Abstract

Prions (PrP) are the main cause of neurodegenerative diseases such as Scrapie in sheep, bovine spongiform encephalopathy, chronic wasting disease in deer, and Creutzfeldt-Jakob disease in humans. Although the cellular PrP (PrP^{C}) is involved in many cellular processes, its precise function still needs to be discovered. The disease is caused by the accumulation of a pathological form of PrP (PrP^{TSE}), which is caused by direct contact of PrP^{TSE} and PrP^{C}. PrP is anchored in the membrane by GPI and can be transmitted by cell-to-cell contact, tunnelling nanotubes, or extracellular vesicles (EVs). EV factions are divided by different biogenesis into exosomes, microvesicles, and apoptotic bodies. PrP^{TSE} was found in exosomes and microvesicles, but these fractions were never compared to each other. The first aim of the doctoral thesis is a comparison of PrP content, prion-converting activity and infectivity in these fractions on CAD5 and N2a-PK1 cellular models of infection. We isolated a fraction of large EVs (20,000× g) and small EVs (110,000× g) by centrifugation from a conditioned medium. We characterised EVs by cryo-electron microscopy and western blot with Alix, TSG-101, CD63, CD9, and HSP70 markers. The contamination from other cellular compartments was checked by calnexin. EV fractions differed in β-1 integrin content. Small EVs were depleted, and large EVs were enriched in β-1 integrin content. Small EV fraction contained vesicles with a mean size of 79 nm, and the size of large EVs started at 100 nm. EV fraction infectivity was studied using two approaches - standardisation on the original volume of conditioned medium (OVS) and standardisation on total protein amount (TPS). The infectivity efficiency was tested by cell blot, western blot, and standard scrapie cell assay. The results show that infection by large EV yields 4× higher prion signals than small EV in OVS infection and more than 20× in TPS infection, which is consistent with the 20× higher prion converting activity in large EVs. These results were verified on the N2a-PK1-RML cell model of infection. Our data indicate that large EVs are more important in the transmission of PrP^{TSE} than small EVs and contain more prion-converting activity.

EVs are currently tested for use in the diagnosis of various diseases, including prions. The second part of the thesis focuses on the optimisation of EV detection from the blood by flow cytometry and their diagnostic potential, which we evaluated in patients with multiple sclerosis (MS) and pre-term newborns. We isolated the EVs from the venous blood of MS patients and labelled them with antibodies against endothelial cells, platelets, leukocytes and red blood cells. EVs from pre-term newborns were isolated from cord blood, and we labelled them with antibodies against endothelial cells and platelets. Standard flow cytometry did not yield differences between MS patients and healthy blood donors nor between pre-term and in-term newborns. We have improved the sensitivity of the cytometer with an upgrade of violet side scatter. After the upgrade, we re-analysed samples from cord blood. We compared the analysis by standard blue laser and violet laser side scatter and obtained significantly correlated results. The reliable analysis of EVs from blood needs thorough optimisation of isolation protocol, labelling and detection. Still, improving cytometer sensitivity does not seem particularly critical in comparative studies of different groups.

Keywords: Prion, Extracellular Vesicles, Exosomes, Microvesicles, PrP, Cell Culture, Flow Cytometry, Blood Plasma