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## The cellular prion protein beyond prion diseases

Manni Giorgia<sup>a</sup>, Lewis Victoria<sup>b</sup>, Senesi Matteo<sup>b</sup>, Spagnoli Giovanni<sup>c</sup>, Fallarino Francesca<sup>a</sup>, Collins Steven J.<sup>bd</sup>, Mouillet-Richard Sophie<sup>e</sup>, Biasini Emiliano<sup>c</sup>

<sup>a</sup> Department of Experimental Medicine, University of Perugia, Italy

<sup>b</sup> Department of Medicine (Royal Melbourne Hospital), The University of Melbourne, Parkville, Victoria, Australia

<sup>c</sup> Dulbecco Telethon Laboratory of Prions and Amyloids, Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Italy

<sup>d</sup> Florey Institute of Neuroscience and Mental Health, Parkville, Victoria, Australia

<sup>e</sup> Centre de Recherche des Cordeliers, INSERM, Sorbonne Université, Université de Paris, France

### Summary

The cellular prion protein (PrP<sup>C</sup>), a cell surface glycoprotein originally identified for its central role in prion diseases (also called transmissible spongiform encephalopathies), has recently been implicated in the pathogenesis of other neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases, by acting as a toxicity-transducing receptor for different misfolded protein isoforms, or in some case by exerting neuroprotective effects. Interestingly, PrP<sup>C</sup> has also been reported to play unexpected functions outside the nervous system, for example by contributing to myelin homeostasis, regulating specific processes of the immune system and participating in various aspects of cancer progression. Collectively, these observations point to a much broader role for PrP<sup>C</sup> in physiological and disease processes than originally assumed. In this manuscript, we provide an overview of what is known about the role of PrP<sup>C</sup> beyond prion disorders and discuss the potential implications of targeting this protein in different diseases.

**Keywords:** cellular prion protein, prion disease, neurodegeneration, immune system, cancer

### Introduction

Aging is accompanied by molecular, cellular and functional changes, which particularly affect the nervous system. Among the physiological processes known to be altered by aging is the protein folding quality control machinery, deputed to monitor and ameliorate protein misfolding. Once present, misfolded proteins typically acquire alternative conformations that can lead to their aggregation and accumulation intracellularly or extracellularly, and eventually initiate a cascade of toxic molecular events, ultimately resulting in cellular dysfunction [1]. A wide range of age-related disorders is indeed linked to protein misfolding and aggregation in the brain. Examples include highly prevalent disorders such as Parkinson's and Alzheimer's diseases, as well as rarer disorders such as prion diseases. Alzheimer's disease is the most common form of dementia in the elderly population, currently affecting almost 40 million individuals worldwide. The number will increase dra-

matically in the coming decades as the population ages, producing challenging medical and socioeconomic consequences [2].

According to the amyloid cascade hypothesis, Alzheimer's disease is a consequence of the accumulation in the brain of the 40–42 amino acid A $\beta$  peptide, a cleavage product of the amyloid precursor protein (APP). The A $\beta$  peptide spontaneously forms polymers ranging from small, soluble oligomers to large, insoluble fibrils [3]. Multiple pieces of evidence suggest that soluble A $\beta$  oligomers, rather than fibrillar aggregates, are primarily responsible for the synaptic dysfunction underlying the cognitive decline in Alzheimer's disease [4]. A $\beta$  oligomers are believed to act, at least in part, by binding to cell surface receptors that transduce their detrimental effects on synapses. Recently, a novel candidate has emerged as a receptor for A $\beta$  oligomers: the cellular form of the prion protein (PrP<sup>C</sup>) [5]. PrP<sup>C</sup>, an endogenous, cell-surface glycoprotein, plays a central role in transmissible neurodegenerative disorders commonly referred to as prion diseases. These diseases, which can be sporadic, inherited or acquired, are caused by the conformational conversion of PrP<sup>C</sup> into a misfolded isoform (called scrapie form of PrP or PrP<sup>Sc</sup>) that accumulates in the central nervous system of affected individuals. PrP<sup>Sc</sup> is an infectious protein (constituting "prions") that propagates itself by binding to PrP<sup>C</sup> triggering its conformational rearrangement ("templating") into new PrP<sup>Sc</sup> molecules [6]. A great deal of evidence indicates a distinction between prion infectivity and toxicity, and suggests that a physiological function of PrP<sup>C</sup> may be altered upon binding to PrP<sup>Sc</sup>, to deliver neurotoxic signals [7]. In fact, the presence of PrP<sup>C</sup> on the neuronal surface has been shown to be critical not only for supporting PrP<sup>Sc</sup> propagation, but also for transducing its neurotoxicity [8–10]. This conclusion recently found unexpected support from data involving other pathogenic protein oligomers. Different studies provided evidence that PrP<sup>C</sup> could mediate the toxicity of oligomeric assemblies of A $\beta$ , alpha-synuclein and other  $\beta$ -sheet-rich protein conformers [5, 11–14]. These results indicate that misfolded assemblies of several different pathogenic proteins could exert their effects by blocking, enhancing or altering the normal activity of PrP<sup>C</sup> [15]. This

### Correspondence:

Emiliano Biasini, PhD, Dulbecco Telethon Laboratory of Prions and Amyloids, Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Via Sommarive 9, 38123 Trento, Italy, [emiliano.biasini\[at\]unitn.it](mailto:emiliano.biasini[at]unitn.it)

conclusion highlights a close connection between the role of PrP<sup>C</sup> in several neurodegenerative diseases and its physiological function. What is this function? Several activities have been attributed to PrP<sup>C</sup> in the nervous system, mostly based on subtle abnormalities detected in mice or cells depleted of PrP<sup>C</sup> [16]. These include roles in neuroprotection, synaptic integrity, neuronal excitability and memory formation. However, most of these observations have not been reproduced in subsequent studies, found little or no physiological or pathological correlates, or were later shown to arise from genetic impurities of the employed mouse models [17, 18]. In fact, a number of previous lines used to study the physiological function of PrP<sup>C</sup> were non-co-isogenic *Prnp*(*-/-*) mice, in some case leading to artifactual conclusions [18]. Curiously, some of the clearest observations regarding PrP<sup>C</sup> function have been collected by studying the protein outside prion diseases. These include roles in the regulation of myelin homeostasis [19], immune processes [20] and in the progression of cancer [21]. Although it seems unlikely that a single protein could be involved in such a wide range of physiological processes, particularly in light of the relatively small number of phenotypic changes observed in PrP<sup>C</sup>-deficient mice, the lack of a clear understanding of the activity of this protein forces us to remain open minded. Thus, in this manuscript we review the most compelling data suggesting a putative role for PrP<sup>C</sup> beyond prion diseases and discuss potential therapeutic implications arising from such observations.

### A role for PrP<sup>C</sup> in other neurodegenerative disorders

Despite the lack of consensus around the normal function(s) of PrP<sup>C</sup> in the central nervous system (CNS), misfolding of PrP<sup>C</sup> with accumulation of altered conformers (PrP<sup>Sc</sup>) in the brain is considered the fundamental pathogenic event in prion diseases [22, 23], with a potential role for PrP<sup>C</sup> in the development of other neurodegenerative diseases such as Alzheimer's disease and the  $\alpha$ -synucleinopathies increasingly described.

#### Alzheimer's disease

In early studies predominantly relying on cell culture models, PrP<sup>C</sup> was described as favourably regulating the activity of  $\beta$ -secretase ( $\beta$ -site APP cleaving enzyme; BACE1), whereby production of neurotoxic A $\beta$  peptides was reduced [24]. This reported capacity of PrP<sup>C</sup> appeared to require PrP<sup>C</sup> localisation in cholesterol-rich lipid rafts and the N-terminal polybasic region [24] thereby allowing direct interaction with Golgi-localised, immature forms of BACE1 causing trapping within the Golgi and reduced BACE1 levels at the cell surface and in endosomes [25]. Of interest, a mutant form of APP (carrying the Swedish mutation) was reported to escape this beneficial regulatory effect of PrP<sup>C</sup>, in keeping with a potential protective effect for sporadic Alzheimer's disease but probably not for at least some types of genetic Alzheimer's disease. Unfortunately, the translational relevance and validity of these early observations has become less clear with the passage of time. In one follow-up study from the same laboratory, Whitehouse and colleagues reported that human brains demonstrated an ~50% reduction of PrP<sup>C</sup> expression in sporadic Alzheimer's disease frontal cortex compared with

age-matched controls, with PrP<sup>C</sup> levels inversely correlated with BACE1 activity, A $\beta$  load, soluble A $\beta$  levels and the Braak neurofibrillary tangle stage of disease [26]. In contrast however, a more recent report from this group, primarily utilising PrP<sup>C</sup> gene ablated (PrP<sup>0/0</sup>) transgenic mice expressing wild-type human APP, the absence of PrP<sup>C</sup> appeared to have no effect on BACE1 activity, with levels of APP proteolytic fragments, cognate A $\beta$  peptides and histopathological findings in the brains of these mice unaltered compared to controls [27]. Further potentially linking PrP<sup>C</sup> to the processing of APP and the generation of deleterious A $\beta$  peptides, another group has reported that the genes influenced by the amyloid intracellular domain transcription regulation fragment produced through  $\gamma$ -secretase processing of  $\beta$ -APP includes the gene encoding PrP<sup>C</sup> (*Prnp*) through a p53-dependent pathway [28], possibly constituting a negative feedback loop. In a subsequent report employing a combination of experimental approaches, however, this putative role for the amyloid intracellular domain in influencing PrP<sup>C</sup> expression levels could not be reproduced, once again leaving uncertainty about the biological validity of the original observations [29]. Additional observations suggesting a potential neuroprotective effect of PrP<sup>C</sup> in Alzheimer's disease have been provided by Rial and co-workers [30]. Utilising a mouse model centred on the effects of a single intracerebroventricular injection of 400 pmol of A $\beta$ 1-40 peptide on spatial learning and memory, these authors demonstrated reduced cognitive impairment in transgenic Tg-20 mice (that overexpress PrP<sup>C</sup> five-fold) compared with wild-type and transgenic PrP<sup>0/0</sup> mice, with the Tg-20 mice also displaying less evidence of apoptosis and cell damage in the hippocampus. The mechanism of neuroprotection was not explored by the authors but other reports raise the possibility that glutamate excitotoxicity may be relevant with PrP<sup>C</sup> able to directly attenuate excessive N-methyl-D-aspartate receptor (NMDAR) activity in a copper-dependent manner, including that induced by the presence of A $\beta$ 1-42 peptide [31–33]. In contrast to any potential neuroprotective effects afforded by PrP<sup>C</sup> in Alzheimer's disease, there is considerable evidence supporting a likely deleterious role in Alzheimer's disease pathogenesis. PrP<sup>C</sup>, through direct binding to residues 95-113, may act to disassemble amyloid fibrils composed of A $\beta$  peptides thereby trapping constituent peptides into an oligomeric state effectively enriching the concentration of putative neurotoxic oligomers [34], but most evidence suggests the harmful behaviour of PrP<sup>C</sup> is through acting as a receptor to transduce the toxic signal of soluble A $\beta$  peptides. Such deleterious effects of this PrP<sup>C</sup> mediated toxic signal transduction include impairment of hippocampal long-term potentiation (LTP), dendritic spine retraction and disruption of rodent spatial memory. In their seminal report, Lauren and co-workers exploited expression cloning to determine that PrP<sup>C</sup> binds with nanomolar affinity to soluble A $\beta$  oligomers (principally through the charge cluster residues 95–110) subserving blockade of hippocampal slice LTP, with synaptic function rescued by anti-PrP antibodies [5]. Despite the inability of early follow-up reports to replicate this implicated pathogenic role for PrP<sup>C</sup> [35–37], subsequent reports have re-affirmed and elaborated this apparent crucial transduction role for mediating soluble A $\beta$  oligomer toxicity. After A $\beta$  oligomers bind PrP<sup>C</sup> at dendritic spines

(possibly also inhibiting constitutive endocytosis and causing clustering of PrP<sup>C</sup> on the cell surface [38]), the A $\beta$  oligomer-PrP<sup>C</sup> complex associates with Fyn causing activation of this Src kinase leading to tau hyperphosphorylation [39], as well as phosphorylation of the NR2B subunit of NMDARs. The kinase activity of Fyn on NMDARs culminates in depletion of these glutamatergic ion channels at the synaptic surface in parallel with loss of dendritic spines [40–42]. In addition to deleterious synaptic changes, axonal and neuronal loss are reported as downstream pathophysiological consequences of A $\beta$  oligomers binding to PrP<sup>C</sup> along with impairment of spatial learning and memory [43, 44]. As a sequitur to these various reports of the importance of PrP<sup>C</sup> as a key transducing mediator of soluble A $\beta$  neurotoxicity, anti-PrP antibodies primarily directed against an epitope within the oligomer binding site have been described as ameliorating or rescuing rodent hippocampal LTP and cognitive function [5, 45–48].

### Alpha-synucleinopathies

Beyond a likely participation of PrP<sup>C</sup> in Alzheimer's disease pathogenesis, the normal form of the prion protein has also recently been suggested to contribute to the pathogenesis of  $\alpha$ -synucleinopathies, such as Parkinson's disease and diffuse Lewy body disease, although discrepancies in findings across reports is noteworthy. Harnessing in vivo and in vitro models, Ferreira and colleagues reported a deleterious interaction of  $\alpha$ -synuclein oligomers (but not  $\alpha$ -synuclein monomers or fibrils) with PrP<sup>C</sup> at the NMDAR causing a failure of LTP in wild-type mouse hippocampal slices; this putative role for PrP<sup>C</sup> was supported by the abrogation of LTP impairment when utilising PrP<sup>0/0</sup> hippocampal slices [12]. Moreover, attempts to block the interaction of  $\alpha$ -synuclein oligomers with PrP<sup>C</sup> using antibodies targeting specific PrP<sup>C</sup> amino acid segments revealed that the integrity of the 93–109 (charge cluster) region was necessary to observe such LTP impairment. The PrP<sup>C</sup> mediated inhibition of synaptic plasticity was also prevented with the use of a specific Fyn inhibitor when co-incubated with the  $\alpha$ -synuclein oligomers, suggesting that an interaction of the  $\alpha$ -synuclein oligomer-PrP<sup>C</sup> complex promotes phosphorylation of the NMDAR through Fyn, thereby causing excessive Ca<sup>2+</sup> influx at the post-synaptic terminal. The interaction between complexes of glycosylphosphatidyl-inositol (GPI)-anchored PrP<sup>C</sup> and  $\alpha$ -synuclein oligomers with cytosolic Fyn appears possible through metabotropic glutamate receptor 5 (mGluR5), as using specific inhibitors of mGluR5-mediated phosphorylation of NMDAR was also able to rescue LTP and cognitive deficits in these mice to levels equivalent to controls. Interestingly, an analogous molecular pathophysiological mechanism has also been observed in ex vivo and in vivo models of Alzheimer's disease assessing synaptic impairment driven by soluble A $\beta$  oligomers, with blockade of the adenosine A2A receptors responsible for mGluR5 activation resulting in the inhibition of the deleterious NMDAR phosphorylation via Fyn [40, 41]. Apparently incongruous with the aforementioned study showing that PrP<sup>C</sup> selectively bound only to  $\alpha$ -synuclein oligomers to subserve their detrimental effects, Aulić and co-workers reported that PrP<sup>C</sup> mediated the cellular uptake and spread of recombinant  $\alpha$ -synuclein amyloid fibrils, with this activity attenuating the propagation of misfolded PrP<sup>Sc</sup> in in

vitro and in vivo scrapie infection models [11]. Although supporting a role for PrP<sup>C</sup> in mediating the movement of  $\alpha$ -synuclein, another group suggested that although the pathological spreading of  $\alpha$ -synuclein may be facilitated by PrP<sup>C</sup>, it is not exclusively dependent on PrP<sup>C</sup> [49]. Despite the reported inability of  $\alpha$ -synuclein oligomers to induce LTP impairment in PrP<sup>0/0</sup> mouse hippocampal slices, the role of PrP<sup>C</sup> in directly mediating neurotoxicity and any direct interaction between  $\alpha$ -synuclein oligomers and PrP<sup>C</sup> are still a matter of controversy. Employing a range of biophysical techniques to assess an intimate interaction between  $\alpha$ -synuclein and PrP<sup>C</sup>, including surface plasmon resonance, La Vitola and colleagues were unable to confirm any direct association of PrP<sup>C</sup> with recombinant  $\alpha$ -synuclein oligomers [50], as well as  $\alpha$ -synuclein monomers and fibrils, although  $\alpha$ -synuclein monomers appeared to suppress PrP<sup>C</sup> concatenation through inhibiting nucleation. In addition, La Vitola and colleagues observed that primary neuronal cultures derived from wild-type and PrP<sup>0/0</sup> mice were equally susceptible to  $\alpha$ -synuclein oligomer neurotoxicity in a dose-dependent manner [46]. Finally, employing an in vivo model using intracerebroventricular injection of  $\alpha$ -synuclein oligomers, they also reported that PrP<sup>0/0</sup> mice displayed similar memory deficits and hippocampal gliosis to wild-type controls. Although the influence of differences in methodology cannot be ruled out, these findings support the likelihood of  $\alpha$ -synuclein oligomer mediated neurotoxicity independent from PrP<sup>C</sup>. Clearly, whereas any role of PrP<sup>C</sup> in non-prion neurodegenerative diseases remains incompletely understood and a subject of contention (especially in  $\alpha$ -synucleinopathies), it appears likely that the normal form of the prion protein may play some part in these other diseases, which for Alzheimer's disease may involve both protective and pathogenic contributions.

### PrP<sup>C</sup> in the immune system and related diseases

Over the last few years the interest of immunologists in PrP<sup>C</sup> and immune diseases has vastly increased. Two main pieces of evidence may justify such interest: firstly, PrP<sup>C</sup> has extensively been studied in the central nervous system but is also widely expressed in cells of the immune system [51]; secondly, immune tolerance to PrP<sup>Sc</sup> has been documented [52–54]. Indeed, immune tolerance may prevent robust immune responses to prions; accordingly, PrP-specific antibodies have not been detected in animals infected with prions. In addition, other studies reported that the immune system may also actively contribute to prion disease pathogenesis, by amplifying prion load in lymphoid compartments, transferring the pathogenic PrP<sup>Sc</sup> to cells and facilitating efficient neuroinvasion [20, 52]. Although it is clear that components of the immune system can contribute to the spread of prions, none of these pieces of evidence have been extensively validated at the molecular level, and conflicting results have often been reported [20, 55]. Overall, based on these observations, two specific roles of the immune system in prion diseases can be identified: immune cells may perform, when properly activated, as a protective shield against prions but, at the same time, they may be involved in the accumulation and spreading of pathogenic PrP<sup>Sc</sup> [56]. For these reasons, manipulation



of the immune system has been envisioned as a potential therapeutic option for prion diseases [57]. Immunotherapy strategies have reported promising results in vitro and in vivo. In particular, three main approaches have been undertaken so far: (i) treatment with antibodies targeting PrP<sup>Sc</sup> [58–61]; (ii) vaccines with antigen-loaded dendritic cells [62, 63]; and (iii) adoptive transfer of PrP-specific CD4<sup>+</sup> T lymphocytes [64]. Although more research into mechanism and safety of these approaches is still required, these immunotherapies may offer potential novel tools to clear the pathological form of PrP. However, because the function of cellular PrP<sup>C</sup> in the lymphoid system and in the CNS remains to be fully elucidated, it is not yet clear how therapies targeting PrP<sup>Sc</sup>, which shows similarities with PrP<sup>C</sup>, will affect immune or other specific endogenous functions. For this reason, uncovering the role of PrP<sup>C</sup> in cells of the immune system may provide novel insights both into its role in the pathogenesis of prion diseases and in specific functions of immune cells in general.

### PrP<sup>C</sup> in immune cells: expression and functions

Its high evolutionary conservation suggests that PrP<sup>C</sup> fulfils ancient and still essential biological functions [65–67]. Notably, PrP<sup>C</sup> is abundantly expressed in neural cells, including neurons and glia [68], as well as in subsets of cells of haematopoietic origin (e.g., myeloid dendritic cells, DCs, and T cells) [69]. In particular, data suggest that PrP<sup>C</sup> is involved in specific immune functions, including T cell development, DC activation, inhibition of macrophage phagocytosis and immunological quiescence [70, 71]. In addition to DCs and T cells, PrP<sup>C</sup> has been detected also in B lymphocytes, natural killer cells, platelets, monocytes and in follicular DCs [72–75]. Within lymphoid cells, B cells express lower levels of PrP<sup>C</sup> compared with T cells and natural killer cells [76]. In addition, it has long been known that PrP<sup>C</sup> is present on the surface of lymphocytes and it is rapidly upregulated upon their activation [77]. Following T cell activation, PrP<sup>C</sup> is redistributed in specific structures such as lipid rafts, together with signalling molecules, leading to immunomodulation [78]. It has been shown that GPI-anchored PrP<sup>C</sup> is enriched at the immunological synapse and can interact with components of the T cell receptor, such as the Fyn tyrosine kinase and the zeta chain-associated protein kinase 70 (ZAP-70), leading to the modulation of T cell receptor signalling cascade [75, 77, 79]. Moreover, PrP<sup>C</sup> expression was reported to be higher in T cells than in B lymphocytes, with CD8<sup>+</sup> cell subsets expressing slightly more PrP<sup>C</sup> than CD4<sup>+</sup> cells [76, 80]. PrP<sup>C</sup> expression is also higher in CD45RO<sup>+</sup> memory compared with CD45RA<sup>+</sup> naive T lymphocytes [75, 81]. Interestingly, data from gene arrays have revealed the murine *Prnp* gene to be up-regulated in T cell [82], via a Stat6-dependent mechanism, during interleukin (IL)-4 driven Th2 differentiation [83] and in CD8<sup>+</sup> memory T cells [84]. In addition, it has been reported that regulatory CD4<sup>+</sup> CD25<sup>+</sup> T cells (Tregs) expressed 4.5 fold higher levels of PrP messenger RNA and showed a 10-fold higher intensity of surface PrP<sup>C</sup> than effector CD4<sup>+</sup> CD25<sup>-</sup> T cells, despite no loss-of-function phenotypes could be recognised in Treg cells from PrP<sup>0/0</sup> mice [85]. Hence, PrP<sup>C</sup> may be more important in certain types of functionally differentiated lymphocytes that operate in particular immune environments. Outside the nervous system, the antigen-presenting

cells, DCs display the highest expression levels of PrP<sup>C</sup>, in both humans and mice [69, 86]. Studies on myeloid DCs showed that PrP<sup>C</sup> levels particularly increase during differentiation and maturation of these cells, in parallel with molecules involved in antigen presentation, such as major histocompatibility complex type II (MHC-II) and costimulatory molecules [69]. Interestingly, it has been demonstrated that important differences of PrP<sup>C</sup> expression exist between different DC subpopulations either analysed after ex vivo isolation or differentiated in vitro. DCs can be classified in two major categories: conventional DCs (cDCs), which include at least two different DC subsets (e.g., cDC1 and cDC2) and plasmacytoid DCs (pDCs) [87, 88]. PrP<sup>C</sup> was found on the surface of bone marrow-derived human and mouse conventional DCs generated in vitro or isolated from the spleen, but not in pDCs [88]. PrP<sup>C</sup> expression in these cDCs was strongly up-regulated after maturation by TLR ligands, such as bacterial lipopolysaccharide and CpG. Interestingly, a study from Ballerini et al. showed that membrane PrP<sup>C</sup> on DCs enhanced the stimulation of specific naïve T cells both in vitro and in vivo [89]. High expression of PrP<sup>C</sup> was also found on the surface of CD8<sup>+</sup> cDC subset, both in the spleen and the lymph-nodes [88]. Although the different PrP<sup>C</sup> expression between cDCs and pDCs could be related to the specific developmental programme of these two cell types, the specific role of PrP<sup>C</sup> in cDC functions still remains to be explored. Related to these issues, specific evidence suggests that absence of PrP<sup>C</sup> in T cells and DCs had different outcomes in T-cell proliferation. Specifically, T cells devoid of PrP<sup>C</sup> exhibited a normal allogenic antigen response, while DCs lacking PrP<sup>C</sup> significantly reduced proliferation of interacting T cells, suggesting that PrP<sup>C</sup> might serve different signalling roles in the two cell types [90]. Another class of dendritic cells, the follicular dendritic cells (FDCs), express high levels of the PrP<sup>C</sup>, although its function in these cells is still uncertain. In fact, it has been shown that PrP<sup>C</sup> is dispensable for the maturation of FDCs and for maintaining antigen-specific antibody responses [91]. PrP<sup>C</sup> has also been found in macrophages and its expression is associated with both the inflammatory M1 phenotypes and with the immunosuppressive M2 types. Interestingly, recent studies have demonstrated that PrP<sup>0/0</sup> mice produce reduced amounts of the anti-inflammatory cytokine IL-10 in response to systemic lipopolysaccharide, potentially suggesting a role for PrP<sup>C</sup> in promoting IL-10 production in M2 macrophages [92]. Moreover, again in macrophages, it has been demonstrated that PrP<sup>C</sup> plays important role in phagocytosis [93, 94]. In particular, Wang and colleagues demonstrated that mouse bone marrow-derived macrophages infected with *Escherichia coli* express high levels of *Prnp* mRNA, leading to inefficient phagocytosis. Conversely, macrophages devoid of PrP<sup>C</sup> internalised bacteria and increased the expression of cytokines such as interleukin-1 $\beta$ , decreasing bacterial proliferation [95]. These data reveal a potentially important role of PrP<sup>C</sup> as a negative regulator of phagocytosis, phagosome maturation, cytokine expression, and macrophage microbicidal activity. Further studies are required to determine how PrP<sup>C</sup> regulates vesicular trafficking associated with phagocytosis and cytokine secretion.

### PrP<sup>C</sup> in immune disorders

The role of PrP<sup>C</sup> in health and homeostatic cell functions is still obscure, but several potential roles have been attributed to this protein in the immune system. Specifically, several studies suggest that PrP<sup>C</sup> may act as a modulator of innate immune responses in pathologies beyond prion diseases [96–98]. The detailed molecular means by which PrP<sup>C</sup> modulates immune signalling pathways contributing to immune modulation are not yet clarified and stand out as a necessary area of future research. Interestingly, PrP<sup>C</sup> is expressed in various organs that, by multiple mechanisms, are relatively protected from inflammation (i.e., immunoprivileged sites) such as the brain, eye, placenta, the pregnant uterus and testes [99, 100]. This high expression in immuno-privileged organs suggest that PrP<sup>C</sup> has an important protective role under inflammatory stress and/or tissue damage [90, 101]. Accordingly, specific reports have shown that the absence of PrP<sup>C</sup> increases inflammatory damage in different models of inflammation such as experimental brain ischaemia, brain trauma and experimental autoimmune encephalomyelitis [102]. For example, experimental autoimmune encephalomyelitis, the animal model for human multiple sclerosis, is worsened in mice lacking PrP<sup>C</sup>. In particular, in the acute stage, the spinal cords, cerebellums and forebrains of *Prn-p*-deficient mice were shown to be more heavily infiltrated with leucocytes and exhibited stronger proinflammatory cytokine gene expression, as compared with those seen in wild-type mice. Remarkably, the persistence of leucocyte infiltration in the forebrain and cerebellum was accompanied by increased pathogenic cytokines, such as interferon (IFN)- $\gamma$  and IL-17 [103]. In this particular model, disease exacerbation has been attributed to T cells that would differentiate into more inflammatory (i.e., Th1 and Th17) and behave more aggressively against the CNS effectors, when deprived of PrP<sup>C</sup> [104]. Thus, based on these results, attenuation of T cell-dependent neuroinflammation may represent a potential novel function of PrP<sup>C</sup>. In addition to experimental autoimmune encephalomyelitis, PrP<sup>C</sup> also appears to be protective in autoimmune colitis. Inflammatory bowel disease, induced by dextran sodium sulphate (DSS), is more severe in PrP<sup>0/0</sup> mice than in wild-type mice. Accordingly, overexpression of PrP<sup>C</sup> greatly attenuates DSS-induced colitis [105]. Again, depletion of PrP<sup>C</sup> was able to skew T cells toward more pronounced Th1 and Th17 inflammatory phenotypes [79]. Based on these data, variations in the human *PRNP* gene or its sequence [106] might have effects on disease susceptibility or the clinical course of autoimmune diseases; however, these specific studies have not yet been performed. Another interesting observation is that PrP<sup>C</sup> may act as antimicrobial peptide. It was demonstrated that synthetic peptides derived from the N-terminal region of PrP<sup>C</sup> are cytotoxic to several bacterial species, including *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* [107]. In addition, in 2013 Ding and co-workers showed that PrP<sup>C</sup> participates in the regulation of microglial response to *Mycobacterium bovis* infection, through the upregulation of pro-inflammatory cytokines and the modulation of apoptosis [108]. In particular, they found a significant increase of *Prnp* mRNA expression upon microglial cell infection with *M. bovis*, and *Prnp* silencing did not alter the expression pattern of anti-inflammatory cytokines IL-10 and

transforming growth factor (TGF)- $\beta$ . PrP<sup>C</sup> was also shown to possess antiviral properties by inhibiting the replication of the human immunodeficiency virus type 1 (HIV-1) and the murine leukaemia virus [109]. In these studies, PrP<sup>C</sup> was able to bind the viral genomic RNA of HIV-1 negatively affecting its translation. Moreover, PrP<sup>C</sup> was found to co-localise with the virus assembly machinery at the plasma membrane and at the virological synapse in infected T cells. Depletion of PrP<sup>C</sup> in infected T cells and microglia favoured HIV-1 replication [109]. Within this conceptual framework, it has been suggested that PrP<sup>C</sup> may serve two principal roles in immune system: to modulate the inflammatory potential of immune cells, and to protect vulnerable parenchymal cells against noxious insults generated through inflammation. The mechanisms lying behind the role of PrP<sup>C</sup> and their significance for pathogenesis and its regulatory roles in specific immune disorders require further investigation.

### PrP<sup>C</sup> and cancer

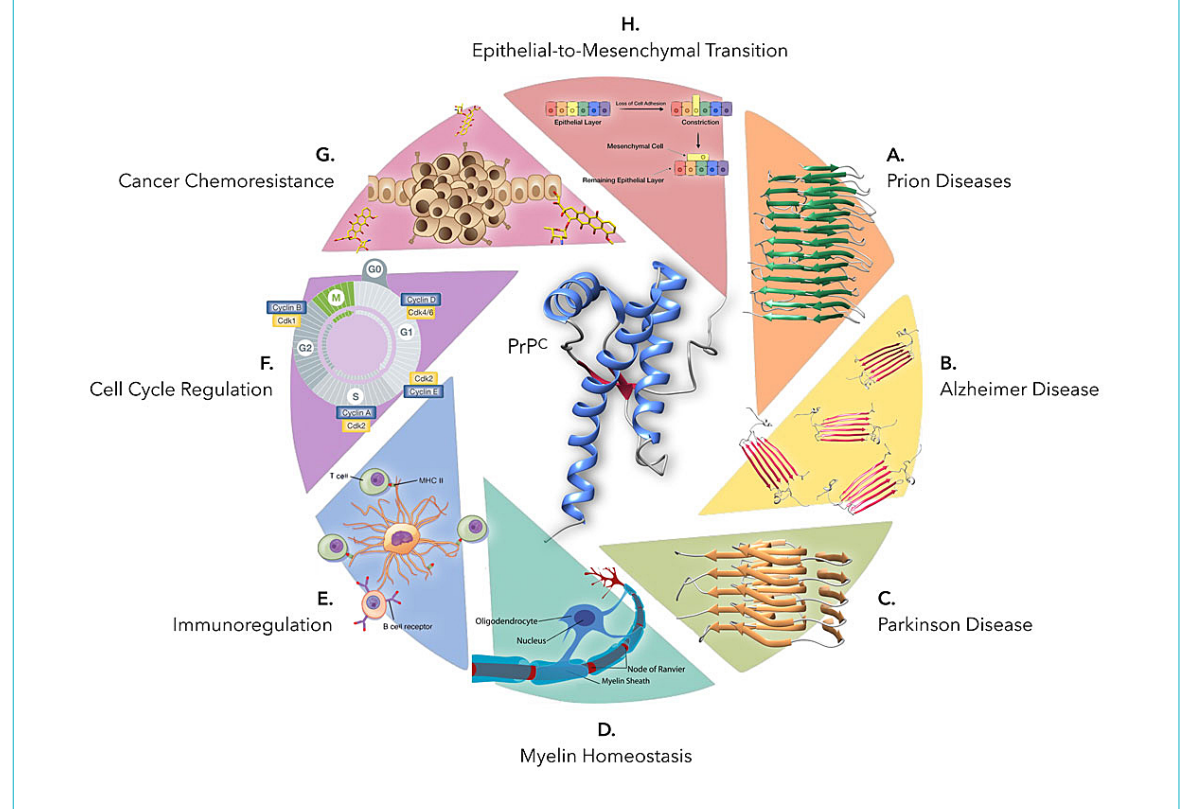
The first hint of a link between PrP<sup>C</sup> and cancer dates back to the early 2000s when *PRNP* was identified as one of the 30 genes most overexpressed in pancreatic cancer cell lines as compared with normal cells [110]. At the same time, PrP<sup>C</sup> was reported to be upregulated in a drug-resistant gastric cancer cell line as compared with the parental cell line [111]. That elevated PrP<sup>C</sup> may confer resistance to anticancer agents was soon confirmed by Diarra-Mehrpour and colleagues, who demonstrated a causal relationship between increased PrP<sup>C</sup> expression and resistance to tumour necrosis factor- $\alpha$  (TNF $\alpha$ ) in a breast cancer cell line [112]. Thereafter, de Wit and colleagues came across PrP<sup>C</sup> when screening for cell surface molecules associated with adenoma to carcinoma transition in colon cancer [113]. Following these pioneering findings, further studies have consolidated the involvement of PrP<sup>C</sup> in four main aspects of cancer biology: proliferation; resistance to anticancer agents; cell migration and invasion; and epithelial to mesenchymal transition. More recently, links between PrP<sup>C</sup> and cancer stem cells (CSCs) (see below), as well as aneuploidy, were also uncovered [114]. Furthermore, although scarce, studies featuring patients globally point to an association between high PrP<sup>C</sup> expression and poor prognosis [115–120]. It is now well established that PrP<sup>C</sup> may sustain cancer cell proliferation in various types of cancers: gastric [121], pancreatic [120] and colon cancer [116, 122, 123], as well as glioblastoma [117, 124] and schwannoma [125]. From a mechanistic point of view, PrP<sup>C</sup> was shown to promote the recruitment of a PI3 kinase (PI3K)-AKT pathway, itself controlling the transcription of CyclinD1 in gastric cancer cells [121] and to activate the MAP kinases ERK1/2 upon interaction with the STI1 chaperone in glioblastoma [117, 126]. In pancreatic cancer cells, the pro-proliferative action of PrP<sup>C</sup> appears to involve activation of the Notch pathway [127]. Moreover, the capacity of PrP<sup>C</sup> to sustain cell proliferation in colon cancer cells may relate to enhanced glucose uptake, as PrP<sup>C</sup>-dependent signalling leads to transcription of the *GLUT1* gene [123]. Overall, the contribution of PrP<sup>C</sup> to cancer cell proliferation fully fits with a gain of its physiological function in normal cells where it controls the activation of several effectors associated with cell growth [128, 129].

A second field of investigation focuses on the correlation between PrP<sup>C</sup> and chemo-resistance. High PrP<sup>C</sup> expression levels is indeed associated with increased resistance to various types of agents in glioblastoma [130], gastric [111, 121, 131, 132], breast [112, 133–135], and colon cancer [122, 136, 137]. According to several studies, the PrP<sup>C</sup>-PI3K-AKT pathway could contribute to drug resistance by enhancing the expression of MDR1 (multidrug-resistance protein 1) [138]. Very recently, PrP<sup>C</sup> was found to confer resistance to doxorubicin in breast cancer cells by directly binding and sequestering the drug via its N-terminal domain [119]. Consistently, the authors found a significant correlation between *PRNP* gene expression levels and resistance to treatment in breast cancer patients, arguing that *PRNP* monitoring could help stratify patients for adequate therapy. A third process to which PrP<sup>C</sup> takes part in cancer cells is invasion/migration. Elevated PrP<sup>C</sup> was shown to confer enhanced migratory and/or invasive properties to glioblastoma [126], gastric [118], breast [133, 139], pancreas [127], colon [140] lung [141] and melanoma [142] cell lines. In pancreatic cancer and melanoma, PrP<sup>C</sup>, which is present as pro-PrP (an isoform retaining its C-terminus instead of a GPI anchor), appears to exert its pro-migratory action by interacting with filamin A, itself connected with the actin cytoskeleton [142, 143]. In colon cancer cells, this is triggered by the binding of PrP<sup>C</sup> with its ligand STII [140]. Of note, the pro-invasive and pro-migratory role of PrP<sup>C</sup> extends to the in vivo situation in animal models. Indeed, Du and colleagues found

that among colon primary tumour cells, only those positive for PrP<sup>C</sup> were able to promote liver metastasis after injection in the caecal wall of immunocompromised mice [115]. Whether this holds true for other types of cancer remains to be investigated.

Metastatic dissemination is highly correlated with epithelial-to-mesenchymal transition (EMT), a process whereby cells lose epithelial markers and cell-cell and cell-matrix contacts, remodel their actin cytoskeleton and acquire mesenchymal hallmarks, favouring cell migration [144]. At a molecular level, EMT induction is controlled by various transcription factors, including ZEB1, ZEB2, SNAIL, SLUG and TWIST [144]. The expression of *PRNP* is highly associated with and EMT signature in colon cancer patients, and PrP<sup>C</sup> controls the expression of ZEB1 in colon cancer cells [116]. Interestingly, EMT appears to be intimately connected with CSC properties [145]. Accordingly, Du and colleagues documented that PrP<sup>C</sup>-positive primary colon cancer cells express high levels of the EMT-associated markers TWIST and N-cadherin and low levels of the epithelial marker E-cadherin and exhibit CSC properties such as expression of the CSC marker CD44 and tumour-initiating capacity [115]. In line with this, PrP<sup>C</sup> was shown to interact with CD44 in multi-resistant breast cancer cells [133]. Furthermore, in primary glioblastoma cells, PrP<sup>C</sup> silencing reduces the expression of the CSC markers SOX2 and NANOG, as well as self-renewal and tumorigenic potential [124]. Similar findings were obtained by Iglesias et al working on glioblastoma cell lines grown

**Figure 1: The physiological and pathological processes in which PrP<sup>C</sup> (PDB 2LFT) may play a role.** In addition to serving as a substrate for PrP<sup>Sc</sup> replication (model depicted in A from [149]), the protein has also been reported to act as a receptor for A $\beta$  oligomers (B, PDB 6RHY) and misfolded  $\alpha$ -synuclein (C, PDB 2N0A). Surprisingly, PrP<sup>C</sup> has also been involved in myelin homeostasis (D), immunoregulatory processes (E) and cancer, mainly at the levels of cell cycle regulation (F), drug resistance (G) and epithelial-to-mesenchymal transition (H), among others.





as neurospheres [126]. As with proliferation, the contribution of PrP<sup>C</sup> to CSC self-renewal may be envisioned as a diversion of its physiological role in normal stem cell maintenance [146]. Collectively, the involvement of PrP<sup>C</sup> in various aspects of cancer progression may be viewed as directly related to its physiological role in normal cells. From a therapeutic perspective, reducing PrP<sup>C</sup> expression through antisense oligonucleotide-based strategies [147] may prove beneficial, as documented for glioblastoma [148] or colon cancer [115]. Besides, alternative opportunities may ensue from a better knowledge of the signals upregulating PrP<sup>C</sup> expression in cancer cells.

## Conclusions

After more than three decades of intense research across numerous research laboratories around the planet there is still much to learn about the biology of PrP<sup>C</sup>. A large amount of data provides solid experimental support for the notion that the simple accumulation of PrP<sup>Sc</sup> in nerve tissues may not explain the whole spectrum of neurotoxic events occurring in prion diseases, which instead is likely to require some poorly understood subversion of PrP<sup>C</sup> function upon binding to PrP<sup>Sc</sup>. Such a role for corruption of PrP<sup>C</sup> as a mediator of prion toxicity has received unexpected support from research in other neurodegenerative disorders, showing that PrP<sup>C</sup> can bind disease-associated misfolded proteins, such as oligomers of A $\beta$  and alpha-synuclein. Research in even more distant fields of biology supports expanded and surprising roles for PrP<sup>C</sup> in several physiological and disease contexts outside the brain, such as myelin homeostasis, immunoregulatory processes and cancer (figure 1). These approaches, which might not appear directly relevant to prion biology and patho-biology, are nevertheless laying the groundwork for a more comprehensive understanding of the physiological function(s) of PrP<sup>C</sup>, and the likelihood of achieving novel insights that could elucidate some fundamental cytotoxic mechanisms potentially shared by prion disorders and several other diseases.

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## Potential competing interests

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