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Differential molecular chaperone response associated with various mouse adapted scrapie strains

Ayodeji A. Asuni[a,*], Joanna E. Pankiewicz[a,c], Martin J. Sadowski[a,b,c,*]

[a] Department of Neurology, New York University School of Medicine, New York, NY 10016, USA
[b] Department of Psychiatry, New York University School of Medicine, New York, NY 10016, USA
[c] Department of Biochemistry & Molecular Pharmacology, New York University School of Medicine, New York, NY 10016, USA

HIGHLIGHTS

- Molecular chaperones (MCs) such as HSPs and Grps are involved in prion diseases.
- Various mouse adapted scrapie strains produce differential MCs response.
- Amyloidogenic and non-amyloidogenic prion strains engage MCs differently.
- MCs response in amyloidogenic prion strains and APPPS1-21 AD Tg mice is similar.

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ABSTRACT

Prionoses are a group of neurodegenerative diseases characterized by misfolding of cellular prion protein (PrP^C) and accumulation of its diseases specific conformer PrP^{Sc} in the brain and neuropathologically, they can be associated with presence or absence of PrP amyloid deposits. Functional molecular chaperones (MCs) that constitute the unfolded protein response include heat shock proteins and glucose-regulated protein families. They protect intracellular milieu against various stress conditions including accumulation of misfolded proteins and oxidative stress, typical of neurodegenerative diseases. Little is known about the role of MCs in pathogenesis of prionoses in mammalian prion model systems. In this study we characterized MCs response pattern in mice infected with various mouse adapted scrapie strains. Rather than uniform upregulation of MCs, we encountered two distinctly different patterns of MCs response distinguishing ME7 and 87V strains from 22L and 139A strains. ME7 and 87V strains are known for the induction of amyloid deposition in infected animals, while in mice infected with 22L and 139A strains amyloid deposits are absent. MCs response pattern similar to that associated with amyloidogenic ME7 and 87V strains was also observed in APPPS1-21 Alzheimer's transgenic mice, which represent an aggressive model of cerebral amyloidosis caused by β-amyloid deposition. Our results highlight the probability that different mechanisms of MCs regulation exist driven by amyloidogenic and non-amyloidogenic nature of prion strains.

1. Introduction

Accumulation of misfolded proteins is an underlying feature of several neurodegenerative diseases, including Alzheimer's disease (AD), tauopathies, Parkinson's disease, polyglutamine diseases, and prion disease (prionoses). Prionoses are characterized by the conformational conversion of cellular prion protein (PrP^C) to its toxic and proteinase K (PK) resistant conformer (PrP^{Sc}). Furthermore, PrP^{Sc} shows reduced solubility and inherent propensity to self-assembly into oligomeric and fibrillar structures. Accumulation of PrP^{Sc} is associated with widespread neurodegeneration; with hallmarks including spongiform changes, intraneuronal vacuolization, astrogliosis, loss of synapses and neuronal bodies; however, Thioflavin-S positive amyloid plaques composed of PrP fragments are present only in a subset of prionoses (reviewed in [9]). Prion strains are defined as clinically and neuropathologically distinct phenotypes of prion disease within given species, with features that can be precisely replicated after transmission. Multiple prion strains have been described in sheep and transmitted into mice resulting in generation of mouse adapted scrapie strains [4]. Examples of mouse adapted prion

* Corresponding authors at: New York University School of Medicine, 450E 29th St., Room 830, New York, NY 10016, USA. Tel.: +1 212 263 0984; fax: +1 646 501 4501.
E-mail addresses: asuni@mac.com (A.A. Asuni), sadowm01@med.nyu.edu (M.J. Sadowski).

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strains include ME7 and 87V, which among other distinguishing phenotypical features both show propensity for PrP amyloid deposition and 139A and 22L in which amyloid deposits are absent [4,6,8].

Elucidating the mechanisms by which misfolded proteins induce neurodegeneration requires information about their relationship with the protein quality control system that chaperones protein folding for proper function, refolds their misfolded conformers, or directs them for degradation. This protein quality control system also dubbed as molecular chaperones (MCs) encompasses two large protein families the heat shock proteins (HSP) and the glucose-regulated proteins [Grp], reviewed in [15]. The HSPs are a family of structurally related proteins classified by their molecular weights including HSP20, HSP40, HSP60, HSP70, HSP90, and HSP100, which are expressed in the cytoplasm, except for Hsp60 which has primarily mitochondrial localization. The Grp family includes Grp58, Grp78, and Grp94 proteins, which are expressed in the endoplasmic reticulum (ER) and are involved in primary folding of nascent proteins translated in the ER. They also serve as ER stress markers in conditions of disturbed proteostasis where they regulate unspecific aggregation and target misfolded proteins for proteasome-mediated degradation (reviewed in [21]).

The role of MCs in the pathogenesis of several neurodegenerative diseases associated with abnormal protein folding has been extensively studied (reviewed in [10]), while information about the role of MCs in mammalian prion model systems has been fragmentary [2,3,12,13,23]. Herein we examined whether a selection of MCs, that collectively represent the major MCs systems protecting neurons against protein unfolding and aggregation, are involved in pathogenesis of various mouse adapted scrapie strains. We provide evidence that amyloidogenic and non-amyloidogenic prion strains engage MCs response differently, which may significantly contribute to phenotypic differences observed among prion strains.

2. Materials and methods

2.1. Animals

Animal experiments were carried out in accordance with the National Institutes of Health guide for the care of laboratory animals and were approved by the Institutional Animal Care and Use Committee. All efforts were made to minimize animal suffering and reduce the number of animals used. This study was performed on the material archived during our previous studies, which encompassed brain hemispheres flash-frozen at the autopsy and stored at −80 °C and corresponding brain hemispheres embedded in paraffin blocks. All brains selected for this were collected within a period of four months and stored for approximately two years prior to commencing this work. We included brain tissue from female CD-1 mice intraperitoneally inoculated with ME7, 22L, 139A mouse adapted scrapie strains [19], control CD-1 mice inoculated with normal brain homogenate (NBH), female MB mice inoculated with 87V strain [18] and female APPPS1-21 transgenic (Tg) mice model of Alzheimer's disease (AD) [17] six of each. All prion inoculated mice were killed in the advanced stage of the disease when they were unable to reach the food bin or water spout or regain posture after being placed on their side. For mice inoculated with ME7, 22L, 139A strains the survival period ranged from 26 to 30 weeks, for mice inoculated with 87V strain it ranged from 38 to 42 weeks, while the control mice remained neurologically intact and were killed 30 weeks following NBH inoculation. APPPS1-21 AD Tg mice overexpress a sequence of human amyloid precursor protein with the double Swedish mutation KM670/671NL and a sequence of human presenilin 1 with the L166P mutation under the control of a neuron-specific Thy1 promoter [17]. APPPS1-21 mice represent one of the most aggressive models of β-amyloid (Aβ) deposition and in these mice the first Thioflavin-S binding Aβ plaques form during the second month of their life. We analyzed brains of APPPS1-21 mice killed at the age of two and eight months old, which both showed no neurological symptoms indicating motor or coordination impairment.

2.2. Western immunoblotting and densitometric analysis

Frozen brain hemispheres were thawed, weighed, and homogenized for downstream biochemical analyses following our published protocols [19]. Protein concentration in the brain homogenate was determined using the bicinchoninic acid method. For PrP^{Sc} analysis aliquots of brain homogenates containing 20 μg of the total protein were PK digested (Roche; Indianapolis, IN) and then resolved on 10% SDS-PAGE as described in our published protocols [16]. For assessment of the total PrP, glial fibrillary acidic protein (GFAP) and various MCs, non-PK treated samples of brain homogenates containing 20 μg of total protein were resolved on 10% SDS-PAGE and electroblotted onto nitrocellulose membranes. The membranes were blocked with 5% nonfat milk in TBS-T for 1 h at room temperature and then incubated with commercially available, previously characterized primary monoclonal antibodies (mAbs) at the indicated dilutions: anti-PrP (clone 6D11 1:3000, [19]), anti-GFAP (1:5000), anti-Grp58 (1:1000), anti-Grp78 (BiP; 1:1000), anti-Grp94 (1:2000), anti-HSP60 (1:4000), anti-HSP70 (1:2000), anti-HSP90 (1:1000). All mAbs were obtained from Stressgen Bioreagents (Farmingdale, NY), except for 6D11 and anti-GFAP, which were provided by Dr. R.J. Kascsak (NYS Institute for Basic Research, Staten Island, NY) and purchased from Sigma (St. Louis, MO), respectively. The antigen–antibody complexes were detected using horseradish peroxidase-conjugated anti-mouse IgG secondary Ab (GE Healthcare Bio-Sciences Corp. Piscataway, NJ) and visualized using SuperSignal (Pierce Chemical; Rockford, IL). For each densitometrically analyzed protein all brain homogenate samples were electrophoresed and immunonoblotted at the same time, rigorously maintaining the same experimental conditions. Western immunoblots were digitized and subjected to densitometric analysis following our published protocols [16,19].

2.3. Histology

Paraffin embedded brain hemispheres were cut into coronal 12 μm-thick sections, which were then carried on histological slides, deparaffinized, and stained with (1) hematoxylin–eosin for general neuropathological evaluation, which included assessment of spongiform pathology, (2) Thioflavin-S for amyloid plaques, or (3) immunostained with anti-GFAP mAbs (1:1000) followed by Mouse on Mouse peroxidase kit (Vector Laboratories, Ltd., Burlingame, CA) for assessment of astrogliosis [19].

2.4. Statistical analysis

Mann–Whitney *U* test was used for pair-wise comparison of brain steady state levels of total PrP, PrP^{Sc}, GFAP, and various MCs among analyzed animal groups.

3. Results

All prion infected mice showed a significant elevation of the total PrP level comparing to the NBH-inoculated control mice. The PrP level ranged from 2.5 ± 0.2 (mean ± standard error of the mean) fold increase over the NBH-inoculated controls in 139A infected animals to 6.8 ± 1.9 fold increase in 22L infected animals ($p < 0.01$ vs. NBH; Fig. 1A). There was also a modest but statistically significant 1.4 fold elevation of the total PrP level in both two and