Available online at www.sciencedirect.com

SCIENCE DIRECT

Blood Cells, Molecules, and Diseases 35 (2005) 143 – 148

BLOOD CELLS, MOLECULES, & DISEASES

www.elsevier.com/locate/ybcmd

Prions and exosomes: From PrPc trafficking to PrPsc propagation

Isabel Porto-Carreiro[a,*], Benoît Février[a], Sophie Paquet[b], Didier Vilette[b], Graça Raposo[a]

[a]*Institut Curie, CNRS UMR 144, 75248 Paris, France*
[b]*Institut National de la Recherche Agronomique, Unité de Virologie Immunologie Moléculaires, 78350 Jouy-en-Josas, France*

Submitted 20 June 2005
Available online 15 August 2005
(Communicated by M. Lichtman, M.D., 21 June 2005)

Abstract

Exosomes are membrane vesicles released into the extracellular environment upon exocytic fusion of multivesicular endosomes with the cell surface. Exosome secretion can be used by cells to eject molecules targeted to intraluminal vesicles of multivesicular bodies, but particular cell types may exploit exosomes as intercellular communication devices for transfer of proteins and lipids among cells. The glycosylphosphatyidylinositol-linked prion protein (PrP) in both its normal (PrP^c) and scrappie (PrP^{sc}) conformation is associated with exosomes. Targeting of exosomes containing the normal cellular PrP could confer susceptibility of cells that do not express PrP to prion multiplication. Furthermore, exosomes bearing proteinase-K resistant PrP^{sc} are infectious, suggesting a model in which exosomes secreted by infected cells could serve as vehicles for propagation of prions. Thus, cells may exploit the nature of endosome-derived exosomes to communicate with each other in normal and pathological situations, providing for a novel route of cell-to-cell communication and therefore of pathogen transmission. These findings open the possibility that methods to interfere with trafficking of such unconventional pathogens could be envisioned from insights on the mechanisms involved in exosome formation, secretion and targeting.

Keywords: Exosomes; Prions; Multivesicular bodies; ESCRT machinery; Glycosylphosphatidylinositol-linked proteins; Transmission

Introduction

Endosomal multivesicular bodies (MVBs) in certain cell types fuse with the cell surface in an exocytic manner. During this process, the small 50–90 nm vesicles contained in their lumen are released into the extracellular environment and are then called exosomes. Exosomes were shown to be secreted from hematopoietic (reticulocytes, B lymphocytes, dendritic cells, mast cells, T cells, platelets) and non-hematopoietic cells (intestinal epithelial cells, melanoma and mesothelioma cells) (reviewed in [1,2]). Exosomes released from these cells harbor functional molecules and can be targeted to other cells in which these molecules can elicit function. Exosomes released from B lymphocytes, dendritic cells (DCs), mast cells, tumor cells and intestinal epithelial cells can be targeted to T cells, bone-marrow-derived and splenic dendritic cells and can induce immunomodulatory functions for exosome-associated antigens [3–11]. Furthermore, vesicles with the hallmarks of exosomes are present, in vivo, at the surface of follicular dendritic cells (FDCs) in germinal centers [12], in malignant effusions [13], in broncho alveolar lavage [14], in urine [15] and in serum [16,17]. These and other studies support the idea that exosomes, in addition to their role in eradication of "unwanted" molecules [18,19], could provide for acellular vehicles to transfer molecules among cells in normal and pathological states [1,2,20].

Recent findings revealed an unexpected role for exosomes in the vehiculation of prions [21]. Prion diseases are invariably fatal neurodegenerative disorders that affect both humans and animals. They are associated with the conversion of the cellular prion protein (PrP^c) into the scrappie PrP (PrP^{sc}), an abnormal conformational state that tends to form amyloid deposits in brain tissue and that is thought to be infectious [22,23]. In the infectious forms of prion diseases, such as Kuru and variant Creutzfeldt–Jakob disease in humans, scrappie in sheep and bovine spongiform encephalopathy in cattle, the infectious agent

* Corresponding author.
E-mail address: Isabel.Porto-Carreiro@curie.fr (I. Porto-Carreiro).

1079-9796/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcmd.2005.06.013

enters the host through the gastrointestinal tract and then replicates in peripheral nerves and usually in lymphoid tissue before invading the central nervous system (CNS) [24,25]. There is increasing evidence that phagocytic mononuclear cells, including bone marrow, spleen-derived and follicular dendritic cells (FDCs), accumulate infectious prions and may play a key role in the transfer of prions from the gastrointestinal tract to the brain and therefore in the onset of disease [26,27]. However, the cellular mechanisms by which infectious prions are transferred from cell to cell are far from being fully unraveled. In agreement with previous studies, our observations provided evidence for a noncellular form of prions [28] corresponding to exosomal membranes bearing PrP^{sc} which are infectious in vitro and in vivo [21]. These observations lead to the hypothesis that exosomes may constitute a potential vehicle for prions [21,29]. Here, we highlight the emerging link between the mode of propagation of this unconventional infectious agent and its intracellular trafficking through the endosomal system. Finally, we further discuss how insights from MVB biogenesis could contribute to design new approaches that interfere with PrP trafficking and secretion.

The prion protein and its trafficking

The glycosylphosphatidylinositol (GPI)-anchored prion protein is ubiquitously expressed, although in higher levels, in neurons, some non-neuronal tissues and in cells from the immune system [22]. Its expression could be associated with a number of cell functions, including copper and/or zinc ion transport or metabolism; protection from oxidative stress; cellular signalling; membrane excitability and synaptic transmission; apoptosis and neurite outgrowth [30,31].

PrP^{c} is synthesized in the endoplasmic reticulum, travels through the Golgi apparatus, before being transferred to the plasma membrane [32]. After synthesis, PrP^{c} processing includes cleavage of the amino (N)-terminal signal peptide, addition of two N-linked oligosaccharide chains, formation of a disulfide bond and the insertion of its GPI anchor, responsible for its association with lipid-rafts at the plasma membrane. Once at the cell surface, PrP^{c} can be constitutively internalized either by clathrin-coated vesicles or caveolae [32,33]. Studies on Chinese hamster ovary (CHO) cells which express caveolin-1 revealed that PrP^{c} is clustered in caveolar-structures at the cell surface as well as in interconnected chains of endocytic caveolae inside the cytoplasm [34]. The choice of internalization pathway is not fully understood and may depend upon the cell type under study and the lipid microenvironment of the plasma membrane where PrP^{c} is inserted. Furthermore, as proposed, PrP endocytosis via clathrin coated pits could be due to its association with a still unidentified accessory protein carrying signals for adaptor and clathrin recruitment [33]. Once internalized by either mechanism, PrP^{c} has been shown to transit through late endosomes and lysosomes [34,35], consistent with the steady state localization of a fraction of PrP^{c} to endocytic MVBs in neurons in situ, in brain and in non-neuronal cell systems [21,34,36].

The infectious prion protein

Based on the protein only hypothesis, the infectious agent is thought to be the PrP^{sc} which arises by a conformational conversion of PrP^{c} [22].

The transconformation implies a switch from a form rich in α-helices to another form where β-sheets predominate. The rate of this conversion can be dramatically increased by the presence of preformed PrP^{sc} as a “seed”. The seed acts as the infectious agent and recruits PrP^{c} molecules into an oligomer that has all the characteristics of an amyloid fibril. Prion can then replicate the altered amyloid conformer through the generation of new protein seeds that can then be transmitted to other cells to nucleate further polymerization and thus propagate the infectious prion form [37].

Several intracellular locations have been proposed as potential sites for conversion of PrP^{c} to PrP^{sc}. Conversion could take place in the endoplasmic reticulum, in lipid-rafts at the cell surface, inside an endocytic organelle or even in the cytosol in the absence of membranes [32,36,38]. It is important to note, however, that the location where normal PrP is actually converted into PrP^{sc} may not reflect the sites of PrP^{sc} accumulation in infected cells. The characterization of the exact site of accumulation of PrP^{sc} has been hampered by the lack of antibodies that recognize the specific conformation of PrP^{sc} using electron microscopy. Nevertheless, visualization of PrP^{sc} after denaturation by guanidinium treatment prior to antibody detection revealed late endosomes and lysosomes of infected neuronal cells as possible sites of accumulation [39–42].

Dissemination of prions, a role for exosomes?

In most natural infections, prions are introduced peripherally and have to find their way to the CNS to produce the disease. A number of cellular players have been identified. For instance, it is thought that prions are transferred from FDCs to peripheral nerve endings within lymphoid organs. A model requiring cell-to-cell contact for intercellular prion transfer would necessitate additional cell types to bridge the gap between FDCs and neurons. Alternatively, prions could be transmitted to the nerves by cell-free short-range diffusion mechanisms. So far, the molecular and cellular mechanisms by which prion-infected cells contaminate their neighbors remain unclear. Work from Kanu and colleagues suggested that cell-to-cell contacts can promote the infection of target cells adjacent to infected cells [43].

Other studies, in contrast, reported on the presence of prion infectivity in the cell culture medium of a prion-