Amyloidogenic prion protein peptides and neurodegeneration:cellular and molecular mechanisms

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Prion diseases are neurodegenerative disorders characterized by spongiform degeneration of the brain, neuronal loss and gliosis, often associated with amyloid deposition. According to the "protein only" hypothesis, all prion diseases share, as crucial pathogenic event, the posttranslational misfolding of a membrane-anchored glycoprotein (cellular prion protein, PrP^{C}) into a protease-resistant, aggregation-prone isoform (PrP^{Sc}). PrP^{C}-PrP^{Sc}conversion, physiologically prevented by energy barrier, can occur as stochastic event, be favoured by mutations in PRNP gene or acquired by infection with exogenous PrP^{Sc}(1).

PrP fragments corresponding to sequences identified in the brain of prion-affected patients match the protease-resistant sequence of PrP^{Sc}, are characterized by a flexible backbone that can undergo conformational rearrangement closely resembling PrP^{C}-PrP^{Sc}conversion. Thus, they represent a model to study the mechanisms of PrP refolding and neurotoxicity. We generated a recombinant protein encompassing amino acid 90-231 of human PrP (PrP90-231). This peptide is a soluble monomer structured as α-helix that after mild thermal denaturation (1 hour at 53°C) is converted in a misfolded ß-sheet-rich conformation that renders the peptide insoluble, highly hydrophobic and partially resistant to proteolysis, features corresponding to PrP^{Sc}characteristics. PrP90-231 misfolding leads to the acquisition of biological activities*in vitro*, inducing glia activation and apoptotic neuronal death with an efficacy comparable to that of PrP^{Sc}extracted from infected Syrian hamster brain (2).

Aim of this study was to define the cellular effects elicited by misfolded PrP90-231 (PrP90-231^{tox}) to induce to neuronal death. After SH-SY5Y neuroblastoma cell treatment, PrP90-$231^{tox}$is rapidly internalized and compartmentalized in large intracellular clusters within lysosomal vesicles. PrP90-231tox-induced cell death was proportional to the amount of internalized peptide, suggesting that neuronal accumulation of PrP90-231^{tox}could trigger apoptosis. The role of PrP90-231 intralysosomial accumulation in SH-SY5Y cell death was addressed analysing the biochemical characteristics the internalized PrP fragment. Internalised PrP90-$231^{tox}$was resistant to proteolysis and aggregated in high molecular weight polymers. By immunoblotting and immunostaining analyses, we observed that PrP90-231^{tox}intracellular accumulation triggers the activation of cathepsin D and caused the cytosolic diffusion of this enzyme. Confocal microscopy analysis of lysosome membrane integrity, using the fluorescent probe Lucifer Yellow (LY), showed that, whereas control cells evidenced a punctate pattern of LY fluorescence (index of dye accumulation into healthy lysosomes), PrP90-231^{tox}-treated cells showed a diffuse signal, indicating a cytosolic redistribution of LY. Analysis of PrP90-231^{tox}intracellular aggregates suggested that a subset of surviving cells, might engulf the peptide into autophagosomes, thus prevailing on its pro-apoptotic activity. Indeed, by immunostaining analyses we observed that PrP90-231 increase the expression of the autophagy marker LC3-II, and that pharmacological activation of autophagy by rapamycin counteracts PrP90-231^{tox}neurotoxicity, suggesting that the presence of autophagic vacuoles containing high amount of the protein could reflect successful cell defence and that insufficient authophagy resulted in cell death. In conclusion, these data suggest that PrP90-231tox accumulates within neuronal lysosomes causing cytosolic release of hydrolytic enzymes, and in the meantime evokes autophagic reaction of the cells to removes damaged cytoplasmic organelles. Thus, neuronal sensitivity to neurotoxic PrP90-231 will results from a balance between lysosomal permeabilization and autophagy.(This work was supported by MIUR-FIRB Accordi di Programma 2011 grant to TF)

1) Corsaro et al. Int J Mol Sci13:8648-869, 2012
2) Corsaro et al. OMICS 16:50-59, 2012