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REVIEW

The role of exosomes in the processing of proteins associated with neurodegenerative diseases

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Abstract Exosomes are small membranous vesicles secreted by a number of cell types and can be isolated from conditioned cell media or bodily fluids such as urine and plasma. Exosome biogenesis involves the inward budding of multivesicular bodies (MVB) to form intraluminal vesicles (ILV). When fused with the plasma membrane, the MVB releases the vesicles into the extracellular environment as exosomes. Proposed functions of these vesicles include roles in cell–cell signalling, removal of unwanted proteins, and the transfer of pathogens between cells, such as HIV-1. Another such pathogen which exploits this pathway is the prion, the infectious particle responsible for the transmissible neurodegenerative diseases such as Creutzfeldt-Jakob disease (CJD) of humans or bovine spongiform encephalopathy (BSE) of cattle. Interestingly, this work is mirrored by studies on another protein involved in neurodegenerative disease, the amyloid precursor protein (APP) which is associated with Alzheimer's disease (AD). Recent work has found APP proteolytic fragments in association with exosomes, suggesting a common pathway previously unknown for proteins associated with neurodegenerative diseases. This review will be discussing the current literature regarding the role of exosomes in secretion of the proteins, PrP and APP, and the subsequent implications for neurodegenerative disease.

Keywords Prion protein · PrP^{C} · PrP^{Sc} · Transmissible spongiform encephalopathy · Amyloid precursor protein · Alzheimer's disease · Exosomes

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Introduction

Exosomes are small membrane vesicles secreted from cells into the extracellular environment and were first reported in association with sheep reticulocytes (Johnstone et al. 1987). The term "exosome" is also used to describe a complex of several exoribonucleolytic and RNA-binding proteins essential for RNA degradation and processing (Vanacova and Stefl 2007). In this review we will be focusing on exosomes as small membrane vesicles secreted by many cell types and discuss their origin and function and particularly on their emerging role in neurodegenerative diseases such as Alzheimer's disease (AD) and the transmissible spongiform encephalopathies (TSE), or prion diseases.

The release of exosomes was suggested to be a cellular mechanism of releasing unnecessary proteins. An example of this is the transferrin receptor (TfR), which becomes obsolete as reticulocytes mature into erythrocytes (Johnstone et al. 1987; Pan et al. 1985). However, recent studies have revealed further functions of exosomes, which appear to be much more important, such as mediating cell

biological function (Abusamra et al. 2005; Fevrier et al. 2004; Gastpar et al. 2005).

A wide variety of cultured cell types have been reported to secrete exosomes, including dendritic cells (Thery et al. 1999, 2001), B lymphocytes (Escola et al. 1998, Wubbolts et al. 2003), mast cells (Skokos et al. 2001a, b), T cells (Blanchard et al. 2002) and several epithelial cell lines (Wolfers et al. 2001; Riteau et al. 2003). The physiological relevance of exosomes was established by the identification of exosomes in vivo, in association with follicular dendritic cells (FDCs) (Denzer et al. 2000), urine (Pisitkun et al. 2004) and malignant tumor effusions (Andre et al. 2002a).

The endosomal pathway

Collectively, the endosomal system consists of primary endocytic vesicles, early endosomes and multi-vesicular bodies (MVBs). These function by co-ordinating vesicular transport between the *trans*-Golgi network (TGN), plasma membrane and lysosomes. Early endosomes are located near the cell membrane where they act as the first port of call for primary endocytosed vesicles. Endocytosed molecules are either recycled to the plasma membrane or targeted to MVBs. Proteins that are sequestered to the limiting membrane of MVBs can be selectively incorporated into intraluminal vesicles (ILV) that are contained within the MVBs. This is possible by invagination of the limiting MVB membrane which creates a membrane enclosed compartment in which the lumen is topologically equivalent to the cytoplasm (Pan et al. 1985; Vidal et al. 1997). Many receptors and transmembrane proteins are targeted to MVB internal vesicles for lysosomal degradation. Degradation occurs via fusing of the MVB with the lysosomal membrane and the release of the MVB vesicles into the lysosome where they are subsequently degraded. This process is important for downregulation of activated cell surface receptors as it allows the cell to remove excessive membrane and transmembrane proteins. Not all proteins are destined for lysosomal degradation. MVBs can also fuse with the plasma membrane, leading to release of ILV into the extracellular environment as exosomes (Fig. 1). These vesicles have also been named dexosomes, texosomes or epididymosomes, depending on their cellular origin.

Intraluminal vesicle formation

At the limiting membrane of MVBs, several mechanism act jointly to allow vesicular formation and protein and lipid sorting, such as ESCRT components, lipids and tetraspanin-enriched microdomains. The main mechanism involves class E Vps proteins which are constituents of

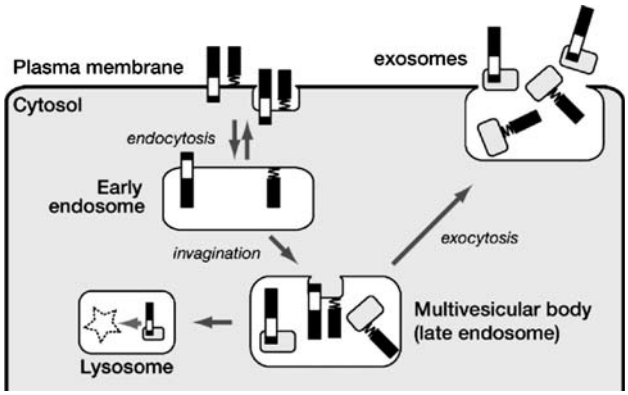

Fig. 1 The exosome pathway. Proteins such as GPI-anchored and lipid raft associated proteins can be sequestered to the limiting membrane of multivesicular bodies (MVBs) from the plasma membrane. These proteins, alongside cytosolic and endosomal proteins can be selectively incorporated into the intraluminal vesicles of the MVB. This is possible by invagination of the limiting MVB membrane which creates a membrane enclosed compartment in which the lumen is topologically equivalent to the cytoplasm (Pan et al. 1985; Vidal et al. 1997). MVBs can fuse with the plasma membrane, leading to release of intraluminal vesicles into the extracellular environment as exosomes

three protein complexes called ESCRT-I, ESCRT-II and ESCRT-III (Endosomal Sorting Complex Required for Transport) (Piper and Luzio 2001). These protein complexes are transiently recruited from the cytoplasm to the endosomal membrane where they function sequentially in the sorting of transmembrane proteins into the MVB pathway and in the formation of MVB vesicles.

The mono-ubiquitinated cargo proteins are recognized by the protein Hrs, which associates in a complex with STAM, Eps15 and Clathrin. Hrs recruits Tsg101 of the ESCRT I complex, leading to activation of ESCRT-II. ESCRT-II in turn activates ESCRT-III sorting cargo proteins at the limiting membrane of the MVB (Babst 2005). The sequential interactions of the ESCRT pathway permit the sequestration of cargo proteins into the inward budding vesicles of MVBs. After protein sorting has been completed AAA-ATPase Vps4 binds to ESCRT-III, dissociating the ESCRT machinery in an ATP-dependent manner (Babst et al. 1998). The involvement of the ESCRT pathway in exosome biogenesis is highlighted by observations that proteins required for the ESCRT pathway such as Tsg101 and Alix can be recovered from exosomes (Thery et al. 2001).

Lipid raft components such as cholesterol and the ganglioside GM1 are enriched in exosomes as are GPI-anchored proteins acetylcholinesterase (Johnstone et al. 1987), CD55, CD58 and CD59 (Piper and Luzio 2001; Rabesandratana et al. 1998). As GPI-anchored proteins associate with lipid rafts at the plasma membrane, it is thought these proteins and other raft associated proteins such as flotillin and stomatin (de Gassart et al. 2003) are sequestered in exosomes due to their presence in lipid raft

like domains. Other proteins such as tetraspanins (TSP) have an affinity for lipid raft domains and may work together with lipid rafts to generate exosomes (de Gassart et al. 2004).

Exosome composition and function

The protein composition of exosomes has been analysed extensively, predominantly by immunoblotting, mass spectrometry and FACS sorting, to reveal a defined subset of cellular proteins common to exosomes originating from a variety of sources and species. The range of exosomal proteins is limited to that of the total cellular proteome in that they do not contain any proteins originating from the mitochondria, nucleus or endoplasmic-reticulum. Exosomal proteins include cytosolic proteins, heat shock proteins, tetraspanins and transmembrane proteins (Thery et al. 2002). The protein composition of exosomes also highlights their endosomal origin, clearly distinguishing them from apoptotic blebs and microvesicles and allowing bona fide classification of extracellular vesicles as exosomes. Other exosomal markers include signal transduction molecules, lipid raft markers such as flotillin, GM, LBPA, phosphatidylserine, sphingomyelin (Fevrier and Raposo 2004; Wubbolts et al. 2003). Recently RNA species (both translatable mRNA and miRNA's) have been isolated from exosomes and termed "exosomal shuttle RNA" (esRNA) for their properties of altering gene expression and protein profiles in neighbouring cells (Valadi et al. 2007). It is clear that secreted exosomes are biologically active and have a role in a variety of biological pathways with the function of exosomes dependent on the cell type from which they are secreted. Some examples of exosome function are discussed below.

Once released from a cell it is proposed that exosomes could fuse with membranes of neighbouring cells, transferring exosomal molecules from one cell to another. It has been postulated that exosome uptake involves clathrin-mediated endocytosis followed by backfusion of exosomes with the limiting membrane of the endosomes (Thery et al. 2002), however this has yet to be formally confirmed. Many studies have examined the physiological function of dendritic cell (DC) derived exosomes in both in vitro and in vivo settings. In murine models, DC derived exosomes can modulate T cell immunity in either a positive or negative manner, depending upon the DC type and state of maturation of the cell (Li et al. 2006). In addition to stimulating immunity, DC-derived exosomes have been shown to be involved in T cell immunosuppression (Morelli et al. 2004; Segura et al. 2005a, b; Xiu et al. 2007; Zitvogel et al. 1998). DC-derived exosomes loaded with the tumor antigens have been shown to stimulate antitumor immunity, leading to phase I clinical trials in human patients with non-small cell lung cancer (Morse et al. 2005) and metastatic melanoma (Escudier et al. 2005). Exosomes can also exhibit immunosuppressive characteristics. Exosomes from bone marrow DCs injected before organ transplantation significantly prolong heart allograft survival and delayed rejection in rats (Peche et al. 2003), moreover exosomes containing MHC antigens can induce regulatory responses that are able to modulate allograft rejection and to induce donor-specific allograft tolerance (Peche et al. 2006). DC derived exosomes can also produce anti-inflammatory responses, resulting in reduced severity of arthritis and autoimmune disease in mouse models (Kim et al. 2005, 2007).

Another function of exosomes is in the proteolytic processing of certain target proteins. For example, the L1 adhesion molecule and CD44 undergo proteolytic ectodomain shedding in exosomes by the protease ADAM10 which is proteolytically active within the exosome (Stoeck et al. 2006). Later studies have demonstrated that soluble tumor necrosis factor receptors (TNFR) released from vascular endothelial cells within exosomes also undergo constitutive ectodomain shedding (Hawari et al. 2004). Moreover, the cleaved exosomal TNFR1 is suggested to be biologically active as it is still able to bind to ligands, implying that exosomes could be important modulators of tumour necrosis factor bioactivity.

Recent studies have demonstrated a role for exosomes in the intercellular trafficking of human immunodeficiency virus (HIV-1) (Loomis et al. 2006; Wiley and Gummuluru 2006). According to the "Trojan exosome hypothesis", retroviruses can exploit the pre-existing pathway of exosomal exchange for the synthesis of retroviral particles and for a low efficiency mechanism of infection (Gould et al. 2003). Lipid rafts are important at various stages of the human HIV-1 replication cycle (Campbell et al. 2001), with the virus preferentially budding from lipid rafts. The enrichment of cholesterol and sphingolipids in MVBS and presence of proteins such as Tsg-101 required for HIV budding, suggest that HIV could bud into MVBs as a viral exosome (Nguyen et al. 2003). Immature dendritic cells were found to mediate HIV-1 trans infection via the release of virus particles in association with exosomes, providing HIV-1 particles an avenue for immune escape (Wiley and Gummuluru 2006).

Neurodegenerative diseases; prion and Alzheimer's disease

It has recently become clear that proteins associated with the neurodegenerative disorders; prion and Alzheimers disease, can be selectively incorporated into ILV of MVBs and subsequently released into the extracellular environment,

enriched within exosomes. These surprising findings brought to light previously un-identified pathways in the processing of the prion protein (PrP) and the amyloid precursor protein (APP) and provide potential explanation for extracellular prion spread and amyloid deposition in the brain, previous unknowns in neurodegenerative disease, which will be discussed below.

The role of exosomes in the processing of PrP

Prion diseases are a group of invariably fatal neurodegenerative diseases that affect both humans and animals. According to the protein only hypothesis, an abnormal isoform of the host encoded prion protein (PrP^{C}), referred to as PrP^{Sc}, is the sole or major component of the infectious agent causing transmissible spongiform encephalopathies (Prusiner 1982). The normal, cellular, form of the prion protein (PrP^{C}) is encoded by the *PRNP* gene and is expressed in all tissues of the human body, with highest expression levels observed in tissues of the central nervous system and brain. PrP^{C} is synthesized in the rough endoplasmic reticulum (ER) and transits the Golgi on its way to the cell surface, in a similar manner to other GPI-linked membrane proteins. In cultured cell lines, PrP^{C} is predominantly found at the plasma membrane associated with lipid rafts (Kaneko et al. 1997; Taylor and Hooper 2006; Vey et al. 1996), microdomains of sphingolipids and cholesterol. PrP is constitutively internalized, targeted to the endocytic compartments and then recycled back to the plasma membrane (Shyng et al. 1993).

Ecroyd et al. were the first group to examine the association of endogenous PrP^{C} with extracellular membrane bound vesicles (Ecroyd et al. 2004). They examined the characteristics of PrP^{C} isoforms present in the male reproductive tract and identified the majority bound to membrane vesicles which they referred to as epididymosomes (Ecroyd et al. 2004).These epididymosomes are presumed to be exosomes (Sullivan et al. 2005) as they are purified in the same manner and have similar physical properties to exosomes. Subsequently, Robertson et al. found PrP^{C} on platelet-derived exosomes (Robertson et al. 2006). The platelets were derived from human blood of normal healthy volunteers. Activation of the platelets led to transient expression of PrP^{C} on the platelet surface and its subsequent release on both microvesicles and exosomes. They proposed that the presence of PrP^{C} on platelet-derived exosomes provided a possible mechanism for PrP^{Sc} spread via blood, however were not able to test for the abnormal isoform of PrP, PrP^{Sc}, to confirm this (Robertson et al. 2006).

The finding of PrP^{C} in exosomes is not unfounded. The distribution of PrP^{C} in vivo in rodent cerebellum is at the plasma membrane, MVB's and other endocytic organelles (Laine et al. 2001). A similar localization of PrP^{C} was also observed in Chinese Hamster Ovary (CHO) cells as revealed by cryoimmunogold electron microscopy (Peters et al. 2003). It was shown that internalized PrP is not directed to the ER and Golgi complex, but to endocytic structures, typically MVB's and lysosomes. The distribution of intracellular PrP^{C} on various organelles and membranes has also been examined in four hippocampal cell populations (Mironov et al. 2003). The highest concentrations of PrP^{C} occur on the plasma membrane and in MVB's (Mironov et al. 2003), consistent with its localization in exosomes.

PrP incorporation into exosomes appears to be dependent on host cell-type. For example, Fisher rat thyroid cells readily release PrP^{C} into cell culture media in a soluble form, not exosome associated (Campana et al. 2007) and pseudo afferent lymph derived exosomes do not contain PrP^{C} (our unpublished observations). The absence of exosomal PrP could signify that incorporation of PrP into exosomes is a cell specific mechanism present in only certain cell types for a particular functional role such as intercellular signaling or PrP transfer (Liu et al. 2002).

The literature regarding cell types that secrete exosomes has flourished since 2001, with identification of exosomes in the culture supernatant of hematopoietic cells (Clayton et al. 2005; Skokos et al. 2001a), intestinal epithelial cells (Van Niel et al. 2001) and tumor cells (Andre et al. 2002b) amongst others. The physiological relevance of exosomes was confirmed by their identification in biological fluids such as urine (Pisitkun et al. 2004) and plasma (Caby et al. 2005). Interestingly, prior to 2005 no one had examined if exosome secretion was possible from brain derived cells presumably due to the technical difficulty of working with large numbers of non-dividing primary cells. In 2005, Potoliccio et al. characterized exosome release by murine primary microglial cells (Potolicchio et al. 2005) followed by Faure et al. who demonstrated, using primary cortical cultures, that neurons and astrocytes can secrete exosomes (Faure et al. 2006). The biochemical composition of primary cortical cell derived exosomes was characterised and in addition to the known exosome protein markers; Tsg101, Alix and Flotillin, PrP^{C} was identified suggesting that exosomes may contribute to PrP^{C} function and prion spread. However, the association of PrP^{Sc} with exosomes was not examined in any of the above studies (Ecroyd et al. 2004; Faure et al. 2006; Gatti et al. 2002; Robertson et al. 2006) leaving uncertain the role of exosomes in PrP^{Sc} trafficking from endogenously expressing PrP cell lines.

PrP^{Sc} and exosomes

The subcellular distribution and localization of PrP^{Sc} has been hard to determine due to the lack of suitable antibodies

that can distinguish the two isoforms. PrP^{Sc} was identified in discrete vesicular foci and some large bodies in the cytoplasm of scrapie-infected cells (McKinley et al. 1991) and in lysosomes (Caughey et al. 1991). Moreover, ME7 scrapie-infected mouse brain has been used to show, both biochemically and by double-labeled immunogold electron microscopy, that PrP^{Sc} is enriched in MVBs (Arnold et al. 1995). These results suggested that in addition to the normal cellular form of prion disease, PrP^{Sc} could associate with the ILV's of MVBs and consequently exosomes.

Fevrier et al. utilised two cell systems that actively replicate sheep prions, to examine extracellular PrP^{C} and PrP^{Sc} (Fevrier et al. 2004) and characterize the localization of PrP^{Sc} in these environments. The two cell lines used were RK13 cells engineered to overexpress the ovine PrP (Rov) and neuroglial cells derived from transgenic mice overexpressing ovine PrP (Mov). They found that both cell lines released PrP^{C} and PrP^{Sc} into the culture medium in association with exosomes, before and after infection with sheep prions respectively. Their findings have important implications and led to much speculation about prion spread throughout an organism and transfer of exosomal infectivity to cells of different origin (Fevrier et al. 2005; Porto-Carreiro et al. 2005). However it was uncertain whether the association of PrP^{Sc} with exosomes was purely a result of PrP overexpression in the cell lines studied. One of the functions of exosomes is the removal of unwanted proteins, so it was plausible that incorporation of PrP^{Sc} into Rov or Mov exosomes was an artifact of overexpression and a mechanism by which cells rid themselves of excess protein.

We have recently demonstrated that both PrP^{C} and PrP^{Sc} are released in association with exosomes from a neuronal cell line (GT1-7) that endogenously expresses PrP (Vella et al. 2007). Our studies provide evidence on the transmission of exosome-associated PrP^{Sc} to heterologous cell types in addition to homologous cell types. Exosomes from the prion-infected neuronal cell line were found to be very efficient initiators of prion propagation in uninfected recipient cells and also to non-neuronal cells. Moreover, the neuronal cell line was highly susceptible to infection by non-neuronal cell-derived exosome PrP^{Sc} suggesting exosomes are very effective at transferring prion infectivity. The finding that exosomes released from infected cells can initiate conversion in cells originating from different tissues is significant for prion pathogenesis, as it may mimic the spread of infectivity around the LRS and to the CNS. These data are supported by those of Schatzl et al. (Schatzl et al. 1997), who showed the transfer of infectivity from infected GT1-7 cell media to N2a cells, albeit at low levels. In these experiments, the recipient cells were infected with conditioned medium that was either filtered or centrifuged at 16,000*xg* before adding to subconfluent recipient cultures. This method would have removed cellular debri or large microvesicles, indicating that the infectious component was small in nature and could be exosomal. In another cell system, culture supernatants from prion-infected mouse cholinergic septal neuronal cell line, SN56 (Baron et al. 2006), and prion infected hippocampal cells (Maas et al. 2007), can initiate infection in recipient cells. While the nature of the infectious entity was not identified in either of these studies, cell debri was removed from the infecting media first, suggesting PrP^{Sc} was released from the cell. In a set of elegant experiments it was shown that retroviral infection of a common fibroblast cell line enhances the extracellular release of PrP^{C} and PrP^{Sc} mediated by exosomes (Leblanc et al. 2006).

How PrP^{Sc} enters the CNS and negotiates the blood brain barrier (BBB) is poorly understood and is a difficult question to investigate (Nakaoke and Banks 2005). Whether exosomes from the lymphoreticular system could cross the blood brain barrier and transmit infection to neuronal cells is unknown. Even the basis for PrP^{Sc} spread within the CNS of a prion disease subject is unclear. In vitro systems mimicking prion trafficking in the CNS are limited and restricted to neuropathological imaging performed subsequent to neurotoxicity. As the brain is an organ in which cell motility is highly restricted, spread of pathological proteins via exosome dissemination is feasible. Combined with our data and the ability of primary cortical neurons to secrete PrP^{C}-containing exosomes (Faure et al. 2006), further investigation into the relationship between prion spread within the CNS and neuronal exosomes is warranted.

Why is PrP in exosomes?

It had been suspected for some time that exosomes contain lipid rafts due to their cholesterol-rich membrane and cholesterol-phospholipid ratio which is similar to lipid rafts in the plasma membrane (Vidal et al. 1989). The presence of lipid raft domains on the surface of exosomes was confirmed by isolation of typical raft components in the detergent-resistant membrane from three types of exosomes (de Gassart et al. 2003). Exosomal raft components include proteins such as flotillin, Lyn, Stomatin, glycolipids (de Gassart et al. 2003) and GPI-anchored proteins such as CD58 and CD59 (Clayton et al. 2003; Rabesandratana et al. 1998). The fact that most secreted exosomes contain lipid rafts suggests that rafts may contribute to the selective sequestion of proteins into exosomes and may play a general role in exosome biogenesis and structure (de Gassart et al. 2003). It has been suggested that molecule incorporation into exosomes may be dependent on the cycling pace of the molecule, be it lipid or protein. Vidal et al. suggests that slow recycling molecules are more

prone to be packaged into ILV's, whilst fast recycling molecules can more easily escape MVB inward budding and subsequently are excluded from incorporation into exosomes (Vidal et al. 1997). Further evidence for selective incorporation of proteins into exosomes was demonstrated by Fang et al. (Fang et al. 2007). Proteins could be directed into exosomes either by exposing cell surface proteins to exogenous cross-linking agents, appending plasma membrane anchors to highly oligomeric cytoplasmic proteins, or adding multiple homo-oligomerization domains to intracellular acylated proteins. Either one of these treatments was sufficient to target a protein, previously not associated with exosomes, to the exosome pathway and consequently into exosomes.

The rate of recycling of GPI-anchored proteins from the early endosomes to the plasma membrane is relatively slow, and is suggested to be because of their presence in lipid rafts. Interestingly, upon depletion of cholesterol or sphingolipids, GPI-anchored proteins recycle much faster (Mayor and Maxfield 1995; Mayor et al. 1994). Fast recycling lipids are known not to be incorporated in reticulocyte exosomes, whilst slow recycling lipids were able to be sorted into exosomes secreted by various cell types (de Gassart et al. 2003). It is possible that lipid rafts slow down the recycling of molecules that have an intrinsic affinity for lipid rafts, such as GPI-anchored proteins and as a consequence, these molecules, such as PrP would be prone to packaging into exosomes.

The association of PrP with exosomes is consistent with PrP being GPI-anchored in lipid rafts at the plasma membrane and constitutive internalization and recycling to the endocytic compartments. The pathway for PrP internalization at the plasma membrane is not fully characterized and still debated however is likely to involve either caveolae (Harmey et al. 1995) or clathrin coated pits (Shyng et al. 1994).

Finding PrP^{Sc} in association with exosomes allows us to speculate that once secreted in exosomes, PrP^{Sc} could be endocytosed or interact with membrane bound PrP^{C} on the recipient cell, initiating PrP conversion. It is not known whether the affiliation of PrP^{Sc} with exosomal lipid rafts aids in the conversion of PrP^{C} on a recipient cell, although we believe it is highly likely as mutant forms of PrP^{C} that are not localized to rafts cannot serve as substrates for PrP^{Sc} formation in scrapie infected N2a cells (Taraboulos et al. 1995, Kaneko et al. 1997). A prion propagation mechanism that is dependent on exosomal seeding would provide a plausible explanation for the movement of prions between distinct host tissues and around the brain. Following transfer of exosomal infection to the recipient cell, cell to cell contact would be the predominant means of transferring infectivity to surrounding cells in a particular tissue (Kanu et al. 2002).

The role of exosomes in processing of the amyloid precursor protein (APP)

Alzheimers disease is the most common form of dementia in humans and is characterised pathologically by the extracellular deposition of insoluble amyloid fibrils as amyloid plaques in the brain. The main component of amyloid is polymerized β-amyloid peptide (Aβ), a 39–43 amino acid residue peptide produced by proteolytic cleavage from the amyloid precursor protein (APP) (Cai et al. 1993; Estus et al. 1992; Golde et al. 1992; Haass et al. 1992; Serpell and Smith 2000). APP is co-translationally translocated into the endoplasmic reticulum (ER) via its signal peptide and then matured during passage through the Golgi. Following modification, a minor percentage of mature APP molecules are transported to the plasma membrane via secretory vesicles (Gandy and Greengard 1994; Selkoe 1996). At the plasma membrane mature APP molecules can undergo proteolytic cleavage by at least three proteases termed α-, β- and γ-secretases, which lead to the generation of a number of proteolytic fragments (Busciglio et al. 1993; Haass et al. 1993), including Aβ .

APP and its C-terminal proteolytic products can undergo rapid re-internalization via clathrin coated vesicles and trafficked to various subcellular locations, including the endoplasmic reticulum/intermediate compartment (Skovronsky et al. 1998), the trans-golgi network and endosomal/lysosomal systems. APP can undergo two separate processing pathways, yielding either the non-amyloidogenic p3 fragment or the Aβ peptide. The accumulation of the longer more amyloidogenic Aβ42 occurs predominantly in MVBs of neurons in normal mouse, rat and human brain and this accumulation increase with age in transgenic mice and human Alzheimer brains (Takahashi et al. 2002). The link between Aβ and its association with exosomes was made recently by Rajendran et al. They proposed that intracellular accumulated Aβ in MVBs is incorporated into exosomes and upon fusion of the MVBs with the plasma membrane, Aβ is released into the extracellular environment in association with exosomes (Rajendran et al. 2006). Albeit, only a minute fraction of total Aβ (<1%) is associated with exosomes.

Further to this work, Vingtdeux et al. reported that APP, APP-CTFs and AICD (amyloid intracellular domain) are integrated and secreted within exosomes from differentiated neuroblastoma and primary neuronal cortical cells (Vingtdeux et al. 2007). Importantly, this neuronal model clarifies that the accumulation of APP and it's proteolytic fragments in exosomes is not caused by the overexpression of the APP gene (Rajendran et al. 2006) and exosomes are not simply removing unwanted, excess protein through lysosomal degradation. Upon treatment of the cells with a γ-secretase inhibitor, they observed abolition of AICD and a

corresponding increase in the amount of APP-CTFs associated with exosomes.

Although APP and some of its proteolytic fragments have been identified in association with exosomes, it remains to be elucidated where APP processing is occurring in the exosomal pathway. APP-CTFs have been shown to accumulate in endosomal compartments upon γ-secretase inhibition (Zhang et al. 2006). AICD has also been shown to interact with the lipid raft-associated protein flotillin-1 which is also a well known exosomal protein. β-secretase associates with lipid rafts whereas α-secretase cleavage occurs outside raft domains. The β-secretase complex is also raft associated. As Aβ, a proteolytic product of β- and γ-secretase cleavage is found in exosomes, it remains to be determined whether cleavage actually occurs in these exosomes or earlier in the endocytic pathway prior to packaging in exosomes.

The identification of Aβ in association with exosomes is an important finding, especially since other exosomal proteins such as Alix and flotillin have been found to accumulate in the plaques of AD patient brains (Rajendran et al. 2006). Exosomes could provide an explanation for transport of Aβ and the equally toxic APP-CTFs around the body, to the brain where they contribute to amyloid deposition; however this hypothesis is unconfirmed. APP and Aβ have been found to circulate in extracellular fluids such as CSF (Hock et al. 1998; Liu et al. 2003; Van Nostrand et al. 1992) and plasma (Mehta et al. 2000; Podlisny et al. 1990) and recent work has confirmed the presence of exosomes in both of these bodily fluids (Vella, Greenwood, Scheerlinck, Cappai and Hill, unpublished observations) (Caby et al. 2005).

It is possible that familial AD patients who generally produce more of the longer, more amyloidogenic Aβ42 peptide, may have altered trafficking of the protein, resulting in more Aβ being localized to MVBs (Takahashi et al. 2002) and ultimately exosomes, which are then released into the extracellular environment for deposition in the brain.

The question remains as to the significance of exosome bound Aβ. Could exosome associated Aβ be the toxic species which starts the seed for amyloid deposition in the brain?

Conclusion

It is intriguing that both PrP and APP fragments are found in association with exosomes. The presence of other neurodegenerative proteins in exosomes, such as alpha-synuclein is yet to be determined, however maybe likely due to the presence of alpha synuclein in extracellular fluids such as culture media and CSF (El-Agnaf et al. 2003; Sung et al. 2005). The role exosomes play in the progression of neurodegenerative disease in in vivo situations is unknown, however one could speculate that exosomes contribute to the amyloidogenic cleavage of APP or that the mildly acidic exosome environment aids in the conversion of PrP^{C} to PrP^{Sc} in prion disease.

It appears that the function of exosomes is much more than just a secretory mechanism for cellular content, but rather a sophisticated means of processing specific molecules. Moreover, the exosome pathway appears to provide proteins such as PrP and APP an escape pathway out of the cell, favoring their spread in the extracellular environment and contributing to subsequent neurodegeneration.

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