

Ceramide transporters in neuroinflammation and neurodegenerative diseases

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Summary

Precise regulation of protein function in the cell can occur by many mechanisms, at any stage in the flow of information from gene to protein. Protein function can be regulated by temporal and spatial control of the gene product which can be targeted to specific cellular compartments as required. Protein activity can also be regulated by the binding of effector molecules, which often work by inducing conformational changes that produce inactive or active forms of the protein. The various strategies of functional control are not mutually exclusive and any one protein may be subject to several modes of regulation. GPBP and its spliced variant CERT, encoded by the collagen gene COL4A3BP, represent a good example of this finely tuned cellular regulation. These two proteins differ solely by a 26 amino acid domain that appears to be sufficient to produce dramatically different biological activities. In **chapter 1** of this thesis we have summarized the current available knowledge of these proteins, attempting to present an integrated view of the background concerning their structure, properties and functions. Throughout, we remain careful to consider each isoform individually, as in much of the literature they can be mistakenly treated as one and the same protein. On the contrary, the two isoforms have many distinguishing properties. The shorter isoform CERT localizes inside the cell and carries out the role of ceramide transporter, while GPBP associates with membranes, can be secreted extracellularly and is likely to regulate molecular organization of structural proteins. Moreover, the additional 26-residue serine rich motif that distinguishes GPBP from CERT results in an increased kinase activity. The role of CERT is intimately linked to ceramide function, whereas a clear function for GPBP remains elusive. However, CERT and GPBP share a common ceramide binding capability and are the only proteins known to transport ceramide. Ceramide plays an important role in a wide variety of physiological neuronal processes, ranging from lipid raft formation in cell membranes to regulation of differentiation and apoptosis (**chapter 2**). By implication, CERT and GPBP must underlie the regulation of many physio-pathological processes. To investigate further, in **chapter 3** we first characterized the cellular localization and expression of these ceramide transporters in normal rat brain. High levels of immunoreactivity were observed throughout the brain, suggesting that GPBP and CERT play an important role in central nervous system (CNS) neuron function. Strong immunoreactivity was found in neurons of the cortex, hippocampus, the basal ganglia, the olfactory bulb and some nuclei of the thalamus, the hypothalamus and the septal area. Glial cells do not show immunoreactivity for GPBP and CERT, suggesting that under basal conditions these proteins have very low levels of expression in non-neuronal cells. In **chapter 4**, in order to further investigate the role of these proteins in the brain, we examined the expression levels of GPBP and CERT in the acute neurodegenerative process of the 6-OHDA rat model of Parkinson's disease (PD). We found that GPBP and CERT expression levels were not altered in diseased animals as compared to the control group. 6-OHDA induces a vigorous inflammation and mimics the selective neuronal degeneration that characterizes PD, however, this model does not produce all of the pathological and clinical features of human parkinsonism. A defining pathological hallmark of PD is the presence of neuronal Lewy body formed by protein aggregates. After 6-OHDA lesioning, deposit of aggregate proteins does not occur. The role of protein aggregation in the pathogenesis of PD has not been fully determined, but altered protein processing in the cell is known to have a negative impact on normal cellular function. Moreover, dysregulation of sphingolipid metabolism, that seems to be strongly involved in many neurodegenerative disorders, is a condition that is missing in a 6-OHDA model. Therefore, in **chapter 5** we have investigated the expression levels of GPBP and CERT in human and transgenic mouse Alzheimer's disease (AD) brain tissues characterized by aberrant protein aggregation and progressive neuronal degeneration. We found GPBP and CERT colocalised in amyloid plaques in post-mortem AD human brain. This led us to study the relationship between these proteins and serum amyloid P component (SAP), a ubiquitous amyloid deposit associated protein. We found that GPBP interacts strongly with SAP,

further supporting a role for GPBP in amyloid disease. Notably, GPBP-SAP binding is reduced by C1q, a well known ligand of SAP, raising the possibility that GPBP and C1q share a common binding site on SAP molecule. SAP binds to proteins involved in immunological responses and can activate the classical complement pathway through interaction with C1q. This might indicate a role for GPBP in the regulation of the complement system by inhibiting the C1q-SAP interaction by steric hindrance. Moreover, we observed GPBP staining in activated microglia of AD tissues, whereas in the non-disease state this protein is found only in neuronal cells. Considering that GPBP associates with amyloid plaques and has been shown to induce intra and extracellular structural protein organization, it seems likely that GPBP has an important role in the molecular organization and self-association processes of amyloid fibrils. Also, GPBP could be involved in the removal of misfolded proteins by activated microglia at the site of the AD plaques. Amyloid plaques are extracellular structures composed of 38–43 amino acid peptides called amyloid beta ($A\beta$) peptides. $A\beta$ -peptides are generated by aberrant proteolysis of a much larger precursor protein, the amyloid precursor protein (APP), a ubiquitous type I cell surface protein of as yet unknown physiological function. In **chapter 6**, we have shown that in the brain GPBP associates with neuronal plasma membranes and is found in the neuropil, conversely the shorter splice variant CERT localizes in the cytoplasm of neuronal cells. We next investigated whether GPBP has a role in the processing of APP using a cell culture model overexpressing APP that results in elevated levels of secreted $A\beta$ peptide. We found that GPBP accumulates at the plasma membrane level in response to APP over-expression, suggesting a regulation that might occur upstream of $A\beta$ production. This suggests that perturbation at the plasma membrane level, due to overexpression of APP and ceramide accumulation, may be sufficient for the upregulation of GPBP levels. However, we cannot exclude the possibility that very low levels of $A\beta$ peptide production may be responsible for the increase in GPBP expression. Secreted GPBP interacts directly with $A\beta$ 1-42 and promotes the formation of high-molecular weight $A\beta$ aggregates while simultaneously reducing the $A\beta$ β -sheet aggregation pathway, interestingly resulting in reduced $A\beta$ 1-42 peptide toxicity in cultured neurons. Importantly, the interaction between GPBP and $A\beta$ 1-42 is not limited to cell culture systems. We have detected a molecular association of GPBP and $A\beta$ aggregates in samples prepared from AD mouse brains demonstrating that the in vitro results have are functionally significant in vivo. Taken together these findings suggest that GPBP has a role in the progression of brain diseases that are characterized by protein misfolding/aggregation rather than merely in response to neuron degeneration or damage. In line with this role, high expression levels of GPBP are found in many autoimmune diseases in which antigens may arise from abnormal protein domain organization. GPBP was first identified as a collagen binding protein in Goodpasture syndrome, an autoimmune disease in which autoantibodies are observed along glomerular and alveolar basement membranes, causing glomerulonephritis and lung hemorrhage. Recent evidence suggests that the pathophysiology of neurodegenerative and inflammatory neurological diseases has an immunological component involving complement, an innate humoral immune defence system. Although historically viewed as an immune-privileged organ, the CNS contains and synthesizes many components of the immune system. Complement components play a central role in the amplification of AD risk factors and neuronal death. C1q, the first component of the complement cascade, is present in fibrillar amyloid plaques and, through activation of complement, has a role in the pathogenesis of AD. Our finding that C1q reduces the binding of GPBP to SAP, could suggest a cross talk between these three molecules at the plaque deposition site. Additionally, in innate immune reactions of the brain, microglial activation is the main cellular response to CNS dysfunction. We found positive GPBP staining in activated microglia in AD human tissue indicating that GPBP may have a role in the immune and/or immunoeffector function of these cells. The rapid clearance of abnormally structured proteins by microglia is important to inhibit inflammation as well as autoimmune responses against neo-antigens.

Together, our findings provide new insight into the physiopathological roles of GPBP protein. We suggest that GPBP may be involved in the innate immune response in the specific context of protein misfolding/aggregation. Through interaction with SAP, GPBP may inhibit SAP-mediated activation of the complement cascade. This raises the possibility that GPBP is able to modulate the innate immune response, placing this protein in a key position in the regulation of autoimmunity, inflammation and neurodegeneration. More research is required to fully characterize the mechanism of action of GPBP; however, the data presented here provide further detail of the increasingly apparent link between chronic inflammatory diseases such as neurodegeneration and autoimmunity.