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Exosomes and their role in the intercellular trafficking of normal and disease associated prion proteins

Lesley Cheng [1], Wenting Zhao [1], Andrew F. Hill*

Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Bundoora, Australia

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ABSTRACT

Over the past decade, small extracellular vesicles called exosomes have been observed to harbour protein and genetic cargo that can assist in health and also cause disease. Many groups are extensively investigating the mechanisms involved that regulate the trafficking and packaging of exosomal contents and how these processes may be deregulated in disease. Prion diseases are transmissible neurodegenerative disorders and are characterized by the presence of detectable misfolded prion proteins. The disease associated form of the prion protein can be found in exosomes and its transmissible properties have provided a reliable experimental read out that can be used to understand how exosomes and their cargo are involved in cell-cell communication and in the spread of prion diseases. This review reports on the current understanding of how exosomes are involved in the intercellular spread of infectious prions. Furthermore, we discuss how these principles are leading future investigations in developing new exosome based diagnostic tools and therapeutic drugs that could be applied to other neurodegenerative diseases.

Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of invariably fatal neurodegenerative diseases characterized by spongiform vacuolation and degeneration of the brain, affecting both animals and humans (Schneider et al., 2008). Based on the protein only hypothesis of prion propagation, infectious prions arise from the misfolding of the normal prion protein, PrP^{C}, which is mainly α-helical in structure, into a disease-associated isoform with enriched β-sheet structure, termed PrP^{Sc} (Baldwin et al., 1994; Gasset et al., 1992; Caughey et al., 1991; Pan et al., 1993). Seeds of multiple PrP^{Sc} molecules promote further conversion of PrP^{C} into PrP^{Sc} in a self-propagating mechanism, thereby multiplying the amount of PrP^{Sc} and leading to aggregation and deposition of PrP^{Sc} in the brain. As PrP^{C} is predominantly expressed in the central and peripheral nervous system (Wopfner et al., 1999; Bendheim et al., 1992), the disease induces neurological symptoms including spongiform change and progressive loss of neuronal structures and functions, resulting in the death of neurons (Tuite and Koloteva-Levin, 2004; Bolton et al., 1982; Telling et al., 1996; Brandner et al., 1996; Forloni et al., 1993). Similar to other neurodegenerative diseases such as Alzheimer's and Parkinson's diseases, these pathological hallmarks cause alterations in the psychological, behavioural and cognitive states of patients. Prion diseases are distinctly different from other neurodegenerative diseases due to their transmissibility among species. Furthermore, the mechanism by which prions spread intracellularly remains to be discovered. This review provides a history on the how prions have been observed to be transmissible from species to species and more recently how exosomes may mediate the intracellular spread of prions upon peripheral exposure or sporadically.

Spread of prion disease in humans and animals

The first case of human prion disease was described in 1920 by German neurologist Hans Gerhard Creutzfeldt. He reported the illness of a female patient with symptoms such as progressive dementia, tremors, spasticity, ataxia and myoclonus (Richardson and Masters, 1995; Creutzfeldt, 1920). Alfons Maria Jakob described similar cases the following year, thus the disease was named Creutzfeldt-Jakob disease (CJD) (Creutzfeldt, 1920). Human prion diseases can occur via three different aetiologies. They can arise sporadically, be inherited through mutations in the *PRNP* (prion)

* Corresponding author.
E-mail address: andrew.hill@latrobe.edu.au (A.F. Hill).
[1] These authors have contributed equally to the review.

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gene, which encodes the cellular prion protein (PrP), or be acquired through exposure to infectious material, including medical procedures such as neurosurgery and blood transfusions using contaminated neurosurgical instruments and materials or consumption of contaminated food (Prusiner, 1998; Aguzzi and Heikenwalder, 2006).

Up to 85% of prion diseases occur sporadically where clinical progression for this disease is rapid and clinical duration is less than 12 months (Collins et al., 2004). Familial human prion diseases account for 15% of cases and are caused by inherited autosomal dominant mutations in the PRNP gene. The three main phenotypic forms of familial prion diseases are Gerstmann-Sträussler-Sheinker syndrome (GSS) (Hainfellner et al., 1995), familial CJD (fCJD) (Brown et al., 1994) and Fatal Familial Insomnia (FFI).

The third aetiology, accounting for less than 1% of cases, is acquired prion disease, including iatrogenic CJD, kuru and variant CJD (vCJD). Acquired CJD is caused by exposure to human prions through medical or surgical procedures, endocannibalism (as in cases of kuru) and consumption of contaminated food, respectively (Brown et al., 2000). Iatrogenic CJD was first observed in 1974 in a patient who developed the disease 18 months after receiving corneal graft transplantation from a patient who later died of CJD (Duffy et al., 1974). Kuru was identified in the 1950s in members of the Fore linguistic group of Papua New Guinea. This disease mainly affected women and young children, with an illness duration spanning from 6 to 36 months and is characterized by progressive ataxia (Gajdusek and Zigas, 1957). The origin of kuru began with the ritualistic consumption of brain tissues from deceased relatives by women and children which was shown to cause transmission of the disease. A key determinant of genetic susceptibility of the population to acquired and sporadic CJD is a common polymorphism on *PRNP*, located at residue 129, which encodes for methionine or valine (Wadsworth et al., 2008; Collinge et al., 1991). vCJD was identified in young adults in the United Kingdom in 1996 and the agent responsible was found to be potentially related to the outbreak of prion disease in cows, known as bovine spongiform encephalopathy (BSE) (Will et al., 1996).

Examples of animal prion diseases include scrapie in sheep, BSE in cattle, chronic wasting disease (CWD) in cervids, transmissible mink encephalopathy (TME) in mink and feline spongiform encephalopathy (FSE) in cats. BSE is one of the most well-known animal prion diseases, also known colloquially as 'mad cow disease'. BSE was first recognised in the United Kingdom in 1985, and rapidly developed into an epidemic (Ducrot et al., 2008). Experiments that exposed mice to either BSE or vCJD prions obtained similar incubation periods and spongiform vacuolation from the two groups of mice, suggesting the link between vCJD and BSE (Will et al., 1996; Collinge et al., 1996; Bruce et al., 1997; Hill et al., 1997).

Peripheral exposure to infectious prions, whether from ingested material or peripheral infection, is first detected in the lymphoreticular system where prions invades the lymphatic tissues before travelling to the sympathetic and parasympathetic nerves to the spinal cord and brain. In 1936, the successful transmission of prion disease in sheep, known as scrapie, was first revealed when recipient animals from scrapie-free flocks were inoculated intraocularly, epidurally, subcutaneously and intracerebrally with suspensions made from brain and spinal cord tissue of scrapie-infected sheep (Schneider et al., 2008). Infected sheep displayed a clinically silent phase before the appearance of symptoms between 11 and 22 months post inoculation. Shortly after, transmission of scrapie within the flock without artificial inoculation was demonstrated, whereby healthy sheep, which had been sharing a grazing paddock with scrapie-infected sheep, also developed the disease (Schneider et al., 2008; Greig, 1950). In comparison, intracerebral inoculation of prions typically leads to a shorter incubation period, inducing an immediate accumulation of prions in neurons and a more rapid onset of neurological symptoms.

Intercellular spread of infectious prions

Studies have demonstrated several mechanisms in which the transfer of infectious prions from cell-cell can occur using cell culture. Three main modes of transmission have been proposed, including cell-cell contact (Kanu et al., 2002), tunnelling nanotubes (Gousset et al., 2009), and/or through exosomes (Fevrier et al., 2004; Vella et al., 2007) (Fig. 1). The most obvious mechanism is the ability of infected cells to spread intracellular PrP^{Sc} to naïve neighbouring cells from cell-cell contact (Kanu et al., 2002). Infectious prions from peripheral sources has also been demonstrated to spread to the CNS via neuroinvasion of the peripheral nervous system (Glatzel and Aguzzi, 2000). The prion protein is synthesized with an N-terminal signal peptide that results in entry into the endoplasmic reticulum (ER). Following the translocation, the C-terminal signal peptide is cleaved off and a glycophosphatidylinositol (GPI) anchor is attached. Removal of the signal peptides results in a mature protein of 208 amino acids (residues 23-230 of the precursor protein). PrP^{C} then moves to the Golgi apparatus where the N-linked glycans mature and the protein is sorted for trafficking to the cell surface. Once there, PrP^{C} is anchored to the plasma membrane via the addition of a GPI anchor and resides specifically in lipid rafts (Stahl and Prusiner, 1991; Riek et al., 1997; Biasini et al., 2012; Naslavsky et al., 1997; Vey et al., 1996), a process that is required for the formation of PrP^{Sc} (Borchelt et al., 1992; Caughey and Raymond, 1991; Taraboulos et al., 1992; Bate et al., 2010). It is plausible to speculate that GPI-anchored PrP^{Sc} is released and inserted into the plasma membrane of neighbouring cells upon contact which is also known as 'GPI-painting' (Kanu et al., 2002; Madore et al., 1999; Stahl et al., 1987; Ilangumaran et al., 1996; Medof et al., 1996; Paquet et al., 2007; Liu et al., 2002). It was demonstrated that *in vitro* GPI-anchored PrP^{C}, rather than non-raft transmembrane PrP^{C}, can be converted to PrP^{res}, the protease-resistant form of PrP (Marshall et al., 2017). However, anchorless PrP was also shown to propagate prion infection but resulted in different disease phenotypes (Rangel et al., 2013). Prion infection on transgenic mice expressing anchorless PrP resulted in an unusual type of slow fatal prion disease distinguished by widespread deposition of PrP^{res} amyloid in the central nervous system (Chesebro et al., 2010). One patient expressing PrP Q227X mutant that lacks GPI-anchor displayed GSS phenotype with multicentric plaques and severe neurofibrillary lesions (Jansen et al., 2010). Furthermore, treating cells with phosphatidylinositol-specific phospholipase C (PI-PLC) and other pharmacological drugs to disrupt lipid rafts or the GPI-anchor from PrP^{C} has prevented the propagation of PrP^{Sc} (Taraboulos et al., 1995).

Lipid rafts containing GPI-anchored proteins enriched with cholesterol, sphingomyelin and sphingolipids are also found in exosomes and are likely to be involved in the packaging of proteins into exosomes. PrP^{C} found at the plasma membrane can undergo internalisation, followed by trafficking back to the cell membrane via recycling endosomes (Harris et al., 1993; Magalhaes et al., 2002; Sunyach et al., 2003), incorporated into intraluminal vesicles (ILVs) by invagination of multi-vesicular bodies (MVB) membranes and released into the extracellular environment within exosomes. Intracellular conversion of PrP^{C} to PrP^{Sc} has been suggested to occur in the MVB rather than the plasma membrane as preventing the maturation of the MVB reduces the level of PrP^{Sc} (Yim et al., 2015). Furthermore, both PrP^{C} and PrP^{Sc} have been found to be associated with exosomes isolated from cellular models infected with prions (Vella et al., 2007; Bellingham et al., 2012; Coleman et al., 2012).

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