66]. Differential Erk1/Erk2 phosphorylation ratios are observed in AD fibroblasts compared to non-AD controls when stimulated with bradykinin. Compared with autopsy validated cases, the overall sensitivity of this difference was found to be 97%, raising the possibility of use as a biomarker [67,68]. Prognostic value has also been suggested in the finding of elevated cutaneous levels of phosphorylated tau protein (p-tau) in AD patients [69].

**FRONTOTEMPORAL DEMENTIA (FTD) AND AMYOTROPHIC LATERAL SCLEROSIS (ALS)**

Frontotemporal dementia (FTD) refers to a heterogeneous group of neurodegenerative symptoms arising from progressive loss of neurons in the frontaltemporal lobe and is a leading cause of dementia in individuals under the age of 65 [70]. FTD can be classified into three typical variants based on the predominant symptoms, which reflects the primary locus of atrophy in the brain area [71]. The behavioral variant is most common and characterized by changes in personality, disinhibition, apathy, along with deficits in executive function. Less common are the language variants, presenting either as impairment in comprehension of speech (semantic dementia) or impairment in production of fluent speech (progressive non-fluent aphasia) [72].

Recent evidence has indicated that FTD shares some clinical and pathological features with amyotrophic lateral sclerosis (ALS) and the two likely exist as a spectrum disorder [73-75]. ALS is a motor neuron disease resulting in muscle atrophy eventually progressing to paralysis. Sporadic and familial cases have been identified, with no major differences in pathology and symptoms of the disease [75]. Individuals in the late stages of the disease lose the ability to speak or swallow and experience breathing issues due to weakening of the muscles, which support respiration. Many ALS patients experience cognitive or behavioral symptoms, with up to 15% of patients meeting the criteria for FTD diagnosis [76-78]. Likewise, cases of FTD can present with motor symptoms such as Parkinsonism, primitive reflexes, weakness, and incontinence [79]. Mutations in the same genes have been implicated in both FTD and ALS [70,73]. Furthermore, the observation of intracellular inclusions containing ubiquitin and transactive response DNA binding protein (TDP-43) in neurons and glial cells of both FTD and ALS patients further supports a pathological link between the two disorders [80,81].

Specific skin alterations have been reported in ALS patients. ALS patients appear to have alterations in the amount, type, and quality of collagen fibers in their skin [82-84]. Patients with ALS have been found to have increased expression of the hormones insulin-like growth factor 1 (IGF-1) and ciliary neurotrophic factor (CNTF) [85,86], as well as increased matrix metalloproteinase 9 (MMP-9) activity [87,88]. These factors are known to promote cell growth and wound healing, respectively, and their upregulation may be an explanation for the changes in collagen and the observation that ALS individuals seem to have a lower incidence of bedsores [89,90].

Immunohistological examination of skin biopsied from living ALS patients reveals increased expression of TDP-43 with higher levels of immunoreactivity depending on longer duration of disease [91]. Cytoplasmic inclusions have also been demonstrated to be present in the skin of ALS patients [92]. Fibroblasts from ALS patients were observed to exhibit abnormalities in the ubiquitin proteasome system and form aggregates containing TDP-43 [93]. Tissue engineered skin derived from ALS patients has been shown to exhibit structural abnormalities as well as TDP-43 containing inclusions. Most remarkably, these pathological features were also shown to be present in engineered skin derived from asymptomatic individuals carrying the ALS-FTD linked mutation in C9orf72 [92], suggesting predictive potential for this method. There exists little literature on the association of skin and FTD, though it has been suggested that skin conductance levels are comparatively lower in behavioral FTD patients [94].

**PRION DISEASES**

As previously mentioned, human prion diseases are a group of sporadic, inherited, and acquired neurodegenerative diseases caused by misfolding of PrPc [1]. The most common human prion disease is Creutzfeldt-Jakob disease (CJD), of which the majority of cases are sporadic, although familial and acquired forms exist. Gerstmann-Sträussler-Scheinker (GSS) syndrome and fatal familial insomnia (FFI) are two other examples of familial prion diseases. In contrast to AD and ALS-FTD, the prion protein gene (PRNP) is the only gene that has been detected so far in the development of prion disease [95].
Acquired human cases of prion disease have been well documented to occur through cannibalistic (kuru), zoonotic (variant CJD and bovine spongiform encephalopathy), or iatrogenic routes [15,96]. Less understood is the mechanism of transmission of the animal prion diseases scrapie and chronic wasting disease (CWD), which appear to be endemically in some animal populations. Scrapie and CWD both exhibit horizontal patterns of transmission, even in the absence of extensive animal-to-animal contact, suggesting environmental reservoirs of the disease. Several studies have identified the persistence and continued infectivity of PrPSc in soil materials [97,98]. There is then a question as to how PrPSc is disseminated into the environment. PrPSc has been detected in range of biological excretions including, the saliva, milk, feces, and urine from animals in clinical and preclinical stages of the disease [98]. There has long been interest in the presence of PrP in dermal tissues, as repetitive scratching resulting in sores and lesions are a prominent symptom of scrapie in sheep [99]. Indeed, the presence of PrPSc has been observed in skin tissue. CWD prions have been found in the shed velvet of developing deer antler, as well as the skin of scrapie and BSE infected animals [100,101]. Juvenile skin from PrPSc infected animals therefore may be a vector for introduction into the environment.

PrP has been identified in normal human skin and skin affected by dermatological disease [102]. PrPSc has also been observed in the skin of a human vCJD patient [103]. Although the level of PrPSc is expected to be substantially lower in the skin compared to the brain, its repeated identification across species raises the potential diagnostic utility of dermal prions, especially with the advancement of the highly sensitive real-time quaking-induced conversion (RT-QuIC) assay, which has been shown to be able to detect PrPSc in femtogram concentrations [104,105]. Indeed, using RT-QuIC assay we recently detected prion-seeding activity in the skin samples of patients with sCJD; moreover, most importantly, sCJD was also transmitted to humanized transgenic mice through intracerebral inoculation of the mice with sCJD skin homogenates [106]. Our new finding seems to be consistent with a previous report that 2 out of 18 mice inoculated with the skin sample of BSE-infected kudu developed prion disease [107]. These findings have significant public health implications for the management of CJD and suspected CJD patients in order to prevent iatrogenic transmission. Furthermore, the demonstrated presence of PrP in the normal human skin raises the possibility of a transdermal route for PrPSc transmission.

CONCLUSION

Neurodegenerative amyloidoses, including Alzheimer’s, Parkinson’s, ALS-FTD, and the prion diseases, are thought to share a common mechanism involving the conversion of a normal protein into a persistent abnormal conformer with the ability to self-replicate in a template directed manner, resulting finally in the formation of pathogenic aggregates which contribute to neuronal cell death. The events leading to the formation of the initial abnormal conformer are not fully understood. A lack of treatment options coupled with the devastating nature of these diseases has motivated the search for reliable biomarkers to which may facilitate earlier diagnosis. Given its shared embryonic origin with the brain and general accessibility, there has attention towards the skin as a potential source of these biomarkers. Indeed, gross and molecular alternations in skin physiology have been demonstrated in patients with neurodegenerative amyloidosis. Due to its extensive connectivity with the CNS, the skin should be explored as a possible route of transmission or even site of origin for these neurodegenerative diseases. Given the persistence of prions and potential infectivity of prionoids, it is crucial to understand the significance of their respective presences in the skin. Research towards this aim will importantly clarify the possible danger of iatrogenic transmission of neurodegenerative protein misfolding diseases via a dermal route.

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health (NIH) NS087588, NS096626, and the CJD Foundation.

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