[Frontiers in Bioscience 13, 6491-6500, May 1, 2008]

The normal cellular prion protein and its possible role in angiogenesis

Marta Turu[1,2], Mark Slevin[3], Priya Ethirajan[3], Ana Luque[1,2], AbdulBaset Elasbali[3], Angels Font[1], John Gaffney[3], Marc Cairols[4], Pat Kumar[3], Shant Kumar[4], Jerzy Krupinski[1,2]

[1]Department of Neurology, University Hospital of Bellvitge (HUB), Fundacio IDIBELL, Barcelona, Spain, [2]Cardiovascular Research Centre, CSIC-ICCC, Hospital de la Santa Creu i Sant Pau,Barcelona, Spain, [3]School of Biology, Chemistry and Health Science, Manchester Metropolitan University, Manchester, UK, [4]Department of Vascular Surgery, University Hospital of Bellvitge (HUB), Barcelona, Spain, [4]Department of Pathology, Stopford building, University of Manchester, United Kingdom

TABLE OF CONTENTS

1. ABSTRACT

Cellular Prion Protein (PrPc) is a ubiquitous glycoprotein present on the surface of endothelial cells. Resting vascular endothelial cells show minimum expression of PrPc and can constitutively release PrPc. PrPc participes in cell survival, differentiation and angiogenesis. During development, neonatal brain endothelial cells transiently express PrPc. Our group recently reported upregulation of PrPc in microvessels from ischemic brain regions in stroke patients. Ischemia/hypoxia induces PrPc expression through the activation of extracellular signal-regulated kinase (ERK). All these data suggest that PrPc plays an important role in angiogenic responses. In addition, PrPc participates in cellular function in the central nervous system, since PrPc is also highly expressed in neurons. PrPc binds copper, suggesting a role in copper metabolism. PrPc also protects cells against oxidative stress and it seems to be involved in neuroprotection. Several studies have demonstrated that PrPc prevents cells from apoptosis and subsequent tissue damage. Moreover, PrPc plays an important role in the immune response. Here, we review the multiple functions of PrPc with a special attention to its recently reported role in angiogenesis.

2. STRUCTURE AND SYNTHESIS OF PRPC

The human cellular prion protein is a highly conserved 32-KDa protein located on chromosome 20 (1). PrPc is a membrane bound glycoprotein of 253 amino acid residues in length that is attached via its glycosyl-phosphatidylinositol (GPI) anchor to the cell surface (2, 3). It has a half-life of ~ 6 hours in the body (4) and it is generally found at the cell membrane associated with cholesterol-rich micro-domains called rafts in polarized cells (5). PrPc is also associated with detergent-resistant micro-domains (6). There are some exceptions to this plasma membrane localization such as the stomach, where PrPc was observed in secretion granules of epithelial cells (7). The caroboxyl terminus (C-terminus) is highly structured and possesses two sites for glycosylation at asparginine residues 181 and 197. Cysteine residues 179 and 214 act as sites for PrPc's disulfide bridge. This disulfide bridge connects two long α-helices (8) and it is important in structure maintenance and abnormal conformation change (9).

PrPc is present in three major subcellular sites when analyzed by immunoelectron microscopy: the Golgi apparatus, early and late endosomes, and the plasma

membrane (10, 11). PrPc is synthesized in the rough endoplasmic reticulum (ER), transits through the Golgi, and is transported to the cell surface. During synthesis of PrPc the protein is subject to a number of post-translational modifications resulting in the mature protein. Direction of the protein to the ER by its 22 amino acid N-terminal signal peptide along with removal of a 23 amino acid C-terminal signal during which a GPI anchor is added. Asn-linked oligosacharides are also attached in the ER and these saccharides are further modified in the Golgi network to become endoglycosidase H resistant. The GPI anchor, which is modified by the addition of sialic acid in the Golgi compartments, facilitates the trafficking of PrPc to the cell surface where endocytosis can occur (12). Correct transport of PrPc is essential for correct processing of PrPc for maintenance of PrPc function. Many of the elements influencing PrPc trafficking are located in the C-terminus of PrPc and include its GPI anchor and the saccharides attached to PrPc in the ER. For PrPc its GPI anchor is fundamental in transportation of PrPc to the cell membrane and for correct glycosylation (13). The functions of GPI anchored proteins are quite diverse ranging from cell adhesion to signalling (14). Recently it was reported that PrPc could be transferred between cells in a GPI anchor dependent manner a process that requires cell to cell contact (15). Lack of anchorage of PrPc also reduces glycosylation of the protein (13), which can exist as an unglycosylated, monoglycosylated and/or diglycosylated molecule.

3. TISSUE LOCALIZATION AND EXPRESSION OF PRPC

PrPc is expressed predominantly in neurons (16) and to a lesser extent in some non-neuronal tissues (17, 18). In central nervous system (CNS), PrPc is concentrated at presynaptic membranes (19) and it seems also to be associated with synaptic vesicles, suggesting a special role of PrPc in synaptic transmition (20). Loss of PrPc in PrPc null mice affects the copper content in synaptosomes, indicating that PrPc is involved in synaptic copper homeostasis (19). Moreover, synaptic transmission disruption in PrPc null mice has been reported previously (21, 22). On this basis, with regard to synaptic transmission, it may be that PrPc is involved in neuronal survival by maintaining the integrity of synaptic function. Normal human lymphocytes and lymphoid cell lines express PrPc mRNA and protein and participate in cell activation (23). PrPc was also detected in circulating leukocytes, heart and skeletal muscle, lung, intestinal tract, spleen, testis, ovary, and some other organs such as peripheral lymphoid tissues (17, 24). PrPc is present in normal adult blood. The majority of blood PrPc is found within the plasma fraction and platelets, although leucocyte subpopulations also express PrPc. Endothelial cells of both macrovascular and microvascular origin seem to express high levels of PrPc which can be constitutively released and contribute to PrPc plasma levels (25). Other study showed that PrPc was expressed on endothelial cells, released during apoptosis on membrane microparticules found in human plasma (26). In contrast, polymorphonuclear leucocytes and red blood cells express little or no PrPc (27).

4 FUNCTIONS OF PRPC

Various studies have demonstrated the existence of many PrPc ligands including metal ligands, adhesion molecules, caveolin, laminin, synapsin Ib, growth factor receptor bound protein 2 (Grb2), Stress-induced protein 1, B-cell leukemia/lymphoma 2 (Bcl-2), glycosaminoglycans, apolipoprotein B, glial fibrillary acidic protein (GFAP), chaperones (Hp60, BiP and STT1) and lipids (28). The most relevant are commented on in the following sections.

4.1 PrPc as a copper ligand

PrPc binds divalent metal ions zinc (Zn) and copper (Cu) via the N-terminal region of the protein which contains octarepeat histidine residues (29, 30). This octarepeat region binds four Cu^{2+} ions cooperatively (31) and a fifth Cu^{2+} binding site centred at residues His-96 and His-111 has also been observed (32, 33). Binding of the first copper ions induces structural organization of the PrPc, which in turn facilities binding of additional copper ions, and results in cooperative binding (34), as well as inducing structural modifications in synthetic peptides of PrPc (35, 36). The observation that PrPc binds cooperatively to copper ions within their physiological range of concentration suggest that it might play a specialized function in the metabolism of copper and other metal ions (35, 37, 38) Binding of copper and Zinc also enhances endocytosis of PrPc, suggesting that PrPc transports copper into the cell (39). Copper binding to PrPc *in vivo* was also observed using PrPc-deficient cells. This study showed a diminished copper content and lower superoxide dismutase activity in these cells, suggesting that PrPc exhibits superoxide dismutase activity (29, 40). The role of PrPc in copper metabolism is controversial. Certain studies have lead to a re-evaluation of the link between PrPc and copper metabolism (41). One study demonstrated that PrPc had no influence on copper delivery at physiological concentrations (42). PrPc might play an indirect role by linking copper metabolism with other cellular functions, similar to the recently described function of X-linked inhibitor of apoptosis (XIAP). Binding of copper to XIAP was shown to induce structural changes in the protein and favour its degradation. This in turn rendered cells more susceptible to apoptosis, thus linking copper homeostasis to the regulation of cell death (43).

Moreover, PrPc has been proposed to act in copper or zinc homeostasis in central nervous tissue (44). Indeed, both copper and zinc, at high micromolar concentrations were found to induce endocytosis of PrPc to early endosomes and Golgi compartments of cultured neuroblastoma cells (39, 44, 45). More specifically, PrPc was proposed to regulate copper level at the synapse, for instance, by sequestering the copper and zinc ions in excess (46, 47). Notably, copper and zinc are concentrated at the presynaptic terminals of glutaminergic synapses, from which they are released after depolarization (48). Therefore, PrPc localized at the presynaptic membrane could re-uptake copper in the synaptic region of the neuron and recycle it through endocytosis (47). It has been proposed that PrPc could reduce captured copper (II) ions and transfer them to copper (I)-specific intracellular copper

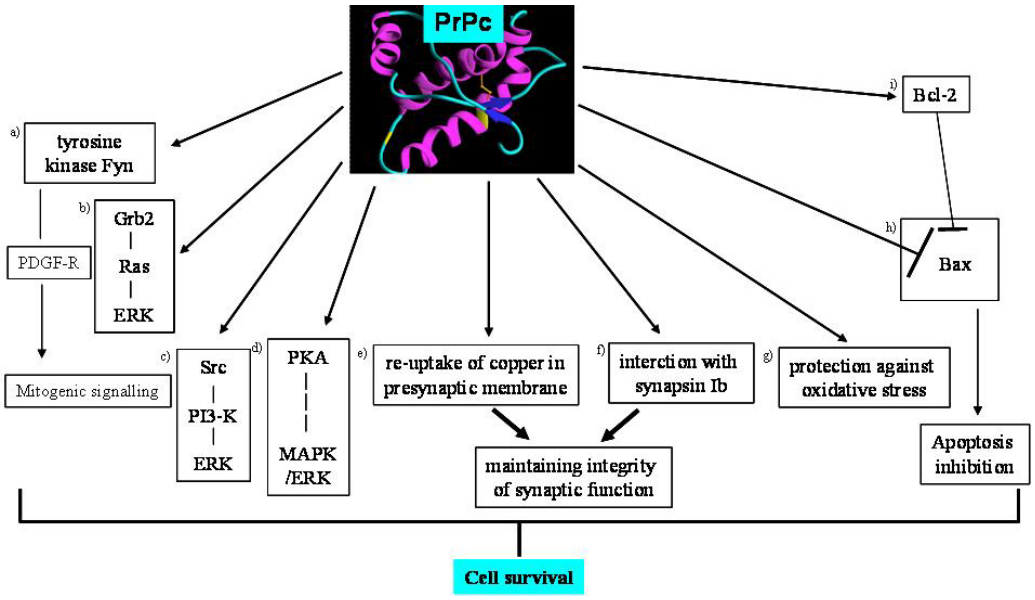

Figure 1. Cell survival and apoptotic pathways of PrPc. a) PrPc produces activation of Fyn kinase in murine differenciated neurons and it is thought to mediate cell survival and proliferation (62). b) PrPc interacts with Grb2 that plays an important role in neuronal survival (20). c, d) Several signal transduction pathways involved in survival are activated by PrPc in mouse primary cerebellar granule neurons, including PKA, Src, PI3K and MAPK/ERK kinases (66). e, f) Interactions between PrPc and synapsin Ib and re-uptake copper at the presynaptic membrane region of the neuron maintain the integrity of synaptic function and neuron survival (20,47). g) PrPc was observed to exhibit protection against oxidative stress (53, 54). h, i) PrPc protects human neurons in primary culture against Bax-mediated cell death (71). It has been demonstrated a strong anti-apoptotic effect of PrPc possibly via up-regulation of Bcl-2 and reduction in expression of Bax (74).

trafficking proteins (49). However, a recent study found no relation between PrPc and copper uptake in isolated mouse synaptosomes (50).

4.2 PrPc protects against oxidative stress

Copper and zinc are essential cofactors for many enzymatic activities, including cytochrome c oxidase, Cu/Zn superoxide dismutase 1 (SOD1), tyrosinase and numerous metalloproteinases (51, 52). Both recombinant and tissue-purified PrPc were observed to exhibit protection against oxidative stress and this was relative to the amount of bound copper (53, 54). The molecular mechanism through which PrPc protects against oxidative stress is still unclear, but it seems to be associated with the ability of PrPc to act as a copper-binding protein and therefore, may reduce copper-mediated oxidative stress (46). Alternatively, it has been proposed that PrPc might modulate the activity of SOD1, a key intracellular antioxidant enzyme (55). However, other studies failed to see variations of SOD1 activity under conditions where the levels of PrPc were varied (41). In addition PrPc-/- cell lines are more sensitive to copper toxicity produced by oxidative stress (40) and cerebellar cell cultures from PrPc null mice are more susceptible to oxidative stress than wild type ones (40, 55). Moreover, it was demonstrated that deletion of PrPc's octapeptide repeats eliminated the antioxidant function of PrPc (53). In fact, it has been demonstrated that PrPc null mice are more susceptible to acute seizures induced by different protocols (56). Considering that oxidative stress plays a role in the pathophysiology during and after cerebral injury, the impairment of brain anti-oxidant defences of PrPc null mice could play an important role in determining their lower threshold to damage (57-59). Furthermore, protein and lipid oxidation is greatly increased in skeletal muscles, heart and liver of PrPc null mice, in association with a lower catalase activity (58). The above data suggest that PrPc may have a role in protecting against oxidative stress, the protection being mediated through reduction in free Cu/Zn, via SOD and also glutathione reductase, not only in the central nervous system, but also in other organs. Additionally, Sauer *et al.* demonstrated that elevation of ROS following treatment with ATP was completely inhibited in PrPc-over-expressing cells. This is in concordance with the hypothesis that PrPc plays a role as a free radical scavenger or a molecular sensor for oxidative stress (60).

4.3 PrPc promotes cellular survival through signal transduction

PrPc could be involved in signal transduction as is the case with other GPI-anchored proteins (61). Blocking PrPc with specific antibodies induced a marked decrease in the phosphorylation level of the tyrosine kinase Fyn in murine differentiated neurons (62). Activation of Fyn in these cells must regulate certain cellular functions modulated by PrPc. As Fyn is associated with cellular proliferation and cellular survival, this study indicates that cell surface PrPc may modulate neuronal survival. Figure 1 shows currently identified survival/anti-apoptotic signalling pathways associated with PrPc.

Consistent with the role of PrPc in modulating intracellular signal transduction activation is the finding that PrPc binds strongly to synapsin Ib, a protein involved in synapse formation, as well as the regulation of neurotransmitter release and therefore which may support neuronal survival (20). Several studies have suggested that PrPc has a synaptic distribution predominantly located at the plasma membrane of neurons (19, 10, 63, 64). Synaptic transmission disruption in PrPc null mice has been reported by some but also disputed by other authors (21, 22, 65). As suggested earlier, it is possible that PrPc is involved in promotion of neuronal survival by maintaining the integrity of synaptic function. Furthermore, PrPc interacts with Grb2, an adaptor protein that mediates growth factor receptor signals and also plays an important role in neuronal survival (20). Several signal transduction pathways involved in survival were activated in mouse primary cerebellar granule neurons grown in PrPc molecule-coated tissue culture plates, including protein kinase A (PKA), Src-related tyrosine kinases, phosphatidylinositol-3-kinase/Akt (PI3K), and MAPK/ERK kinases (66). Among downstream targets, increased Bcl-2 levels and decreased Bax levels were observed, consistent with PrPc triggering survival signals. These studies clearly support a role for PrPc in cellular survival through signal transduction activation.

4.4 PrP and antiapoptotic function

Given the similarity between the PrPc octapeptide repeats and the BH2 domain of Bcl-2 family members, it was suggested that PrPc might function as a member of the Bcl-2 family of proteins playing a role in survival or cell death (67). The BH2 domain was shown to mediate the interaction between Bcl-2 and Bax protein and also to be responsible for Bcl-2 protection against Bax-mediated cell death (68). Bax is the major pro-apoptotic protein of neurons and, once activated, undergoes a conformational change, oligomerization, and translocation to the mitochondria, where it allows the release of apoptotic factors from the mitochondria (69). Therefore, it is possible that PrPc acts as member of the Bcl-2 family of proteins. Furthermore, transfection with Bcl-2 or PrPc constructs rescued PrPc null cells (70). Later, it was shown that PrPc protected human neurons in primary culture against Bax-mediated cell death (71). Co-expression of PrPc with Bax completely abolished Bax-mediated cell death (71). In addition, a PrPc-null Hpl3-4 cell line from mice, was more sensitive to apoptosis induced by serum deprivation than its parental wild type (70, 72). Further characterization showed that PrPc specifically inhibits the mitochondrion-dependent apoptotic process (72). Overproduction of PrPc also prevents Bcl-2-associated protein X (Bax)-mediated cell death, which induces caspase-3 activation and cytochrome c release from the mitochondrion (71, 73). Moreover, Liang *et al*, demonstrated a strong anti-apoptotic effect of PrPc associated with up-regulation of Bcl-2 and reduction in expression of p53/Bax in poorly differentiated gastric adenocarcinoma cells (74). The inhibitory effect of PrPc on Bax-mediated apoptosis was specific as it did not prevent apoptosis induced by other pro-apoptotic inducing agents including Bax or staurosporine (STS) (73). In contrast, there are reports showing that over-expression of PrPc sensitizes cells to apoptosis induced by pro-apoptotic agents such as STS (75, 76). Additionally, cells devoid of PrPc expression appeared to be resistant to STS-induced apoptosis, suggesting PrPc plays a synergic role in STS-mediated apoptosis. Other studies suggested that PrPc regulates neuronal cell death through a p53-dependent and caspese-3-mediated mechanism (76).

Interestingly, deletion of the octapeptide repeats eliminates PrPc neuroprotective function. However, the GPI anchor of PrPc is not required for the anti-Bax function, indicating that the presence of PrPc at the plasma membrane is not essential (71). Although cytosolic PrPc is toxic in neuroblastoma N2a cells and in mouse cerebellar granule neurons, in human neurons, cytosolic PrPc is not only non-toxic but also protects against Bax-mediated cell death, similar to the role full-length PrPc plays against Bax in human neurons (77, 71).

PrPc expression is also increased following brain ischemia. Mitsios *et al.* showed increased levels of PrPc both in the plasma and in peri-infarted brain tissue following acute stroke (Figure 2). It appears to have a protective role against cell death produced after stroke (78). Similarity, other groups demonstrated that cerebral PrPc is up-regulated early in response to focal cerebral ischemia. The extent of up-regulation seems to depend on the severity of ischemia and may therefore reflect the extent of ischemia-induce neuronal damage. Given the anti-apoptotic function of PrPc *in vitro* and *in vivo*, this up-regulation may be involved in the adaptive cellular response to ischemic brain injury (79). Furthermore, Andrew *et al.* demonstrated that PrPc plays an important role in neurogenesis and differentiation (80). This data suggests that PrPc has an important protective role against cellular stress leading to apoptotic cell death in ischemic disease.

4.5 Role of PrPc in immune response

Components of the immune system participate in chronic inflammation and also express PrPc (81). PrPc is expressed in haematopoietic stem cells (HSCs) (82). Human CD34+ HSCs express PrPc, but this is down-regulated upon granulocytes differentiation (83). In contrast, maturation of monocytes and dendritic cells (DCs) leads to PrPc up-regulation (84-86), and PrPc expression also increases during human NK cells differentiation (84). Studies in mice show a trend towards down-regulation of PrPc with B and T cell maturation, and mature T lymphocyte expression during quiescence is low (87, 88). PrPc has been detected on human T and B lymphocytes, natural killer (NK) cells, platelets, monocytes, dendritic cells and follicular dendritic cells (23, 83-85, 89-92). Its expression may be somewhat higher in peripheral blood T cells than in B lymphocytes, while CD8+ cells express slightly more PrPc than CD4+ cell (84, 90). Hence, PrPc may be more important in certain types of functionally differentiated lymphocyte that operate in particular immune environments. PrPc is up-regulated within a few hours in T cells following mitogenic activation (23, 89, 93, 94). Under inflammatory conditions activated lymphocytes express lymphotoxins triggering PrPc up-regulation. Chronic inflammation can expand the expression of PrPc (95), and

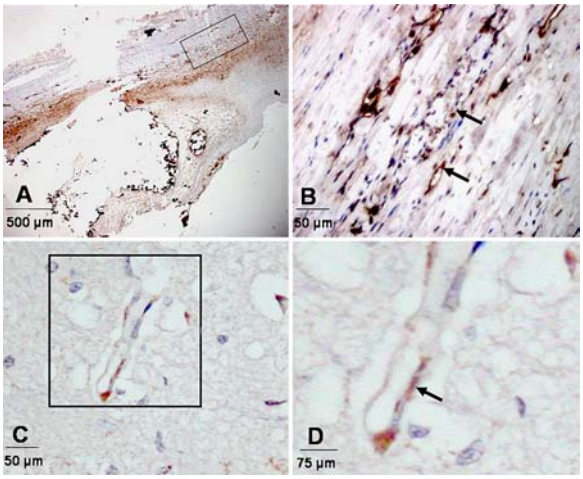

Figure 2. A, B) Representative immunostaining for PrPc in unstable carotid plaques. Positive PrPc immunostaining corresponded to a neovessel-rich and inflammatory cell-rich area. C, D) Representative immunostaining for PrPc in in microvessels from ischemic brain regions in stroke patients (78).

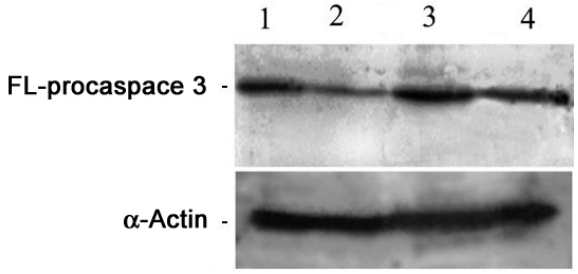

Figure 3. Western blot showing PrPc protection against apoptosis. 1) Control coronary artery endothelial cells, 2) Cells incubated with staurosporine (125 nM 12h), 3) Cells incubated with PrPc (5µM 4h), 4) Cells incubated with staurosporine and PrPc. Pre-addition of PrPc resulted in a reduction in staurosporine-induced caspase-3 cleavage (band 4).

infection with H.Pylori leads to the up-regulaton of gastric PrPc expression (96). This is possibly a consequence of pro-inflammatory cytokine expression especially Il-1 and TNF which are known to up-regulate PrPc. Up-regulation of PrPc *in vitro* was shown in various cells stimulated with IL-1 or heat shock proteins (97-101). Signalling via elements of the T cell receptors (TCR) may be required to induce changes in PrPc up-regulation in activated T cells and treatment with lipopolysaccharide (LPS) does not increase PrPc expression on B cells (94). PrPc-/- mice have been reported to have normal MHC class I and II expression, dendritic cells maturation and numbers of haematopoietic stem cells, CD4+, CD8+ and B cells (86, 88, 102, 103), suggesting that they are not grossly immuno-deficient. Other studies showed that macrophages from mice with deletion of the PrPc gene observed higher rates of phagocytosis than wild-type macrophages *in vitro* assays. They also showed that the elimination of GPI-anchored proteins from the cell surface of macrophages from wild type mice rendered these cells as efficient as macrophages derived from knockout mice. The same study showed a raised phagocytic activity in PrPc minus mice. In addition, leukocyte recruitment was altered in knockout mice, as compared with wild type. This data demonstrated that PrPc modulates phagocytosis *in vitro* and *in vivo*. PrPc was also shown to down-regulate phagocytosis in macrophages (104). Furthermore, Starke *et al* showed that expression of PrPc increased in megakaryocyte differentiation and that PrPc was located within platelet α-granules (105). Taking together, PrPc seems to play a important role in regulation of the immune response.

4.6 Role of PrPc in angiogenesis: PrPc expression in endothelial cells

PrPc is expressed and present on the surface of endothelial cells (25). Resting vascular endothelial cells have minimal or no PrPc expression *in vivo*, i.e. normal resting endothelial cells of umbilical cord and adult blood vessels (aorta, saphenous vein and our normal transplant endothelial cells) did not appear to express demonstrable quantities of PrPc (106). Endothelial cells of the blood capillaries in the intestinal wall of the digestive tract and renal capillaries, however, expressed the PrPc reporter gene (107). Other studies showed an increased expression of PrPc in endothelial cells, astrocytes and neurons in penumbra regions in a rat model of cerebral ischemia (108). Furthermore, endothelial cells can constitutively release PrPc, both as a soluble protein and bound to micro-particles, whilst vascular endothelium may be a source of plasma PrPc within the blood (25, 26, 109). PrPc has been identified as a constituent of caveolae, the flask-shaped membrane invaginations abundant in endothelial cells, which participate in signal transduction events connected to cell survival, differentiation and angiogenesis (110). Other evidence suggesting that caveolae plays a role in angiogenesis is indicated by the fact that the caveolae have been implicated in VEGF signalling machinery in endothelium (111). This work supports a central role for caveolae and possibly PrPc in modulation of angiogenic events (110). Furthermore, Satoh *et al.* observed that disruption of the PrPc gene resulted in an aberrant regulation of a battery of genes important for cell proliferation, differentiation and survival, including those located in the Ras and Rac signalling pathways associated with angiogenesis (112). During the development, neonatal brain endothelial cells transiently express PrPc transcripts suggesting a role in central nervous system angiogenesis and blood-brain barrier maturation (113, 114). PrPc expression maybe modulated by different growth factors via protein-protein interactions with normal protease sensitive PrPc (60, 115, 116).

In addition, our group recently published identification of the up-regulation of PrPc in microvessels from ischemic brain regions in stroke patients (78). Up-regulation of PrPc on endothelial cells within the areas of neovascularization might be the result of ischemic/hypoxic conditions within the damaged brain tissue after stroke (79). Several studies have demonstrated that ischemia/hypoxia induces the expression of PrPc through the activation of extracellular signal-regulated kinase

(ERK) (108, 117, 118). Shyun *et al* demonstrated that increased expression of PrPc occurs through a pathway involving ERK1/2, since enhanced expression of PrPc was inhibited by addition of an ERK1/2 inhibitor to the primary cortical culture medium (108). Interestingly, this might be the same pathway which up-regulates CD105, marker of angiogenic EC, expression during hypoxia (119) or atherogenesis (120). Shyu *et al.* proposed that HSTF-1, when phosphorylated by ERK1/2, could interact with HSE in the promoter of PrPc resulting in increased PrPc gene expression (108).

Krupinski *et al* observed an abnormal morphology of newly formed PrPc-positive microvessels demonstrating features characteristics of tumour-like microvessels which are prone to leak (78, 121). In our unpublished, recent studies we found PrPc expression in neovessels of carotid atherosclerotic plaques. PrPc expression on endothelial cells may be involved in modulation of apoptosis and in support of this, was mainly observed in advanced ulcerated complicated and non-complicated atherosclerotic plaques with notable intra-plaque haemorrhage (26). In our *in vitro* experiments apoptotic stimuli (STS) were able to up-regulate PrPc gene and protein expression in cultured bovine aortic endothelial cells and PrPc peptide also reduced caspase-3 cleavage following STS treatment, and therefore may be protective against apoptosis (Figure 3). These results are in concordance with results showed by Zhang *et al* which demonstrated down-regulation of PrPc sensitised neuro-2a cells to apoptosis induced by STS (122). Maintenance of a cellular population in atherosclerotic plaques through the pro-angiogenic and anti-apoptotic properties of PrPc could help to reduce the formation of unstable a cellular, haemorrhagic plaque regions.

5. REFERENCES

1. Puckett, C., P. Concannon, C. Casey & L. Hood: Genomic structure of the human prion protein gene. *Am J Hum Genet*, 49, 320-329 (1991)
2. Madore, N., K. L. Smith, C. H. Graham, A. Jen, K. Brady, S. Hall & R. Morris: Functionally different GPI proteins are organized in different domains on the neuronal surface. *Embo J*, 18, 6917-6926 (1999)
3. Stahl, N., M. A. Baldwin, R. Hecker, K. M. Pan, A. L. Burlingame & S. B. Prusiner: Glycosylinositol phospholipid anchors of the scrapie and cellular prion proteins contain sialic acid. *Biochemistry*, 31, 5043-5053 (1992)
4. Caughey, B., R. E. Race, D. Ernst, M. J. Buchmeier & B. Chesebro: Prion protein biosynthesis in scrapie-infected and uninfected neuroblastoma cells. *J Virol*, 63, 175-181 (1989)
5. Vey, M., S. Pilkuhn, H. Wille, R. Nixon, S. J. DeArmond, E. J. Smart, R. G. Anderson, A. Taraboulos & S. B. Prusiner: Subcellular colocalization of the cellular and scrapie prion proteins in caveolae-like membranous domains. *Proc Natl Acad Sci U S A*, 93, 14945-14949 (1996)
6. Sarnataro, D., S. Paladino, V. Campana, J. Grassi, L. Nitsch & C. Zurzolo: PrPC is sorted to the basolateral membrane of epithelial cells independently of its association with rafts. *Traffic*, 3, 810-821 (2002)
7. Fournier, J. G., F. Escaig-Haye, T. Billette de Villemeur, O. Robain, C. I. Lasmezas, J. P. Deslys, D. Dormont & P. Brown: Distribution and submicroscopic immunogold localization of cellular prion protein (PrPc) in extracerebral tissues. *Cell Tissue Res*, 292, 77-84 (1998)
8. Zahn, R., A. Liu, T. Luhrs, R. Riek, C. von Schroetter, F. Lopez Garcia, M. Billeter, L. Calzolai, G. Wider & K. Wuthrich: NMR solution structure of the human prion protein. *Proc Natl Acad Sci U S A*, 97, 145-150 (2000)
9. Lee, S. & D. Eisenberg: Seeded conversion of recombinant prion protein to a disulfide-bonded oligomer by a reduction-oxidation process. *Nat Struct Biol*, 10, 725-730 (2003)
10. Fournier, J. G., F. Escaig-Haye & V. Grigoriev: Ultrastructural localization of prion proteins: physiological and pathological implications. *Microsc Res Tech*, 50, 76-88 (2000)
11. Laine, J., M. E. Marc, M. S. Sy & H. Axelrad: Cellular and subcellular morphological localization of normal prion protein in rodent cerebellum. *Eur J Neurosci*, 14, 47-56 (2001)
12. Shyng, S. L., M. T. Huber & D. A. Harris: A prion protein cycles between the cell surface and an endocytic compartment in cultured neuroblastoma cells. *J Biol Chem*, 268, 15922-15928 (1993)
13. Walmsley, A. R., F. Zeng & N. M. Hooper: Membrane topology influences N-glycosylation of the prion protein. *Embo J*, 20, 703-712 (2001)
14. McConville, M. J. & M. A. Ferguson: The structure, biosynthesis and function of glycosylated phosphatidylinositols in the parasitic protozoa and higher eukaryotes. *Biochem J*, 294 (Pt 2), 305-324 (1993)
15. Liu, T., R. Li, T. Pan, D. Liu, R. B. Petersen, B. S. Wong, P. Gambetti & M. S. Sy: Intercellular transfer of the cellular prion protein. *J Biol Chem*, 277, 47671-47678 (2002)
16. Sales, N., K. Rodolfo, R. Hassig, B. Faucheux, L. Di Giamberardino & K. L. Moya: Cellular prion protein localization in rodent and primate brain. *Eur J Neurosci*, 10, 2464-2471 (1998)
17. Bendheim, P. E., H. R. Brown, R. D. Rudelli, L. J. Scala, N. L. Goller, G. Y. Wen, R. J. Kascsak, N. R. Cashman & D. C. Bolton: Nearly ubiquitous tissue distribution of the scrapie agent precursor protein. Neurology, 42, 149-156 (1992)
18. Fournier, J. G.: Nonneuronal cellular prion protein. *Int Rev Cytol*, 208, 121-160 (2001)
19. Herms, J., T. Tings, S. Gall, A. Madlung, A. Giese, H. Siebert, P. Schurmann, O. Windl, N. Brose & H. Kretzschmar: Evidence of presynaptic location and function of the prion protein. *J Neurosci*, 19, 8866-8875 (1999)
20. Spielhaupter, C. & H. M. Schatzl: PrPC directly interacts with proteins involved in signaling pathways. *J Biol Chem*, 276, 44604-44612 (2001)
21. Collinge, J., M. A. Whittington, K. C. Sidle, C. J. Smith, M. S. Palmer, A. R. Clarke & J. G. Jefferys: Prion protein is necessary for normal synaptic function. *Nature*, 370, 295-297 (1994)

22. Whittington, M. A., K. C. Sidle, I. Gowland, J. Meads, A. F. Hill, M. S. Palmer, J. G. Jefferys & J. Collinge: Rescue of neurophysiological phenotype seen in PrP null mice by transgene encoding human prion protein. *Nat Genet*, 9, 197-201 (1995)
23. Cashman, N. R., R. Loertscher, J. Nalbantoglu, I. Shaw, R. J. Kascsak, D. C. Bolton & P. E. Bendheim: Cellular isoform of the scrapie agent protein participates in lymphocyte activation. *Cell*, 61, 185-192 (1990)
24. Brown, K. L., D. L. Ritchie, P. A. McBride & M. E. Bruce: Detection of PrP in extraneural tissues. *Microsc Res Tech*, 50, 40-45 (2000)
25. Starke, R., O. Drummond, I. MacGregor, J. Biggerstaff, R. Gale, R. Camilleri, I. Mackie, S. Machin & P. Harrison: The expression of prion protein by endothelial cells: a source of the plasma form of prion protein?. *Br J Haematol*, 119, 863-873 (2002)
26. Simak, J., K. Holada, F. D'Agnillo, J. Janota & J. G. Vostal: Cellular prion protein is expressed on endothelial cells and is released during apoptosis on membrane microparticles found in human plasma. *Transfusion,* 42, 334-342 (2002)
27. Barclay, G. R., J. Hope, C. R. Birkett & M. L. Turner: Distribution of cell-associated prion protein in normal adult blood determined by flow cytometry. *Br J Haematol*, 107, 804-814 (1999)
28. Marc, D., R. Mercey & F. Lantier: Scavenger, transducer, RNA chaperone? What ligands of the prion protein teach us about its functions. *Cell Mol Life Sci*, 64, 815-829 (2007)
29. Brown, D. R., K. Qin, J. W. Herms, A. Madlung, J. Manson, R. Strome, P. E. Fraser, T. Kruck, A. von Bohlen, W. Schulz-Schaeffer, A. Giese, D. Westaway & H. Kretzschmar: The cellular prion protein binds copper *in vivo*. *Nature*, 390, 684-687 (1997)
30. Thompsett, A. R., S. R. Abdelraheim, M. Daniels & D. R. Brown: High affinity binding between copper and full-length prion protein identified by two different techniques. *J Biol Chem*, 280, 42750-42758 (2005)
31. Viles, J. H., F. E. Cohen, S. B. Prusiner, D. B. Goodin, P. E. Wright & H. J. Dyson: Copper binding to the prion protein: structural implications of four identical cooperative binding sites. Proc Natl Acad Sci U S A, 96, 2042-2047 (1999)
32. Jackson, G. S., I. Murray, L. L. Hosszu, N. Gibbs, J. P. Waltho, A. R. Clarke & J. Collinge: Location and properties of metal-binding sites on the human prion protein. *Proc Natl Acad Sci U S A*, 98, 8531-8535 (2001)
33. Kramer, M. L., H. D. Kratzin, B. Schmidt, A. Romer, O. Windl, S. Liemann, S. Hornemann & H. Kretzschmar: Prion protein binds copper within the physiological concentration range. *J Biol Chem*, 276, 16711-16719 (2001)
34. Garnett, A. P. & J. H. Viles: Copper binding to the octarepeats of the prion protein. Affinity, specificity, folding, and cooperativity: insights from circular dichroism. *J Biol Chem*, 278, 6795-6802 (2003)
35. Hornshaw, M. P., J. R. McDermott & J. M. Candy: Copper binding to the N-terminal tandem repeat regions of mammalian and avian prion protein. *Biochem Biophys Res Commun*, 207, 621-629 (1995)
36. Wells, M. A., C. Jelinska, L. L. Hosszu, C. J. Craven, A. R. Clarke, J. Collinge, J. P. Waltho & G. S. Jackson: Multiple forms of copper (II) co-ordination occur throughout the disordered N-terminal region of the prion protein at pH 7.4. *Biochem J*, 400, 501-510 (2006)
37. Flechsig, E., I. Hegyi, M. Enari, P. Schwarz, J. Collinge & C. Weissmann: Transmission of scrapie by steel-surface-bound prions. *Mol Med*, 7, 679-684 (2001)
38. Thackray, A. M., R. Knight, S. J. Haswell, R. Bujdoso & D. R. Brown: Metal imbalance and compromised antioxidant function are early changes in prion disease. *Biochem J*, 362, 253-258 (2002)
39. Pauly, P. C. & D. A. Harris: Copper stimulates endocytosis of the prion protein. J Biol Chem, 273, 33107-33110 (1998)
40. Brown, D. R., W. J. Schulz-Schaeffer, B. Schmidt & H. A. Kretzschmar: Prion protein-deficient cells show altered response to oxidative stress due to decreased SOD-1 activity. *Exp Neurol*, 146, 104-112 (1997)
41. Waggoner, D. J., B. Drisaldi, T. B. Bartnikas, R. L. Casareno, J. R. Prohaska, J. D. Gitlin & D. A. Harris: Brain copper content and cuproenzyme activity do not vary with prion protein expression level. *J Biol Chem*, 275, 7455-7458 (2000)
42. Rachidi, W., D. Vilette, P. Guiraud, M. Arlotto, J. Riondel, H. Laude, S. Lehmann & A. Favier: Expression of prion protein increases cellular copper binding and antioxidant enzyme activities but not copper delivery. *J Biol Chem*, 278, 9064-9072 (2003)
43. Mufti, A. R., E. Burstein, R. A. Csomos, P. C. Graf, J. C. Wilkinson, R. D. Dick, M. Challa, J. K. Son, S. B. Bratton, G. L. Su, G. J. Brewer, U. Jakob & C. S. Duckett: XIAP Is a copper binding protein deregulated in Wilson's disease and other copper toxicosis disorders. *Mol Cell*, 21, 775-785 (2006)
44. Watt N.T, a. H. N. M.: The prion protein and neuronal zinc homeostasis. Trends Bioche. Sci, 28, 406-410 (2003)
45. Brown, L. R. & D. A. Harris: Copper and zinc cause delivery of the prion protein from the plasma membrane to a subset of early endosomes and the Golgi. *J Neurochem*, 87, 353-363 (2003)
46. Vassallo, N. & J. Herms: Cellular prion protein function in copper homeostasis and redox signalling at the synapse. *J Neurochem,* 86, 538-544 (2003)
47. Kretzschmar, H. A., T. Tings, A. Madlung, A. Giese & J. Herms: Function of PrP (C) as a copper-binding protein at the synapse. *Arch Virol Suppl*, 239-249 (2000)
48. Frederickson, C. J., J. Y. Koh & A. I. Bush: The neurobiology of zinc in health and disease. *Nat Rev Neurosci*, 6, 449-462 (2005)
49. Miura, T., S. Sasaki, A. Toyama & H. Takeuchi: Copper reduction by the octapeptide repeat region of prion protein: pH dependence and implications in cellular copper uptake. *Biochemistry*, 44, 8712-8720 (2005)
50. Giese, A., M. Buchholz, J. Herms & H. A. Kretzschmar: Mouse brain synaptosomes accumulate copper-67 efficiently by two distinct processes independent of cellular prion protein. *J Mol Neurosci*, 27, 347-354 (2005)
51. Tapiero, H., D. M. Townsend & K. D. Tew: Trace elements in human physiology and pathology. *Copper. Biomed Pharmacother,* 57, 386-398 (2003)

52. Tapiero, H. & K. D. Tew: Trace elements in human physiology and pathology: zinc and metallothioneins. *Biomed Pharmacother,* 57, 399-411 (2003)
53. Brown, D. R., B. S. Wong, F. Hafiz, C. Clive, S. J. Haswell & I. M. Jones: Normal prion protein has an activity like that of superoxide dismutase. *Biochem J*, 344 Pt 1, 1-5 (1999)
54. Brown, D. R., C. Clive & S. J. Haswell: Antioxidant activity related to copper binding of native prion protein. J *Neurochem*, 76, 69-76 (2001)
55. Brown, D. R. & A. Besinger: Prion protein expression and superoxide dismutase activity. *Biochem J*, 334 (Pt 2), 423-429 (1998)
56. Walz, R., O. B. Amaral, I. C. Rockenbach, R. Roesler, I. Izquierdo, E. A. Cavalheiro, V. R. Martins & R. R. Brentani: Increased sensitivity to seizures in mice lacking cellular prion protein. *Epilepsia*, 40, 1679-1682 (1999)
57. Dal-Pizzol, F., F. Klamt, M. M. Vianna, N. Schroder, J. Quevedo, M. S. Benfato, J. C. Moreira & R. Walz: Lipid peroxidation in hippocampus early and late after status epilepticus induced by pilocarpine or kainic acid in Wistar rats. *Neurosci Lett*, 291, 179-182 (2000)
58. Klamt, F., F. Dal-Pizzol, M. J. Conte da Frota, R. Walz, M. E. Andrades, E. G. da Silva, R. R. Brentani, I. Izquierdo & J. C. Fonseca Moreira: Imbalance of antioxidant defense in mice lacking cellular prion protein. *Free Radic Biol Med*, 30, 1137-1144 (2001)
59. Pereira, G. S., R. Walz, C. D. Bonan, A. M. Battastini, I. Izquierdo, V. R. Martins, R. R. Brentani & J. J. Sarkis: Changes in cortical and hippocampal ectonucleotidase activities in mice lacking cellular prion protein. *Neurosci Lett*, 301, 72-74 (2001)
60. Sauer, H., K. Wefer, V. Vetrugno, M. Pocchiari, C. Gissel, A. Sachinidis, J. Hescheler & M. Wartenberg: Regulation of intrinsic prion protein by growth factors and TNF-alpha: the role of intracellular reactive oxygen species. *Free Radic Biol Med*, 35, 586-594 (2003)
61. Jacobson, K. & C. Dietrich: Looking at lipid rafts? *Trends Cell Biol*, 9, 87-91 (1999)
62. Mouillet-Richard, S., M. Ermonval, C. Chebassier, J. L. Laplanche, S. Lehmann, J. M. Launay & O. Kellermann: Signal transduction through prion protein. *Science*, 289, 1925-1928 (2000)
63. Haeberle, A. M., C. Ribaut-Barassin, G. Bombarde, J. Mariani, G. Hunsmann, J. Grassi & Y. Bailly: Synaptic prion protein immuno-reactivity in the rodent cerebellum. *Microsc Res Tech*, 50, 66-75 (2000)
64. Moya, K. L., N. Sales, R. Hassig, C. Creminon, J. Grassi & L. Di Giamberardino: Immunolocalization of the cellular prion protein in normal brain. *Microsc Res Tech*, 50, 58-65 (2000)
65. Lledo, P. M., P. Tremblay, S. J. DeArmond, S. B. Prusiner & R. A. Nicoll: Mice deficient for prion protein exhibit normal neuronal excitability and synaptic transmission in the hippocampus. *Proc Natl Acad Sci U S A*, 93, 2403-2407 (1996)
66. Chen, S., A. Mange, L. Dong, S. Lehmann & M. Schachner: Prion protein as trans-interacting partner for neurons is involved in neurite outgrowth and neuronal survival. *Mol Cell Neurosci*, 22, 227-233 (2003)
67. LeBlanc, A.: Unravelling the controversy of prion diseases. In: Wang E, Snyder, S editors. hanbook of the aging brain. . New York: Academic Press, 202-214 (1998)
68. Yin, X. M., Z. N. Oltvai & S. J. Korsmeyer: BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. *Nature*, 369, 321-323 (1994)
69. Martinou, J. C. & D. R. Green: Breaking the mitochondrial barrier. *Nat Rev Mol Cell Biol*, 2, 63-67 (2001)
70. Kuwahara, C., A. M. Takeuchi, T. Nishimura, K. Haraguchi, A. Kubosaki, Y. Matsumoto, K. Saeki, Y. Matsumoto, T. Yokoyama, S. Itohara & T. Onodera: Prions prevent neuronal cell-line death. *Nature*, 400, 225-226 (1999)
71. Bounhar, Y., Y. Zhang, C. G. Goodyer & A. LeBlanc: Prion protein protects human neurons against Bax-mediated apoptosis. *J Biol Chem*, 276, 39145-39149 (2001)
72. Kim, B. H., H. G. Lee, J. K. Choi, J. I. Kim, E. K. Choi, R. I. Carp & Y. S. Kim: The cellular prion protein (PrPC) prevents apoptotic neuronal cell death and mitochondrial dysfunction induced by serum deprivation. *Brain Res Mol Brain Res*, 124, 40-50 (2004)
73. Roucou, X., P. N. Giannopoulos, Y. Zhang, J. Jodoin, C. G. Goodyer & A. LeBlanc: Cellular prion protein inhibits proapoptotic Bax conformational change in human neurons and in breast carcinoma MCF-7 cells. *Cell Death Differ*, 12, 783-795 (2005)
74. Liang, J., Y. L. Pan, X. X. Ning, L. J. Sun, M. Lan, L. Hong, J. P. Du, N. Liu, C. J. Liu, T. D. Qiao & D. M. Fan: Overexpression of PrPC and its antiapoptosis function in gastric cancer. *Tumour Biol*, 27, 84-91 (2006)
75. Paitel, E., R. Fahraeus & F. Checler: Cellular prion protein sensitizes neurons to apoptotic stimuli through Mdm2-regulated and p53-dependent caspase 3-like activation. *J Biol Chem*, 278, 10061-10066 (2003)
76. Paitel, E., C. Sunyach, C. Alves da Costa, J. C. Bourdon, B. Vincent & F. Checler: Primary cultured neurons devoid of cellular prion display lower responsiveness to staurosporine through the control of p53 at both transcriptional and post-transcriptional levels. *J Biol Chem*, 279, 612-618 (2004)
77. Roucou, X., Q. Guo, Y. Zhang, C. G. Goodyer & A. C. LeBlanc: Cytosolic prion protein is not toxic and protects against Bax-mediated cell death in human primary neurons. *J Biol Chem*, 278, 40877-40881 (2003)
78. Mitsios, N., M. Saka, J. Krupinski, R. Pennucci, C. Sanfeliu, M. Miguel Turu, J. Gaffney, P. Kumar, S. Kumar, M. Sullivan & M. Slevin: Cellular prion protein is increased in the plasma and peri-infarcted brain tissue after acute stroke. *J Neurosci Res*, 85, 602-611 (2007)
79. Weise, J., O. Crome, R. Sandau, W. Schulz-Schaeffer, M. Bahr & I. Zerr: Upregulation of cellular prion protein (PrPc) after focal cerebral ischemia and influence of lesion severity. *Neurosci Lett*, 372, 146-150 (2004)
80. Steele, A. D., J. G. Emsley, P. H. Ozdinler, S. Lindquist & J. D. Macklis: Prion protein (PrPc) positively regulates neural precursor proliferation during developmental and adult mammalian neurogenesis. *Proc Natl Acad Sci U S A*, 103, 3416-3421 (2006)
81. Heikenwalder, M., N. Zeller, H. Seeger, M. Prinz, P. C. Klohn, P. Schwarz, N. H. Ruddle, C. Weissmann &

A. Aguzzi: Chronic lymphocytic inflammation specifies the organ tropism of prions. *Science*, 307, 1107-1110 (2005)
82. Isaacs, J. D., G. S. Jackson & D. M. Altmann: The role of the cellular prion protein in the immune system. *Clin Exp Immunol*, 146, 1-8 (2006)
83. Dodelet, V. C. & N. R. Cashman: Prion protein expression in human leukocyte differentiation. Blood, 91, 1556-1561 (1998)
84. During, J., giese A, Schulz-Schaeffer, W: Differential constitutive and activation-dependent expression of prion protein in human peripheral blood leucocytes. *Br J Haematol*, 108, 3733-3738 (2000)
85. Burthem, J., B. Urban, A. Pain & D. J. Roberts: The normal cellular prion protein is strongly expressed by myeloid dendritic cells. *Blood*, 98, 3733-3738 (2001)
86. Ballerini, C., P. Gourdain, V. Bachy, N. Blanchard, E. Levavasseur, S. Gregoire, P. Fontes, P. Aucouturier, C. Hivroz & C. Carnaud: Functional implication of cellular prion protein in antigen-driven interactions between T cells and dendritic cells. *J Immunol,* 176, 7254-7262 (2006)
87. Liu, T., R. Li, B. S. Wong, D. Liu, T. Pan, R. B. Petersen, P. Gambetti & M. S. Sy: Normal cellular prion protein is preferentially expressed on subpopulations of murine hemopoietic cells. *J Immunol*, 166, 3733-3742 (2001)
88. Kubosaki, A., S. Yusa, Y. Nasu, T. Nishimura, Y. Nakamura, K. Saeki, Y. Matsumoto, S. Itohara & T. Onodera: Distribution of cellular isoform of prion protein in T lymphocytes and bone marrow, analyzed by wild-type and prion protein gene-deficient mice. *Biochem Biophys Res Commun*, 282, 103-107 (2001)
89. Li, R., D. Liu, G. Zanusso, T. Liu, J. D. Fayen, J. H. Huang, R. B. Petersen, P. Gambetti & M. S. Sy: The expression and potential function of cellular prion protein in human lymphocytes. *Cell Immunol*, 207, 49-58 (2001)
90. Politopoulou, G., J. D. Seebach, M. Schmugge, H. P. Schwarz & A. Aguzzi: Age-related expression of the cellular prion protein in human peripheral blood leukocytes. *Haematologica*, 85, 580-587 (2000)
91. Antoine, N., J. Y. Cesbron, B. Coumans, O. Jolois, W. Zorzi & E. Heinen: Differential expression of cellular prion protein on human blood and tonsil lymphocytes. *Haematologica*, 85, 475-480 (2000)
92. Thielen, C., N. Antoine, F. Melot, J. Y. Cesbron, E. Heinen & R. Tsunoda: Human FDC express PrPc *in vivo* and *in vitro*. *Dev Immunol*, 8, 259-266 (2001)
93. Mabbott, N. A., K. L. Brown, J. Manson & M. E. Bruce: T-lymphocyte activation and the cellular form of the prion protein. *Immunology*, 92, 161-165 (1997)
94. Kubosaki, A., Y. Nishimura-Nasu, T. Nishimura, S. Yusa, A. Sakudo, K. Saeki, Y. Matsumoto, S. Itohara & T. Onodera: Expression of normal cellular prion protein (PrP (c)) on T lymphocytes and the effect of copper ion: Analysis by wild-type and prion protein gene-deficient mice. *Biochem Biophys Res Commun*, 307, 810-813 (2003)
95. Seeger, H., M. Heikenwalder, N. Zeller, J. Kranich, P. Schwarz, A. Gaspert, B. Seifert, G. Miele & A. Aguzzi: Coincident scrapie infection and nephritis lead to urinary prion excretion. *Science*, 310, 324-326 (2005)
96. Plonka, M., W. Bielanski, S. J. Konturek, A. Targosz, Z. Sliwowski, M. Dobrzanska, A. Kaminska, E. Sito, P. C. Konturek & T. Brzozowski: Helicobacter pylori infection and serum gastrin, ghrelin and leptin in children of Polish shepherds. *Dig Liver Dis*, 38, 91-97 (2006)
97. Clerk, A., J. G. Harrison, C. S. Long & P. H. Sugden: Pro-inflammatory cytokines stimulate mitogen-activated protein kinase subfamilies, increase phosphorylation of c-Jun and ATF2 and upregulate c-Jun protein in neonatal rat ventricular myocytes. J *Mol Cell Cardiol*, 31, 2087-2099 (1999)
98. Wang, H., L. Xu, S. Venkatachalam, J. M. Trzaskos, S. M. Friedman, G. Z. Feuerstein & X. Wang: Differential regulation of IL-1beta and TNF-alpha RNA expression by MEK1 inhibitor after focal cerebral ischemia in mice. *Biochem Biophys Res Commun*, 286, 869-874 (2001)
99. Brzozowski, T., P. C. Konturek, A. P. Moran, S. Kwiecien, R. Pajdo, S. J. Konturek, D. Drozdowicz, A. Ptak, W. Pawlik & E. G. Hahn: Enhanced resistance of gastric mucosa to damaging agents in the rat stomach adapted to Helicobacter pylori lipopolysaccharide. *Digestion*, 67, 195-208 (2003)
100. Lasmezas, C. I.: Putative functions of PrP (C). *Br Med Bull*, 66, 61-70 (2003)
101. Derrington, E. A. & J. L. Darlix: The Enigmatic Multifunctionality of the Prion Protein. *Drug News Perspect*, 15, 206-219 (2002)
102. Bueler, H., M. Fischer, Y. Lang, H. Bluethmann, H. P. Lipp, S. J. DeArmond, S. B. Prusiner, M. Aguet & C. Weissmann: Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature*, 356, 577-582 (1992)
103. Zhang, C. C., A. D. Steele, S. Lindquist & H. F. Lodish: Prion protein is expressed on long-term repopulating hematopoietic stem cells and is important for their self-renewal. *Proc Natl Acad Sci U S A*, 103, 2184-2189 (2006)
104. de Almeida, C. J., L. B. Chiarini, J. P. da Silva, E. S. PM, M. A. Martins & R. Linden: The cellular prion protein modulates phagocytosis and inflammatory response. *J Leukoc Biol*, 77, 238-246 (2005)
105. Starke, R., P. Harrison, I. Mackie, G. Wang, J. D. Erusalimsky, R. Gale, J. M. Masse, E. Cramer, A. Pizzey, J. Biggerstaff & S. Machin: The expression of prion protein (PrP (C)) in the megakaryocyte lineage. *J Thromb Haemost*, 3, 1266-1273 (2005)
106. Sivakumaran, M.: The expression of prion protein (PrPc) by endothelial cells: an *in vitro* culture-induced artefactual phenomenon? *Br J Haematol*, 121, 673-674 (2003)
107. Lemaire-Vieille, C., T. Schulze, V. Podevin-Dimster, J. Follet, Y. Bailly, F. Blanquet-Grossard, J. P. Decavel, E. Heinen & J. Y. Cesbron: Epithelial and endothelial expression of the green fluorescent protein reporter gene under the control of bovine prion protein (PrP) gene regulatory sequences in transgenic mice. *Proc Natl Acad Sci U S A*, 97, 5422-5427 (2000)
108. Shyu, W. C., S. Z. Lin, M. F. Chiang, D. C. Ding, K. W. Li, S. F. Chen, H. I. Yang & H. Li: Overexpression of PrPC by adenovirus-mediated gene targeting reduces ischemic injury in a stroke rat model. J Neurosci, 25, 8967-8977 (2005)
109. Starke, R., P. Harrison, R. Gale, I. Mackie, O. Drummond, I. MacGregor & S. Machin: Endothelial cells

express normal cellular prion protein. *Br J Haematol*, 123, 372-373 (2003)
110. Massimino, M. L., C. Griffoni, E. Spisni, M. Toni & V. Tomasi: Involvement of caveolae and caveolae-like domains in signalling, cell survival and angiogenesis. *Cell Signal*, 14, 93-98 (2002)
111. Feng, Y., V. J. Venema, R. C. Venema, N. Tsai, M. A. Behzadian & R. B. Caldwell: VEGF-induced permeability increase is mediated by caveolae. *Invest Ophthalmol Vis Sci*, 40, 157-167 (1999)
112. Satoh, J., Y. Kuroda & S. Katamine: Gene expression profile in prion protein-deficient fibroblasts in culture. *Am J Pathol*, 157, 59-68 (2000)
113. Li, A., S. Sakaguchi, K. Shigematsu, R. Atarashi, B. C. Roy, R. Nakaoke, K. Arima, N. Okimura, J. Kopacek & S. Katamine: Physiological expression of the gene for PrP-like protein, PrPLP/Dpl, by brain endothelial cells and its ectopic expression in neurons of PrP-deficient mice ataxic due to Purkinje cell degeneration. *Am J Pathol*, 157, 1447-1452 (2000)
114. Adle-Biassette, H., C. Verney, K. Peoc'h, M. C. Dauge, F. Razavi, L. Choudat, P. Gressens, H. Budka & D. Henin: Immunohistochemical expression of prion protein (PrPC) in the human forebrain during development. *J Neuropathol Exp Neurol,* 65, 698-706 (2006)
115. Kuwahara, C., A. Kubosaki, T. Nishimura, Y. Nasu, Y. Nakamura, K. Saeki, Y. Matsumoto & T. Onodera: Enhanced expression of cellular prion protein gene by insulin or nerve growth factor in immortalized mouse neuronal precursor cell lines. *Biochem Biophys Res Commun,* 268, 763-766 (2000)
116. West, D. C., C. G. Rees, L. Duchesne, S. J. Patey, C. J. Terry, J. E. Turnbull, M. Delehedde, C. W. Heegaard, F. Allain, C. Vanpouille, D. Ron & D. G. Fernig: Interactions of multiple heparin binding growth factors with neuropilin-1 and potentiation of the activity of fibroblast growth factor-2. *J Biol Chem*, 280, 13457-13464 (2005)
117. Hung, T. H., J. N. Skepper & G. J. Burton: *In vitro* ischemia-reperfusion injury in term human placenta as a model for oxidative stress in pathological pregnancies. *Am J Pathol*, 159, 1031-1043 (2001)
118. Schneider, B., V. Mutel, M. Pietri, M. Ermonval, S. Mouillet-Richard & O. Kellermann: NADPH oxidase and extracellular regulated kinases 1/2 are targets of prion protein signaling in neuronal and nonneuronal cells. *Proc Natl Acad Sci U S A,* 100, 13326-13331 (2003)
119. Zhu, Y., Y. Sun, L. Xie, K. Jin, N. Sheibani & D. A. Greenberg: Hypoxic induction of endoglin via mitogen-activated protein kinases in mouse brain microvascular endothelial cells. *Stroke*, 34, 2483-2488 (2003)
120. Piao, M. & O. Tokunaga: Significant expression of endoglin (CD105), TGFbeta-1 and TGFbeta R-2 in the atherosclerotic aorta: an immunohistological study. *J Atheroscler Thromb*, 13, 82-89 (2006)
121. Krupinski, J., P. Stroemer, M. Slevin, E. Marti, P. Kumar & F. Rubio: Three-dimensional structure and survival of newly formed blood vessels after focal cerebral ischemia. *Neuroreport*, 14, 1171-1176 (2003)
122. Zhang, Y., K. Qin, J. Wang, T. Hung & R. Y. Zhao: Dividing roles of prion protein in staurosporine-mediated apoptosis. *Biochem Biophys Res Commun,* 349, 759-768 (2006)

Abbreviations: PrPc: Cellular Prion Protein, ERK: extracellular signal-regulated kinase, GPI: glycosyl-phosphatidylinositol, ER: endoplasmic reticulum, Grb2: growth factor receptor bound protein, B-cell , Bcl-2: leukemia/lymphoma 2, GFAP: glial fibrillary acidic protein, Zn: zinc, Cu: copper, XIAP: X-linked inhibitor of apoptosis, SOD1: superoxide dismutase 1, PKA: protein kinase A, PI3K: phosphatidylinositol-3-kinase/Akt, STS: staurosporine, HSCs: haematopoietic stem cells, NK: natural killer, TCR: T cell receptors, LPS: lipopolysaccharide.

Key Words: Cellular Prion Protein, angiogenesis, Review

Send correspondence to: Jerzy Krupinski., Department of Neurology, Stroke Unit, University Hospital of Bellvitge, C/Feixa Llarga s/n, 08907 L´Hospitalet de Llobregat (Barcelona), Spain, Tel: 34932607711, Fax: 34932607882, E-mail: krupinski@csub.scs

http://www.bioscience.org/current/vol13.htm