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Significance of Prion and Prion-Like Proteins in Cancer Development, Progression and Multi-Drug Resistance

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Abstract: Prions are renowned for their role in neurodegenerative diseases in humans and animals. These are manifested as transmissible spongiform encephalopathies (TSEs) that result from the conversion of the normal glycosylphosphatidylinositol (GPI) anchored cellular prion protein (PrP^{c}) to a misfolded, aggregated and pathogenic form, prion protein scrapie (PrP^{Sc}) *via* a post-translational process followed by the accumulation of PrP^{Sc} within the central nervous system.

New research in this area has demonstrated that PrP is over-expressed in a variety of cancers including gastric, pancreatic and breast cancers, affecting the growth and invasiveness of these cancers as well as playing an important role in the acquisition of multi-drug resistant (MDR) gastric cancer. Prion-like doppel protein (Dpl), sharing 25% amino acid sequence homology to PrP and whose function remains elusive, has also been shown to exhibit a high level of expression in a number of cancers including acute myeloid leukemia's, myelodysplastic syndromes, gastric adenocarcinoma, anaplastic meningioma and astrocytomas**.** Furthermore, the tumour suppressor protein, p53, already known for its involvement in cancer development, has recently been shown to display prion-like tendencies.

This review provides an overview of prions and prion-like proteins in mammals discussing their structure, function and role in cell function and disease. Furthermore, current research progress on the role of prion/prion-like proteins in the development, progression, and drug resistance of various cancers will be summarized. Potential implications for future development of new therapeutic treatments targeting prion and prion-like proteins will be discussed.

Keywords: Epigenetic, PRND, PRNP, protein misfolding, tumour marker.

INTRODUCTION

Cancer is fundamentally a disease of abnormal and uncontrolled cell proliferation beyond natural boundaries [1]. Familial or inherited cancers, resulting from known or still unknown predisposing genes, make up only a small percentage (5-10%) of cancer incidence [2, 3]. The greater percentage of cancer is sporadic, meaning there is no apparent inherited cause. Sporadic cancers are thought to be initiated by an accumulation of oncogenic mutations at the somatic level as a result of exposure to carcinogens. Carcinogens comprise both external and internal factors such as environmental pollutants, chemicals, ionizing radiations, hormones, and infectious diseases (e.g. by viruses). Cell division allows progressive passage of a DNA mutation within one cell to new daughter cells, thus amplifying its effect when at the somatic level. Accumulation of several such mutations may then complete the oncogenic transformation of a normal cell [4].

Epigenetics is emerging as an important contributing factor in human diseases, including cancer. Epigenetic effects are broad and not restricted to alterations in gene expression (e.g. due to changes in DNA methylation) as they also encompass post-translational changes in protein function, e.g. the conversion of normal cellular proteins into prions. The prion protein (PrP), is well known for its causative role in many neurodegenerative diseases such as Creutzfeldt-Jakob disease, scrapie and bovine spongiform encephalopathy [5]. These prion diseases are caused by the conversion of normal cellular prion protein (PrP^{c}) into the pathogenic isoform Prion Protein Scrapie (PrP^{Sc}). Although the normal biological function of PrP^{c} remains elusive, its expression in non-neuronal tissues has suggested a role in multiple cellular pathways and signaling processes throughout the body [6]. It was also noted that PrP^{c} expression is up-regulated in the mucosa of patients infected with *Helicobacter pylori* while it was absent or weak in uninfected gastric mucosa [7]. More recently, *H pylori* infection has also been linked to carcinogenesis of gastric mucosa, prompting investigations into the relationship between PrP^{c} and gastric cancer [8]. Current literature now suggests PrP^{c} is involved in a number of cancers such as colorectal [6, 9], gastric [8-10], pancreatic [11], breast [12, 13] and multi-drug resistant (MDR) phenotypes of gastric [14-16] cancers. Surprisingly, these reports do not confirm the conformational state of PrP (i.e. whether PrP^{c} or PrP^{Sc}) in the cancer tissues. Determining the role of PrP and other proteins displaying

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prion-like tendencies may provide a potential new target for therapeutic treatment of cancer.

Here we discuss the role of prions and prion-like proteins in mammals describing their structure, function and role in cell function and disease. Furthermore, current research progress on the role of prion/prion-like proteins in the development, progression, and drug resistance of various cancers will be summarized and the potential implications for future diagnostic techniques and their use as a potential target for therapeutic treatment will be discussed.

PRION DISEASE

Prions, an abbreviation of proteinaceous infectious particle [6], are renowned for their role in many neurodegenerative diseases in humans and animals. In 1929, Creutzfeldt and Jakob initially described the disease as a progressive dementia associated with astrocytic gliosis, gait abnormalities and vacuolation within the brain [17], now known to be a prion disease known as Creutzfeldt-Jakob disease (CJD). Since this time, many other prion diseases in both humans and other animals have been identified including Gerstmann-Straussler-Scheinker disease (GSS), familial fatal insomnia (FFI) and kuru in humans, scrapie in sheep and bovine spongiform encephalopathy (BSE) in cattle [5, 18]. This group of prion diseases is also known as transmissible spongiform encephalopathies (TSEs) due to their ability to be transmitted to humans and animals, and the characteristic vacuolar degradation of the gray matter [19].

Over the past few decades, the molecular mechanisms of prion diseases have begun to be uncovered. It was discovered that the infectious particle that may cause scrapie, is a 27-30kDa protease-resistant protein designated as PrP 27-30 [20]. PrP was found to be encoded by a single copy mammalian gene (located on chromosome 20 in humans) [21], designated as PRNP in humans and Prnp in animals. Furthermore, it was discovered that PrP27-30 was derived from a 30-35kDa protein in scrapie-infected animals, designated as PrP^{Sc}. PrP^{Sc} was discovered to be a derivative of the normal protease sensitive form of PrP that was designated as PrP^{c} [22]. TSEs result from the conversion of PrP^{c}, to the misfolded aggregated and pathogenic form, PrP^{Sc} [23] in a post-translational process,followed by an accumulation of PrP^{Sc} within the central nervous system resulting in disease [5].

As in prion diseases, protein aggregation is a hallmark of other neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's diseases resulting from the misfolding of proteins [24, 25]. Recent experimental findings suggest that the protein aggregates in these diseases have the ability to move from the affected areas of the brain into the unaffected areas. These findings suggest that there may be a prion-like mechansim of the misfolded proteins underlying these diseases [26, 27]. This further infers that prion-like mechanisms may be present in diseases other than the already known TSEs, and are therefore a potential target for therapeutic treatment.

THE NORMAL CELLULAR PRION PROTEIN ($PRP^{C)}$

Due to its central role in the development of many neurodegenerative diseases in humans and animals, the normal PrP^{c} has been extensively studied. The structure and cellular localizations of PrP^{c} have been elucidated from various studies. Owing to these and its level of expression in various tissues and organs, many putative functions have also been proposed for PrP^{c}, although the exact function still remains in debate.

Structure, Cellular Localisation and Function of PrP^{c}

PrP^{c} is generally located on the cell membrane associated with cholesterol-rich microdomains (rafts) in cultured non-neuronal and neuronal cells [28]. It is also associated with detergent-resistant microdomains with a basolateral localization in polarized cells such as epithelial cells [29]. The immature PrP^{c} protein is 253amino acid residues long, 32-35kDa in mass and comprises of an unstructured N-terminal region and a globular C-terminal domain. The C-terminal domain consists of 3 α-helices (α-1: aa144-153, α-2: aa172-192, and α-3: aa200-225), a β-sheet comprising two antiparallel β-stands (β1: aa129-130, and β2: aa162-163) [30] and a signal sequence for the GPI anchor (aa231-253)[6] as shown in Fig. (**1A**). In humans, the PRNP gene encoding PrP^{c} protein contains 2 exons and is 20kb in length. The PRNP locus also includes two other genes, PRND and PRNT. The PRNP locus spans 55kb in the p12/p13 region of chromosome 20 [31].

In order to form a mature protein, PrP^{c} undergoes a number of posttranslational modifications, initiated by the removal of the N-terminal and C-terminal signal peptides (Fig. **1A**). The nascent chain is then imported into the endoplasmic reticulum where two N-linked glycans and the GPI anchor are attached, followed by disulfide bonding between Cys179 and Cys214 [32, 33]. The disulfide bond thus connects the C-terminal α-helices and serves to stabilize the fold of the protein [34]. The 210 amino acid PrP^{c} proprotein [32] is then targeted to the outer leaflet of the plasma membrane [32, 33].

PrP^{c} can also undergo two endoproteolytic cleavage events [35]. The normal constitutive cleavage, known as α-cleavage [36] (Fig. **1A**), occurs between residues 110 and 111 and has been demonstrated in the brain and in cultured cells. This cleavage is stimulated by agonists of the protein kinase C pathway [37] and results in the formation of a 9kDa soluble N-terminal fragment and a 17kDa C-terminal fragment that remains attached to the cell membrane *via* the GPI anchor [38-40]. The second cleavage, known as β-cleavage [36] (Fig. **1A**), is mediated by reactive oxygen species (ROS) [36, 41] and leads to the formation of a 19kDa GPI-anchored C-terminal fragment and a 7kDa N-terminal fragment [38, 40, 42].

As mentioned above, the precise normal function of PrP^{c} remains unknown [6]. Nevertheless several evidences suggest that it plays a role in the regulation of intracellular calcium and presynaptic copper concentrations, signal transduction, lymphocyte activation and has antiapoptotic and antioxidant properties [14]. There is also increasing evidence that PrP^{c} plays a role within the central nervous system (CNS), e.g. in neuronal differentiation and neuro-protection [43] .

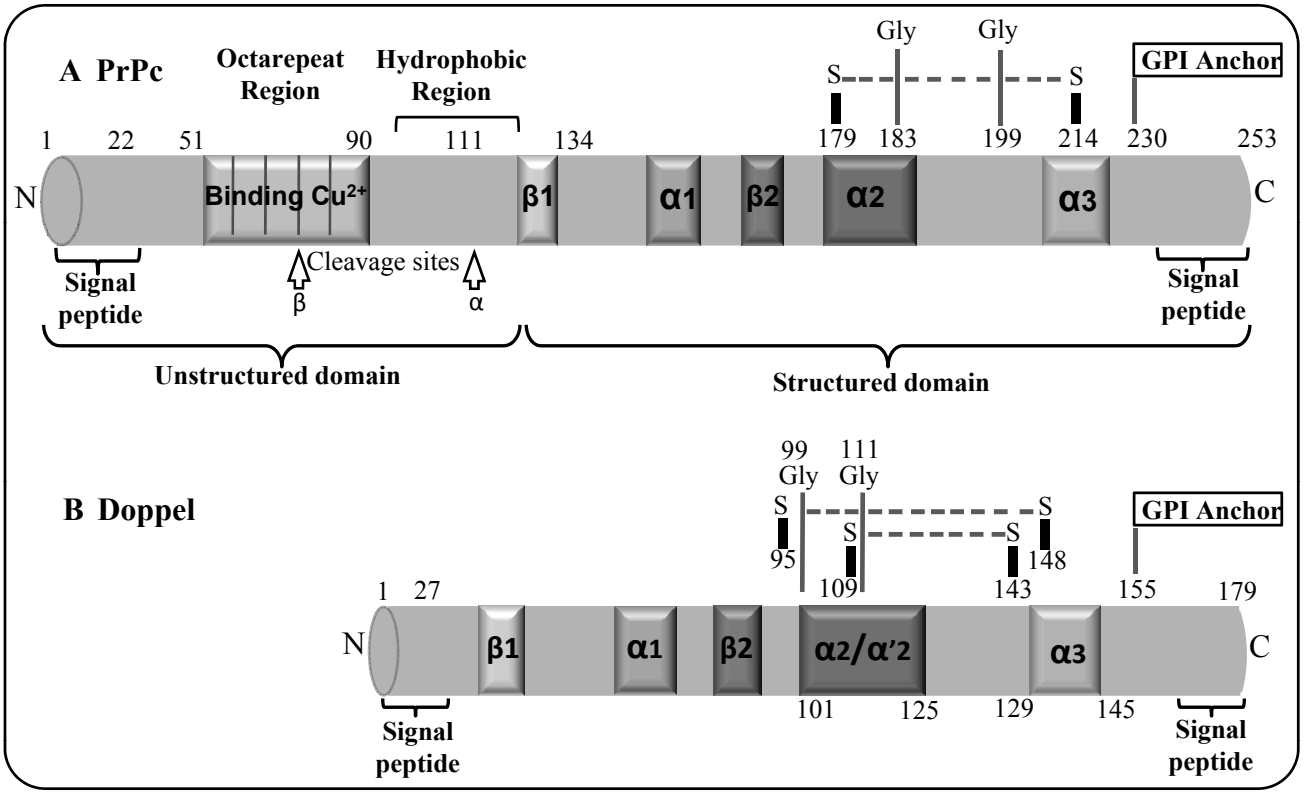

Fig. (1). Domain structure of human PrP^c, Doppel. **A**) PrP^c contains an N-terminal unstructured or 'variable' and C-terminal structured or 'core' domain. The unstructured domain consists of N-terminal signal peptide, octarepeat region, hydrophobic region and two cleavage sites labelled α and β. The structured domain of PrP^c contains three α-helices and two β-sheets along with signal peptide, GPI anchor and disulfide bond. **B**) Domain structure of Dpl is similar to the structured domain of PrP^c, however it has an additional disulfide bond and a split α2 helix [6].

The Level of Expression of PrP^c in Normal Human Tissues

Human PrP^c is expressed early in embryogenesis. In adults, the highest level of PrP^c expression is observed in neurons of the brain and spinal cord [44], specifically in those of the cerebellum, cerebral cortex, thalamus, hippocampus and medulla oblongata [31, 45, 46]. It is primarily concentrated within the glia and neuronal synapses [47] and has also been found on the surface of the neuron [48]. However, PrP^c is not only expressed in the nervous system. It is also expressed at low levels in many normal peripheral tissues including secondary lymphoid organs, and at even lower levels in the kidney and liver. PrP^c expression has also been detected in muscle cells, nonciliated lung epithelial cells, endothelial cells, immature T cells and dendritic cells [14].

HIGH LEVEL OF EXPRESSION OF PRP IN CANCER

Recent studies have shown the potential involvement of PrP (whether PrP^c or PrP^{Sc} has not been conclusively proven in any study) in a number of cancers including gastric, pancreatic, breast and prostate cancers as well as multi-drug resistant forms of gastric and breast cancer. The potential role of PrP in each cancer (discussed below) suggests PrP may be a potential target for drug treatment.

The Level of Expression of PrP^c in Gastric Cancer

Over-expression of PrP^c has been reported in a number of human gastric cancer cell lines including SCG7901 and AGS [8, 9, 14]. Du, *et al.* [14], using northern blot and western blot analysis, confirmed the expression of PrP^c in gastric carcinoma cell lines; SGC7901 and SCG7901/ADR. Through the use of immunofluorescence staining, it was found that PrP^c is clustered within the cytoplasm or plasma membrane of SGC7901 and AGS cells [9, 10]. It was also confirmed that the over-expressed PrP in these cell lines were proteinase K sensitive form PrP^c [8]. Further western blot analysis, showed that PrP is widely expressed in several other gastric cancer cell lines including MGC803 and KATOIII [9].

PrP was also found to be expressed in gastric carcinoma tissues using immunohistochemical assays [9, 14]. Du *et al.* [14] discovered that PrP^c is expressed more strongly in gastric adenocarcinoma tissues than in adjacent non-tumorous tissues and is weakly, or not expressed, in normal gastric mucosa. Liang *et al.* [9] then examined clinical pathological parameters including age, sex, tumour, node, metastasis (TNM) stage and histological differentiation. While there was no correlation between PrP^c expression level and the patient's sex or age ($p>0.05$), there was a significant correlation ($p<0.05$) with histological differentiation (47.2%

well differentiated, 74.3% moderately differentiated and 84.9% poorly differentiated) and TNM stage (~92% expressing PrP^c for TNM stage III and IV compared to 62.5% for TNM stages I and II) [9], a result similar to that of Comincini *et al*. [49] in relation to the prion-like Dopple gene (PRND) in human gliomas (discussed further below). The high expression of PrP in both gastric cancer cell lines and tissue samples suggest PrP may have a potential role in the development and progression of gastric cancer and would therefore be a potential drug target.

PrP influence on the invasive and metastatic properties of gastric cancer was assessed by Pan *et al.* [10]. While PrP was found to be highly expressed in metastatic cancer compared to non-metastatic cancer, there was no significant correlation between the primary site and the metastatic site of the same metastatic gastric cancer, suggesting that an increase in PrP expression is an early determinant of metastasis and may be useful as a prognostic factor [10]. PrP was also shown to promote adhesive, invasive and *in vivo* metastatic properties of gastric cancer cell lines SGC7901 and MKN45. The effect of PrP on the adhesive ability of gastric cancer cell lines was investigated through the use of adhesive assay, comparing the ability of the cell lines, with and without down-regulated PrP, to adhere to a solubulized basement membrane (matrigel). The ability to adhere to matrigel was decreased in the cell lines with down-regulated PrP. The effect of PrP on the invasive ability was investigated using an *in vitro* invasion assay where is was shown that invasion of cell lines SGC7901 and MKN45 was inhibited at a rate of 56.4% and 43.5% respectively when transfected with PrP siRNA. To further investigate the *in vivo* influence of PrP on metastatic ability of gastric cancer cells, tail vein metastatic assays comparing gastric cancer cells lines transfected with PrP siRNA and control cell lines. It was shown transfected cell lines led to significantly less ($p<0.05$) tumours in liver surface. The results obtained from this investigation suggested PrP has the potential to promote metastasis in gastric cancer [10] and therefore may be a potential target for therapeutic treatment to potentially help decrease or inhibit the promotion of metastasis of gastric cancer.

The mechanism(s) that underlie the biological actions of PrP was further investigated by Liang *et al.* [8, 9]. When poorly differentiated cancer cell line AGS was transfected with PrP^c cDNA, the release of reactive oxygen species (ROS) was significantly reduced and apoptosis was decreased in the transfectants. Anti-apoptosis protein Bcl-2 was also expressed at a higher level while the expression of apoptosis induced proteins p53, Bax and cytochrome c was lower. The opposite effect was observed when cells were transfected with a PrP^c siRNA (small interfering RNA), further suggesting that PrP may play an anti-apoptotic role like Bcl-2protein by interfering with apoptotic pathways in gastric cancer cells [9]. PrP was also further implicated in stimulating cell proliferation by promoting the transition from G1 to S phase in the cell cycle and by elevating the transcription of cyclin D1. Levels of cyclin D1 were found to be up regulated in cells transfected with PrP cDNA at both mRNA and protein levels as assessed by microarray and western blot analysis. Furthermore, it was demonstrated that PrP^c increases the level of phosphorylated Akt. This suggests that the PI3K/Akt signal may mediate the transactivation of cyclinD1 gene transcription induced by PrP^c [8]. Akt is a serine-threonine protein kinase involved in the regulation of a number of cellular functions such as survival, proliferation, migration and metabolism [50]. PI3K, mediated by Akt, is a signalling cascade involved in the regulation of cellular homeostasis such as glucose homeostasis and cell growth and proliferation [51]. When Akt was blocked by LY294002, an Akt-specific inhibitor, the proliferating effect of PrP^c was inhibited. This further indicates that the PI3K/Akt pathways play a role in transducing the proliferation-promoting signal of PrP^c in gastric cancer [8].

The Role of PrP in Multi-Drug Resistant Gastric Cancer

Multi-drug resistance in cancer has a significant influence on the effectiveness of chemotherapeutic treatment and therefore patient survival rates [52], making the discovery of a potential cause of such drug-resistance a high priority. Primary studies on differential gene expression profiles revealed that the PrP gene was up-regulated in adriamycin-resistant gastric carcinoma cell line (SGC7901/ADR) when compared to its adriamycin-sensitive parental cell line SGC7901 [53]. This indicated that PrP may have a role in the development of multi-drug resistant (MDR) phenotypes in gastric carcinomas and led to a focus on PrP expression levels in gastric cancer and the mechanisms of PrP action within MDR gastric carcinoma [14]. Using northern and western blot techniques, PrP was found to be highly expressed and promoting the MDR phenotype *via* up-regulation of P-gp (P-glycoprotein) expression and suppression of apoptosis [14]. P-gp is a plasma membrane glycoprotein that functions as an energy dependent multi-drug efflux pump [54]. The up-regulation of P-gp was inferred from *in vitro* drug sensitivity assays of the SGC7901/ADR cells. A greater increase was observed in drug resistance to P-gp substrates such as ADR, vincristine (VRC) and etoposide (VP-16) while only a slight increase was seen in drug resistant to non-P-gp substrates like 5-fluorouracil and cisplatin (CDDP). Verapamil, a p-gp inhibitor, partially reversed the MDR phenotype of cell lines highly expressing PrP further suggesting the up-regulation of P-gp may be an important mechanism of PrP-related MDR resistance phenotype [14]. More importantly, this study also demonstrated that the suppression of PrP expression *via* RNAi technology could partially reverse ADR resistance in SGC7901/ADR and that PrP could, in an indirect manner, expel drugs from cells [14].

Investigation into the role of the PI3K/Akt pathway in PrP upregulation in MDR gastric cancer demonstrated that the inhibition of the PI3K/Akt signalling pathway using LY2940002 (an AKT-specific inhibitor), or AKT siRNA, inhibited the PrP-induced drug resistance as well as the up-regulation of P-gp in gastric cancer cells [15]. Using *in vitro* experiments, it was inferred that PrP over-expression led to drug resistance by decreasing apoptosis in gastric cancer cells through synergistic effects with Bcl-2. Furthermore, patients with gastric cancer, shown to over-express PrP, displayed poor sensitivity to chemotherapy and decreased 2-year survival rates. On the other hand, those with negative PrP expression showed high sensitivity to chemotherapy and therefore increased 2-year survival rate [55], thus suggesting,

like previous results, that PrP may have an involvement in developing MDR in gastric cancer and is therefore a potential target for therapeutic treatment.

The octarepeat region (aa51-91) of PrP (Fig. **1A**), is known to be functionally important for its Cu^{2+}/Zn^{2+} transport and antioxidant mechanisms. This domain consists of a histidine rich nonapeptide (R1) and four octapeptide repeats (R1-R4) [56]. Owing to the importance of antioxidative mechanisms in MDR development, a recent study investigated the role of the octarepeat peptides in gastric cancer MDR and found that PrP octarepeat peptides play a role in drug tolerance and stress response in gastric cancer cells through a regulatory effect on the superoxide dismutase (SOD), and glutathionine/glutathione S-transferase (GSH/GST) families (reactive oxygen species scavengers). This was shown using an *in vitro* drug sensitivity assay where the octarepeat region of PrP was silenced using siRNA. It was shown anti-apoptotic ability of gastric cancer cells decreased when octarepeat region was silenced. Additionally, cells absent of ocatrepeat region had a diminished response to stress [52]. These results indicate that the octapeptide repeat of PrP may be an important contributing factor for the MDR phenotype of gastric cancer, and therefore a potential target for therapeutic treatment.

PrP in Pancreatic Cancer

PRNP was found to be over-expressed in pancreatic ductal adenocarcinoma (PDAC) in a microarray study by Han *et al*. [57]. Another study investigated seven human PDAC cell lines to determine whether PrP is over-expressed. While PrP is expressed in the PDAC cell lines, it exists in the form of a pro-protein (Pro-PrP) and was neither glycosylated nor GPI anchored. It was further determined that the pro-PrP binds filamin A (FLNa), which is a cytoskeletal linker protein that links cell surface receptors and integrates cell mechanics and signalling [11]. This occurs through the presence of a FLNa binding motif on the GPI peptide signal sequence [58]. Interaction of pro-PrP was shown to interfere with the normal function of FLNa, increasing aggressiveness and therefore resulting in a growth advantage for the PDAC cells. This was confirmed by the reduction in proliferation and invasiveness of PDAC with down-regulation of PrP [11]. More importantly, the expression of PrP was shown to be drastically associated with a faster disease progression independent of other factors, such as age and gender of the patients, as well as the size or differentiation stage of the tumour. This is because, like in the PDAC cell lines, PrP also promotes a growth advantage and aggressiveness in *in vivo* PDAC tumour models [11]. Thus, Pro-PrP, could serve as a marker for early detection of PDAC. Moreover, the physical interaction between pro-PrP and FLNa could serve as a target for therapeutic intervention in PDAC.

PrP in Breast Cancer

Studies comparing the tumour necrosis factor-α (TNFα)-sensitive breast cancer cell line MCF7 and a TNFα- resistant clone showed that PrP was relatively over-expressed only in the TNFα-resistant clone. It was demonstrated that PrP over-expression in MCF-7 protects the cell line from induced cell death converting TNFα-sensitive cells into TNFα-resistant cells. This occurs, in part, *via* alterations in the cytochrome c release from mitochondria and in nuclear chromatin condensation [12]. Further research showed that resistance to adriamycin and tumour necrosis factor related apoptosis inducing ligand/-TRAIL-induced apoptosis, is associated with over-expression of PrP. This was suggested in the finding that silencing of PrP in a adriamycin-resistant and TRAIL-resistant breast carcinoma cell lines with PrP siRNA by transfection was shown to sensitize the cells to TRAIL-mediated apoptosis. Furthermore, this increased sensitivity was shown not to be associated with increased recruitment of receptors and signalling molecules to the DISC (death inducing signaling complex) [59]. Li *et al.* [60] demonstrated that not only is PRNP over-expressed in MCF7/Adr cells in comparison to MCF7 cells, but at the protein level it interacts with P-glycoprotein (P-gp) playing a role in the resistance to Paclitaxel (P-gp substrate) and the anti-apoptotic activity of MCF7/Adr cells.

In another study, Roucou and colleagues [61] have shown that PrP prevents Bax-mediated cell death in MCF-7 cells by inhibiting the Bax pro-apoptotic conformational change. This mechanism appears to be functionally analogous to the interactions with the Akt pathway that affect the functions of Bcl-2 and Bax in gastric cancer by up-regulating anti-apoptotic properties of cells (i.e. Bcl-2 upregulation to increase antiapoptotic functions and Bax down-regulation to inhibit pro-apoptotic functions).

PrP in Colorectal Cancer

RT-PCR analysis conducted on surgically removed colorectal specimens found that PRNP expression was up-regulated ($p<0.001$) in colorectal carcinoma (median expression 0.1874) when compared to normal colorectal tissue (median expression 0.1266). This suggested a role for PrP in the development of colorectal cancer. The expression level of PRNP was found to be unrelated to age, gender, grade or stage of the carcinomas. However, it was found that expression levels associated with primary tumour site with higher expression in the rectum and left colon in comparison to the right colon [62].

Further examination of PrP expression in colorectal cancer was conducted using formalin-fixed paraffin-embedded colonic neoplastic tissue samples from 110 patients. This study also found that PrP (whether PrP^c or prion form was not assessed) protein expression increased in cancerous colorectal tissues in comparison to normal tissues [63], and the differential expression in these tissues was even greater than that observed in PRNP mRNA levels of the previous study [62]. This over-expression of PrP correlates to that found in gastric carcinomas [9] as discussed previously. Furthermore, PrP expression was found to correlate with recurrence of disease, where patients with high PrP expression levels relapsed earlier than those with low PrP expression levels [63]. Overall, the investigation of PrP expression in colorectal cancer further provided evidence that PrP has potential as a new target for therapeutic and prognostic techniques. In fact, an *in vitro* study demonstrated that PrP antibodies could be an effective anticancer therapy. Different antibodies of PrP were shown to have varying

degrees of anti-proliferative activity with an increased efficiency in combination chemotherapy [64]. Another recent study has identified that the glycosylation state of PrP is critical for its anti-apoptotic functions in colon cancer cells and therefore could be a potential therapeutic target [65]. However, further studies are required to evaluate the state of PrP (whether PrP or prion-form) and its role in cancer development.

PrP in Prostate Cancer

PrP expression has been detected in the androgen-independent prostate cancer cell line Du-145. A role for PrP in the oxidative defence stress is also evident from the correlation between increased PrP expression level and an intracellular redox state in the prostate tumour spheroids [66]. This suggests that PrP potentially acts as a sensor molecule and/or free radical scavenger for oxidative stress in tumour cells. *In vitro* studies of these tumour spheroids, show that the level of ROS correlated with an increase of PrP, Cu/Zn SOD-1 and catalase in small spheroids relative to the larger spheroids. This suggests that not only does PrP expression level correlate with redox state, but also with tumour size [66]. However, further research is required to confirm this role of PrP.

THE PRION DOPPEL PROTEIN

Structure and Function

The doppel protein (Dpl), encoded by the PRND gene in human and Prnd in mouse, is a glycoprotein attached to the cell surface *via* a GPI anchor [67]. In humans, PRND has been shown to be expressed highly in the testis and in lower levels in other peripheral organs and brain [31, 68, 69]. PRND is believed to have arisen from the duplication of a single ancestral gene [70] as it shares 24% amino acid sequence similarity to the C-terminal of PrP^c encoded by the PRNP that lies 27kb downstream. While structurally similar to PrP, it is not yet known if Dpl has the ability to form a prion like PrP.

Despite the structural similarities, there are a few differences between Dpl and PrP (Fig. **1B**). For example, Dpl has an N-terminal octarepeat region like PrP but it also contains an extra disulfide bond compared to PrP^c; the first between Cys 109 and Cys 143 analogous to that in PrP and a second disulfide bond between Cys95 and Cys148. This second disulfide bond not only helps to stabilize the Dpl protein, but helps prevent conformational change [67]. Other differences include parallelity between the β-sheet plane and helix B and C axes in Dpl (compared to the perpendicularity in PrP) [71] and the presence of a kinked helix B in Dpl (which results in a separation into two regions; B and B').

Similar to PrP, the N-terminal signal sequence is removed upon entry into the secretory pathway and its C-terminal sequence is replaced with a GPI anchor (Fig. **1B**). It is further modified with the addition of N-linked sugars and O-linked carbohydrate moieties at Thr43 [67, 72].

While the function of the Dpl protein remains unresolved, it is believed that it may have functions in neurons antagonistic to PrP and the deregulation of the balance between them may result in neuropathology. Prnd-null mice have been shown to survive until adulthood, with no obvious phenotype, suggesting that the Dpl protein may not play an essential role in embryogenesis and postnatal development [73].

ROLE OF DOPPEL PRION PROTEIN IN CANCER

Since the discovery of the PRND gene which encodes for the Dpl protein, expression of these at both gene and protein levels have been assessed in a number of cancers. These include astrocytomas, gastric adenocarcinomas, anaplastic meningiomas [49], actuate myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) [74]. While the mechanism of involvement of PRND in these cancers is yet to be elucidated, the results obtained from the various studies indicate that PRND could be a possible tumour marker, or a potential new target for therapeutic treatment[49].

Astrocytomas, Gastric Adenocarcinoma and Anaplastic Meningioma

Comincini and colleagues [49] studied the expression of PRND and Dpl in various histopathological grades of primary and secondary astrocytomas obtained from patients along with cultured astrocytoma cell lines. This research discovered a high level of PRND expression as well as a high expression of the corresponding Dpl protein in glioblastoma multiforme (GBM). This expression appeared to be related to the malignancy of the GBM. Immuno-histochemical studies on GBM suggested that Dpl is produced by a variety of cells including neoplastic cells, endothelial cells and lymphocytes. Comincini and colleagues [49] further investigated the expression of PRND in 6 gastric adenocarcinoma samples and 6 anaplastic meningioma specimens. High levels of PRND expression were also found in these specimens. Further examination into the role of Dpl in progression and development of astrocytomas, gastric adenocarcinomas and anaplastic meningiomas is necessary, however, these studies suggest that Dpl may be a candidate target for diagnostic and therapeutic strategies.

Acute Myeloid Leukaemia and Myelodysplastic Syndrome

Acute myeloid leukaemia (AML) is a group of hematopoietic stem cell disorders resulting in the accumulation of non-functional cells known as myeloblasts [75]. The cause of this group of disorders remains unknown [75]; although it is thought to results from the mutation of one or more genes controlling blood cell development [76].

Travaglino and colleagues [74], discovered that while Dpl protein was barely detectable or not expressed in bone marrow from healthy subjects, Dpl was expressed in the bone marrow samples from patients with AML and myelodysplastic syndrome (MDS). Furthermore, Dpl expression in normal bone marrow specimens was restricted to $CD34^+$ stem cells and down-regulated in stem cell differentiation. Travaglino and colleagues [74] further found that Dpl expression localized on the cell surface or in the cytoplasm of the cells that look morphologically similar to blast cells (confirmed

by the correlation between the percentage of Dpl positive cells and the percentage of bone marrow blast cells). Bone marrow cells from advanced MDS were found to have higher Dpl protein reactivity in comparison to early forms of MDS which may be explained by the localization of Dpl in blast cells [74]. The cytoplasmic localization of Dpl in some cases is hypothesized to be caused by either inadequate surface anchoring as a result of glycosylphosphatidylinositol deficiency (frequently observed in MDS) or by abnormal Dpl cellular trafficking resulting from structural modifications of Dpl. Dpl patterns obtained from western blot reveals a band 29-35kDa in size in normal human testis [72] however, bands of 55-65kDa were obtained in MDS, possibly indicating differing glycosylated isoforms [74].

Even though Dpl may be highly expressed in AML and MDS, its role in the pathophysiology of the disease and the mechanisms responsible for the high protein expression remain elusive. Over-expression of the Dpl protein may be due to changes in gene methylation patterns and thereby transcriptional activity patters observed in tumours [77]. High Dpl expression may also be explained by the immaturity of the leukemic and dysplastic cells since most normal $CD34^+$ cells express Dpl. Overall, the differences in expression of the Dpl protein in AML cells and MDS cells in comparison to healthy cells has indicated that Dpl may be a possible leukaemia-associated antigen therefore making it a target for diagnostic and/or therapeutic strategies [74].

P53: A POTENTIAL PRION-LIKE PROTEIN

Recently, p53 the tumour suppressor protein p53 (a transcriptions factor [78] involved in the regulation of the cell cycle [79]) has been shown to display prion-like tendencies in cancer cells. Approximately 50% of tumours harbour mutation in their p53 proteins [79] with the remaining percentage possessing a faulty component in either the post translational modification pathway of p53 or the annulment of the p53 signalling pathway [80]. Recently, it has been shown that cancer may be considered as an aggregation-associated disease through studies of mutant p53. It was shown that oncogenic p53 has an increased aggregation propensity achieved by exposure of an aggregation-nucleation sequence by structural destabilization of the DNA binding domain. Exposure results in the coaggregation of mutant p53 with wild-type p53 into cellular inclusion, eliminating wild-type p53 activity. Mutant p53 was also demonstrated to co-aggregate with p63 and p73, family members of p53. Furthermore, aggregated p53 was shown to increase the expression of number of heat-shock proteins such as Hsp70, an antiapoptotic agent [79]. Furthering this research, a recent study set out to determine is wild-type p53 and hot-spot mutant R248Q have the ability to aggregate as amyloids under physiological conditions and if the mutant p53 could seed aggregates of wild type p53. They found that r248Q had a greater tendency to aggregate that wild-type p53. Furthermore, they found that full length p53 aggregated into amyloid-like species in a similar pattern to that of the p53 core domain [81]. With the known knowledge of p53 involvement in cancer development and its new found prion-like capabilities, p53 may be a potential target for therapeutic treatment.

CONCLUDING REMARKS

The theory of prion involvement in cancer development and progression suggests that genetic inheritance is only one mechanism that underlies cancer susceptibility and there may also be cytoplasmic inheritance of cancer-causing proteins. This highlights that these proteins may be a new potential target for therapeutic treatment of cancer.

The over-expression of PrP and Dpl in various cancers has been established, with evidence supporting their involvement in a number of cellular processes such as apoptosis and kinase signalling, whose dysfunction may possibly promote excessive proliferation and drug-resistance phenotypes. With new evidence of other proteins, such as p53, exhibiting prion –like capabilities, as well as evidence of prion-like protein aggregates in other disease types such as neurodegenerative diseases, further suggests that prion or prion-like proteins may also play a role in the cancer development and progression and are therefore a potential target for future therapeutic treatment. Determining the state of PrP, Dpl and p53 (whether these proteins exist in the prion or normal form) in cancer cells and their role in apoptotic pathways is an essential next stage of understanding if prion/prion-like proteins play a role in cancer development.

Overall, defining the role of PrP, Dpl, and other prion/ prion-susceptible proteins in cancer development and progression, will lead to their assessment as potential new targets for cancer prognostic, preventative and especially therapeutic treatment. For example, it might be possible to determine whether inhibition of PrP, Dpl or other prion-like proteins through drug targeting can be combined with current methods such as chemotherapy, surgery and/or radiotherapy, to improve patient survival rates.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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ABBREVIATIONS

AML	=	Acute myeloid leukaemia
ADR	=	adriamycin resistance
BSE	=	bovine spongiform encephalopathy
CDDP	=	Cisplatin
Cdk5	=	cyclin-dependent kinase 5
CNS	=	central nervous system
DISC	=	death inducing signalling complex
DNA	=	deoxyribonucleic acid
Dpl	=	Dopple prion protein

ERK	=	Extracellular-signal-regulated kinase
FFI	=	familial fatal insomnia
FLNa	=	Filimin A
GABAa	=	gamma-aminobutyric acid type A
GBM	=	glioblastoma multiforme
GPI	=	Glycosylphosphatidylinositol
GSS	=	Gerstmann-Straussler-Scheinker disease
MAPK	=	Mitogen-activated protein kinase
MDR	=	multiple drug resistance
MDS	=	myelodysplastic syndrome
MMP11	=	matrix metalloproteinase 11
NCAM	=	neural cell adhesion molecule
NMDAR	=	N-methyl-D-aspartate receptor
PDAC	=	Pancreatic ductal adenocarcinoma
P-gp	=	P-glycoprotein
PrP	=	Proteinacious infectious particle
PrP^{c}	=	Cellular prion protein
PrP^{Sc}	=	Prion protein scrapie
RNA	=	ribonucleic acid
siRNA	=	small interfering RNA
TNF	=	Tumour necrosis factor- α
TNM	=	Tumour, node, metastasis
TRAIL	=	Tumour necrosis factor related apoptosis inducing ligand
VP-16	=	etoposide
VRC	=	vincristine

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