Functions of MAPR (membrane-associated progesterone receptor) family members as heme/steroid-binding proteins

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ABSTRACT

Progesterone receptor membrane component 1 (PGRMC1), PGRMC2, neudesin, and neuferricin all contain a cytochrome b5-like heme/steroid-binding domain and belong to the membrane-associated progesterone receptor (MAPR) family. Their amino acid sequences are well conserved among vertebrates, from humans to zebrafish. MAPR family genes are abundantly expressed in the central nervous system and exhibit neurotrophic effects in neural cells. During lipid metabolism, PGRMC1 regulates cholesterol synthesis, and neudesin plays a role in adipogenesis. Their bioactivities are dependent on the binding of heme to their cytochrome b5-like heme/steroid-binding domains. Conversely, it has been reported that the binding of steroids to MAPR family proteins induces biological responses that are unrelated to the nuclear steroid receptors. The interaction between PGRMC1 and progesterone promotes cell survival and damage resistance by progesterone. Moreover, MAPR family proteins exhibit a unique expression pattern in breast cancer, indicating the possibility of using MAPR family members as drug target in breast cancer. In this review, we summarize the identification, structure, and bioactivity of members of the MAPR family, and present an essential overview of the current understanding of their physiological roles.

Key words: MAPR, heme-binding protein, PGRMC1, PGRMC2, neudesin, neuferricin

Running title: Functions of MAPR (membrane-associated progesterone receptor) family members

INTRODUCTION

We recently identified two novel extracellular heme-binding proteins and named them neudesin and neuferricin [1, 2]. Both belong to the membrane-associated progesterone receptor (MAPR) family, a subfamily of the cytochrome b5 (cyt-b5) family, which consists of heme-binding proteins with cyt-b5-like heme/steroid-binding domains [3]. Progesterone receptor membrane component 1 (PGRMC1) and the closely related PGRMC2 are also as members of the MAPR family in mammals [4, 5]. Members of the MAPR family have a highly homologous primary structure (Table 1), but have distinct localization patterns, that is, neudesin and neuferricin are both secreted proteins, while PGRMC1 and PGRMC2 are single transmembrane proteins. All of them possess a heme-binding domain, and their bioactivities are dependent on the binding of heme to their cyt-b5-like heme/steroid-binding domains. Several heme-binding proteins have been isolated and characterized recently [6-9]. Heme is a key component of many biochemical reactions. Heme serves as a prosthetic group in the heme-binding proteins, such as the hemoglobins, cytochromes, and guanylate cyclases. It has important transportation, catalytic, electron transfer, and signaling functions. Heme-binding proteins are also involved in many important cellular functions. For example, hemoglobin and myoglobin are among the most abundant heme-binding proteins associated with oxygen transportation [6]. while the cytochromes are involved in electron transport and steroidogenesis [7], and the guanylate cyclases catalyze the conversion of GTP to cGMP [8]. In addition, it has been reported that the binding of heme to DGCR8, another heme-binding protein, is related to microRNA processing [9]. Thus, heme-binding proteins may play important roles in biological functions, and some unidentified heme-binding proteins that have novel functions may exist. Here, we provide an overview of the structure and function of this unique heme-binding protein family.

1. PGRMC1 (sigma-2 receptor, Hpr6.6, 25-Dx)

1.1. Structure and binding studies

PGRMC1 is a member of a multi-protein progesterone-binding complex that co-purifies with progesterone-binding proteins in the liver and ovary [10, 11]. PGRMC1 is a 26-28 kDa vertebrate protein with a single transmembrane domain. The protein has been identified from porcine liver firstly as putative membrane receptor of progesterone [10]. Subsequently, homologous proteins were cloned in rats (25-Dx), mice (PGRMC1) and humans (Hpr.6) [12]. However, PGRMC1 does not bind directly to progesterone [13] and shares no homology with the nuclear or other membrane-associated steroid receptors [3]. The only currently known biochemical function of PGRMC1 is that it binds to heme [14, 15]. It also shares key structural motifs with cyt-b5 [3]; the 134-amino acid human cyt-b5 molecule posses a heme-binding domain in which His44 and His68 act as the ligands for the heme iron [16]. The 195-amino acid human PGRMC1 possesses a heme-binding domain in which Tyr107 and Tyr113 act as the expected binding sites for the heme iron [13] (Fig. 1). On the other hand, PGRMC1 complex binds progesterone with a similar affinity as other steroids such as corticosterone, testosterone and cortisol [10], which suggests limited hormone-binding specificity. Curiously, progesterone binding to PGRMC1 complex can be competed with haloperidol, an anti-psychotic drug, with a Ki of 20 nM [10]. Progesterone binding is also inhibited by fluphenazine, carbetapentane citrate and R(-)-N-(3-phenyl-1-propyl)-1-phenyl-2 aminopropane HCl (PPAP HCl) [10]. These suggests that the PGRMC1 complex is a mixed-function steroid/drug-binding complex (S/D-BP). In addition, PGRMC1 contains phosphorylation sites at serine-56, tyrosine-138, tyrosine-179, and serine-180 [17] (Fig. 1). Recently, PGRMC1 was identified as the sigma-2 receptor, a putative type of opioid receptor; sigma-2 receptors are reported to be induced in cancers [18, 19].

1.2. Expression

PGRMC1 is highly expressed in the liver and kidney in humans, with lower expression observed in the brain, lung, heart, skeletal muscle, and pancreas [12]. In rodents, PGRMC1 is present in the liver, lung, kidney and brain [20, 21]. PGRMC1 is overexpressed in breast tumors and in cancer cell lines from the colon, thyroid, ovary, lung, and cervix [22]. In ovarian cancer, PGRMC1 expression has been found to increase in advanced-stage tumors, where it is expressed homogeneously throughout the tumor [23]. Microarray analyses have identified PGRMC1 expression in colon, lung and breast tumors [24-26]. The non-genotoxic carcinogen 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces PGRMC1 expression in the rat liver [21], but the induction is found only in males [27]. PGRMC1 is expressed in the ovary and corpus luteum, where expression is induced by progesterone [28] and during pregnancy [29], respectively. PGRMC1 is expressed in the various areas of the brain that are involved in CSF production (choroid plexus) [30], osmoregulation (hypothalamus, circumventricular organs, meninges) [30], and female reproductive behavior lordosis (ventromedial hypothalamus) [20]. PGRMC1 is expressed at a basal level in the rat cerebellum at birth; the mRNA and protein levels of PGRMC1 increased neonatally and decrease thereafter without a significant gender difference [31]. PGRMC1 is expressed primarily in Purkinje and external granule cells in the cerebellum of neonate rats, while it is expressed in Purkinje cells only in the adult cerebellum [31]. Cerebellar Purkinje cells are the sites of de novo synthesis of progesterone from cholesterol, where the steroid may promote dendritic growth, synaptogenesis, and spinogenesis.

1.3. Signal transduction

PGRMC1 binds and activates the P450 proteins [13, 32, 33], which are involved in drug, hormone and lipid metabolism. It also interacts with insulin-induced gene-1 (Insig-1) [34], which regulates

synthesis of cholesterol [35]. In cancer cells, PGRMC1 promotes cell death after oxidative damage, possibly related to Akt and IκB degradation but does not itself appear to act directly on Akt and IκB molecules [36]. On the other hand, progesterone inhibits apoptosis in immortalized granulosa cells, which requires PGRMC1 and its binding partner plasminogen activator inhibitor RNA-binding protein-1 (PAIR-BP1) [11]. The component of PGRMC1 complex that binds to progesterone remains unknown because PGRMC1 and PAIR-BP1 do not directly interact with progesterone.

1.4. Physiological functions

The physiological roles of PGRMC1 have been reported so far in steroid synthesis and metabolism [37], cholesterol regulation [32, 38-40], axonal guidance [41], endocytosis, and alteration of reproductive behaviors [20]. It also acts together with progesterone as a component of a progesterone receptor, and plays roles in intracellular signal transduction and/or membrane trafficking as an adapter protein [42]. PGRMC1 is induced by progesterone after traumatic brain injury, effecting neuroprotective activities in a number of model systems of brain lesions [43, 44]. Additionally, pseudopregnant rats display higher PGRMC1 expression [30], which also corresponds with neuroprotection. However, it is unclear whether PGRMC1 regulates neuroprotection directly. Very recently, progesterone-PGRMC1 signaling has been reported to directly and rapidly suppress GnRH neuronal activity via inhibition of intracellular calcium oscillation in GnRH neuron [45]. The PGRMC1 yeast homologue, damage associated protein 1 (Dap1), binds heme [14, 38] through a penta-coordinate mechanism [14, 46]. Yeast cells lacking the DAP1 gene are sensitive to DNA damage [39], and heme binding is necessary for damage resistance [38]. Dap1 is also necessary for a crucial step in cholesterol synthesis, where the P450 protein Erg11/Cyp51 removes a methyl group from lanosterol [32, 38-40]. Erg11/Cyp51 is the target of the azole antifungal drugs. Accordingly, yeast cells without the DAP1 gene are highly sensitive to antifungal drugs [32, 38, 39]. Dap1 also

regulates the metabolism of iron in yeast [40]. In yeast and humans, PGRMC1/DAP1 binds directly to P450 proteins, including CYP51A1, CYP3A4, CYP7A1 and CYP21A2 [32]. PGRMC1 also activates Cyp21 when the two proteins are co-expressed in COS-7 cells [13, 33], indicating that PGRMC1 promotes progesterone turnover. Just as Dap1 is required for the action of Erg11 in the synthesis of ergosterol in yeast, PGRMC1 regulates the Cyp51-catalyzed demethylation step in human cholesterol synthesis [32]. Therefore, PGRMC1 and its homologues bind and regulate P450 proteins, and have been likened to "a helping hand for P450 proteins" [47]. The yeast PGRMC1 homologue is required for resistance to damage [39]. PGRMC1 also promotes survival in human cancer cells after chemotherapy treatment [11, 15]. Conversely, it promotes cell death in cancer cells after oxidative damage [48]. Moreover, a recent study has shown that PGRMC1, as well as its homologue neudesin, is secreted in the plasma and to promotes proliferation of lung cancer cells [49].

1.5. Disease

PGRMC1 is up-regulated in many types of cancer. PGRMC1 is part of a protein signature that predicts hypoxia in breast cancer [25], which is reminiscent of its induction during hypoxia in *S. pombe* [32]. In contrast, in ovarian cancer, PGRMC1 is inactivated by hypermethylation of the PGRMC1 promoter [50]. PGRMC1 is also implicated as a biomarker in other steps during cancer progression. Nie et al. identified a carcinogenicity signature in rats that included PGRMC1 and five genes in response to 52 known carcinogenic compounds [51]. Hokaiwado et al. performed a similar analysis and identified PGRMC1 [52]. PGRMC1 was originally cloned as 25-Dx in the rat liver because of TCDD-induced modification of gene expression [21]. TCDD also induces P450 proteins such as Cyp1A1 [53]. It is reported that P450 proteins like Cyp1A1 trigger tumor formation, a possible mechanism of which may be P450-mediated oxidative damage [54]. It is noteworthy that

human PGRMC1/25-Dx promotes cell death in cancer cells after oxidative damage [48]. The effect of TCDD on PGRMC1/25-Dx expression is gender-specific, so that the expression is induced in male rats but repressed in females [27]. TCDD induces liver tumors in females but not in males [55], which suggests that the gender-specific expression patterns may contribute to the hepatocarcinogenicity of TCDD. PGRMC1/25-Dx expression is repressed by progesterone and estrogen in murine neurons [20]. It is possible that the steroids mediate the TCDD-induced reduction of PGRMC1/25-Dx expression in female rat livers.

Thus, as summarized in Table 2, the progesterone/heme-binding protein PGRMC1 is widely expressed in several tissues including the central nervous system and tumor lesions and cancer cells, and has a number of functions such as neuroprotection and cell survival.

2. PGRMC2 (DG6)

PGRMC2 was first cloned by Gerdes et al. using the sequence of a porcine progesterone receptor as a probe, and designated DG6 [12]. Human PGRMC2 protein comprises 247 amino acids, with the transmembrane region and the cyt-b5 domain at its N- and C-terminus, respectively (Fig. 1). The cyt-b5 domain of PGRMC2 has 67% identity and 82% similarity to that of human PGRMC1. Northern blot analysis revealed PGRMC2 expression in all tissues examined, with highest expression in the placenta. Detailed analyses of the function of PGRMC2 have not been reported, but several studies suggest a possibility that PGRMC2, as well as PGRMC1, are involved in physiologically important activities. Recent reports show that PGRMC2 inhibits migration of SKOV-3 ovarian cancer cell, but has no effect on cell survival [56]. Moreover, Liu et al. reported that PGRMC1 and PGRMC2 were expressed in neural precursor cells and regulate cell proliferation through ERK activation [57]. In this report, analyses of PGRMC1 were performed, but not of

PGRMC2, other than the expression profiles. Additionally, PGRMC2 was identified as one of the genes associated with ovarian reserve status in young women [58]. In this report, expression levels of PGRMC2 in membrana granulosa cells were validated in a group comprising normal and diminished ovarian reserve patients. PGRMC2 is also reported to be involved in advanced endometriosis [59]. The expression level of PGRMC2 was revealed to be markedly increased during the secretary phases of the endometrium, and PGRMC2 mRNA expression was dramatically decreased in endometriosis, exhibiting an alteration of intracellular staining patterns for the PGRMC2 protein when compared with disease-free animals.

As shown in Table 3, PGRMC2, which shows progesterone-binding and putative heme-binding properties, is widely expressed in several tissues including the placenta, and some cell types, including neural cells and cancer cells, and inhibits cell migration. However, Further analyses are needed to elucidate the detailed biological functions of PGRMC2.

3. Neudesin (NENF)

3.1. Structure

We identified neudesin as a novel secreted protein by analysis of the mouse embryonic cDNA database using a computer program for the prediction of the protein localization site in cells (PSORT) [1]. Mouse neudesin cDNA encodes a secreted 171-amino acid protein with a putative signal sequence at its N-terminus. The amino acid sequence of human neudesin (172 amino acids) is highly similar (~91% identity) to that of mouse neudesin. Zebrafish neudesin cDNA encodes 158 amino acids. Zebrafish neudesin protein is significantly similar (~60% identity) to the mouse neudesin protein. As opposed to vertebrates, no neudesin gene is found in invertebrate genomes including *C. elegans*, *D. melanogaster*, and *C. intestinalis*.

Mouse and human neudesins contain a heme/steroid-binding domain similar to those of PGRMC1 and cyt-b5, and belong to the MAPR family, a subfamily of the cyt-b5 family, along with PGRMC1 [60]. The 45-143-amino acid sequence in mouse neudesin is a predicted heme-binding domain, in which Tyl-81 and Tyl-87 are the putative binding sites for the heme iron (Fig. 1). Indeed, recombinant mouse neudesin was found to bind hemin; however, recombinant mouse mutant neudesin, which lacks a crucial part of the heme-binding domain (neudesinΔHBD), does not bind hemin [60]. This binding of hemin to neudesin potently encourages the neuroprotective activity of the neudesin protein in primary cultured neurons, while neudesinΔHBD exhibited no neuroprotective effect. Additionally, in neuroblastoma Neuro2a cells, neudesin-hemin, but not neudesin or hemin alone, exhibited neurotrophic and neuroprotective activities. These data indicate that the binding to hemin is essential for the biological activity of neudesin.

3.2. Expression

In mouse embryos, although neudesin is expressed in several discrete regions, it is expressed most abundantly in the developing brain and spinal cord [1]. In the embryonic cerebral cortex, neudesin is mostly expressed in the preplate, which mainly consists of postmitotic neural cells, but not in the subventricular/ventricular zone, which mainly consist of self-renewing neural precursor cells [61]. In contrast to the expression pattern in embryos, neudesin in adult mice is detected in various tissues, including the brain, heart, lung, and kidney. In the brain, it is expressed in most neurons but not in glial cells, and in the cerebral cortex, hippocampus, thalamus, and hypothalamus [1]. In cultured neural precursor cells, it is observed that neudesin expression gradually decreases along with differentiation [61]. This temporal expression profile of neudesin *in vitro* is not consistent with the expression in neurons of adult mice *in vivo.* The reason behind the difference between *in vivo* and *in vitro* expression remains to be elucidated. Neudesin is also detected in white adipose tissue (WAT)

but not brown adipose tissue [62]. WAT mainly consists of white adipocytes, which contain large lipid droplets. Adipogenesis is the process by which preadipocytes differentiate into mature adipocytes [63]. Several transcription factors, which are necessary for adipogenesis, have been identified through the study of an immortalized preadipocyte cell lines: 3T3-L1 cells. These factors include members of the CCAAT/enhancer-binding protein beta (C/EBPb), C/EBPa, and peroxisome proliferator-activated receptor gamma (PPARg). Neudesin is expressed in 3T3-L1 preadipocytes before the addition of inducers for adipogenesis; this expression subsequently decreases in the early stage of adipogenesis as the expression of C/EBPb increases [62]. In contrast, neudesin expression is increased in the late stage of differentiation parallel to the expression of PPARg and C/EBPa [62].

3.3. Signal transduction

The signal transduction of neudesin is mainly studied by using cultured neural precursor cells and neurons [1, 61]. The activity of neudesin in primary cultured neurons is mediated through the MAPK and phosphatidylinositol-3 kinase (PI-3K) pathways [1]. The Gi/Go protein inhibitor pertussis toxin (PTX) significantly inhibited the phosphorylation of ERK1/2 by neudesin, which indicates that the activity of neudesin may be mediated by the activation of a Gi/Go protein–coupled receptor [1]. Neudesin also promoted the phosphorylation of ERK, Akt, and CREB in neural precursor cells, but the effect of neudesin was not inhibited by PTX, unlike in the case of the primary neurons [61]. As neudesin increased cAMP levels in neural precursor cells, a Gs protein–coupled receptor might be involved in the activation of the MAPK, PKA, and PI-3K signal pathways [61]. The signal transduction pathway of neudesin appears to change in the course of development. To date, specific receptor(s) for neudesin has not yet been reported. Identification of the neudesin receptor(s) may provide a clue for the understanding of the unique signal transduction system of neudesin.

3.4. Physiological functions

As described in Section 3.2, neudesin is expressed in neural precursor cells, adult neurons, and white adipocytes. Recombinant neudesin significantly enhanced survival of primary cultured mouse neurons by decreasing apoptosis but exhibited no mitogenic activity in primary cultured mouse astrocytes. In contrast, FGF2 had both neurotrophic and mitogenic activities, indicating that the activity of neudesin is distinct from that of FGF2 [1]. Recombinant neudesin induced the differentiation of neural precursor cells into MAP-2-positive neurons through the PI-3K and PKA pathways, and transiently promoted neural cell proliferation in the dividing neural precursor cells through the ERK and PKA pathways, but not the PI-3K pathway [61]. Interestingly, Neuro2a cells produce endogenous hemin-bound neudesin [60]. Transfection of neudesin siRNA, but not of negative control siRNA, significantly reduced cell survival and proliferation in Neuro2a cells, which produce neudesin independently, suggesting that endogenous neudesin-hemin is expected to play physiological roles in development and maintenance of the nervous system [60]. Neudesin-hemin significantly inhibited the late stage of adipogenic inducer-dependent adipogenesis in 3T3-L1 preadipocytes, but not the early stage [62]. In contrast, transfection of neudesin siRNA enhanced the expression of C/EBPa and PPARg and promoted adipogenesis in 3T3-L1 preadipocytes [62]. These results and the expression profile during adipogenesis suggest that the decrease in the neudesin level in the early stage may be a triggering event for adipogenesis, and neudesin may play potential roles in the development of WAT and obesity. Studies of human diseases also provide another approach to the physiological functions of neudesin. Expression of progesterone receptor (PR) in estrogen receptor (ER)-positive breast tumors correlates with increased probability of tamoxifen responsiveness and provides a good prognosis as compared to tumors that express only ER [64]. Neudesin protein was shown to be more abundant in ER^+/PR^+ tumors than ER^+/PR^- tumors [65]. An increase in neudesin expression was observed in SV40-transformed human embryonic kidney

cells immediately following a proliferative crisis [66]. Such information suggests that neudesin may be involved in immortalization of tumor cells and/or resistance to carcinogenesis. A recent study has shown that the ectopic expression of neudesin promotes invasiveness and tumorigenicity in MCF-7 breast cancer cells [67].

As summarized in Table 4, the heme-binding protein neudesin is expressed widely in several tissues, including the central nervous system and breast tumor cells, and it has a number of functions such as cell differentiation and survival.

4. Neuferricin (CYB5D2)

4.1. Structure

In 2010, we reported neuferricin as the second secretary member of the MAPR family [2]. In 2011, Xie et al. also identified it as the cyt-b5 domain containing 2 (CYB5D2) [68]. Neuferricin was identified using a homology-based search with the cyt-b5-like heme/steroid-binding domain of neudesin. Phylogenetic analysis has indicated that neuferricin and neudesin are two paralogue genes generated from one identical gene of *C. elegans* by gene duplication during metazoan evolution. The human neuferricin has 264 amino acids, and its sequence is conserved from fish to primate. Neuferricin contains a signal sequence at its N-terminus, and it was confirmed that this protein is effectively secreted into the culture media from High Five cells and Neuro2a cells. The protein contains a heme-binding domain at its N-terminal half region, while the other members of the MAPR family possess a cyt-b5 domain at C-terminus (Fig. 1). Indeed, endogenous neuferricin is secreted in a heme-binding form into the culture medium of Neuro2a cells, although it remains unclear how neuferricin binds heme.

4. 2. Expression

During the mouse embryonic stage, neuferricin mRNA is primarily expressed in the central nervous system, particularly in subventricular zone and ventricular zone of the cerebral cortex and olfactory bulb [2]. The expression level of neuferricin gradually increases along with brain development. In the postnatal brain, neuferricin mRNA is abundantly expressed, especially in pyramidal cells around the CA3 region of Ammon's horn in the hippocampus. In addition to the brain, neuferricin mRNA is widely expressed in several tissues in the postnatal period, including those of the heart, adrenal gland, and kidney[2].

4.3. Signal transduction and physiological function

So far, neuferricin has been reported to function in two physiological activities: neurogenesis [2] and protection from etoposide-induced cytotoxicity [68]. As described in Section 4.2., neuferricin is mostly expressed in the brain during the developmental stage and in the hippocampus in the adult brain. The suppression of neuferricin by RNA interference in Neuro2a cells promotes cell survival and inhibits neurite outgrowths. Furthermore, the neuferricin protein, but not the recombinant form of neuferricin lacking the heme-binding domain, significantly suppressed cell survival in Neuro2a cells in a concentration-dependent manner. These data suggest that neuferricin is expressed in a region of neural precursor cells and promotes neurogenesis by suppressing the self-renewal and proliferation of neural stem cells. Heme binding to neuferricin may be essential for the biological activity of neuferricin as well as that of the other member of the MAPR family [60]. In Neuro2a cells, siRNA-induced knockdown of neuferricin increased the expression of Bcl-2, a molecule involved in apoptosis suppression, but exerted no effect on the expression of p53 and Bax, molecules involved in apoptosis promotion. This result indicates that the suppression of cell survival by neuferricin appears to be associated with apoptosis due to the inhibition of Bcl-2 expression. In contrast to the neurotrophic activity of neudesin being transduced via the MAPK signaling pathway

[61], neuferricin did not exert any effect on the phosphorylation of ERK1/2 [2]. Additionally, neuferricin and neudesin have opposite effects on cell proliferation in Neuro2a cells; neuferricin suppresses it [2], while neudesin promotes it [60]. These results suggest that the members of the MAPR family have different functions through their distinct intracellular transduction pathways in neurotrophic activity.

Xie et al. reported the protection activity conferred by neuferricin from etoposide [68]. Just as PGRMC1 and Dap1 protect cells from DNA damage-induced toxicity [15, 38], neuferricin enhances the survival of HeLa cells suffering from cytotoxicity induced by the topoisomerase II inhibitor etoposide. This activity requires the neuferricin N-terminus signal sequence and cyt-b5 domains. Since cyt-b5 domain-mediated heme binding is necessary for PGRMC1-mediated resistance, the association with heme may contribute to neuferricin-mediated resistance to this cytotoxicity. However, neuferricin has no significant effects on etoposide-induced phosphorylation of ataxia telangiectasia mutated (ATM) kinase. Neuferricin may protect cells from etoposide-induced cytotoxicity independently of ATM-dependent DNA damage response modulation. This idea is also supported by the observation that neuferricin does not resist UV-induced apoptosis, which is mediated primarily through the ataxia telangiectasia mutated related (ATR) kinase pathway [68, 69]. Moreover, neuferricin also does not affect the expression level of Bcl-2 as well as other major components of apoptosis in HeLa cells. Taken together, neuferricin may modulate cell survival against etoposide-induced cytotoxicity through an unidentified pathway. One possibility is that neuferricin may protect heme from chemical-induced damage, as suggested for PGRMC1 [4]. It has been demonstrated that PGRMC1 interacts with the P450 protein CYP51A, which is known to detoxify toxic compounds [32]. The interaction between neuferricin and CYP51A may contribute to neuferricin-mediated resistance to etoposide-induced cytotoxicity.

Thus, as shown in Table 5, the heme-binding protein neuferricin is expressed widely in several

tissues including the central nervous system and is associated with the promotion of neurogenesis and protection from cytotoxicity.

CONCLUSION

As member of the MAPR family, PGRMC1, PGRMC2, neudesin, and neuferricin contain a cyt-b5-like heme/steroid-binding domain. MAPR family genes are expressed widely in the central nervous system and in several tissues. They have a number of functions, including neurotrophic effects, as shown in Table 6. Although their functions are slightly different, all MAPR family members, except PGRMC2, appear to share some activities relating to cell differentiation and survival. Their bioactivities depend on the binding of heme to their cyt-b5-like heme/steroid-binding domains. Thus, the MAPR family members exert some novel, interesting effects as heme-binding proteins. Although several published reports have described how progesterone binding to MAPR family proteins induces biological responses, their exact function in progesterone signaling remains obscure. The knockout of MAPR family genes in mice has also not been reported thus far. Therefore, the *in vivo* functions of these proteins remain unclear. In the future, MAPR family gene knockout mice will provide much information on the physiological functions of the MAPR family proteins. Elucidation of the MAPR family members' precise functions may provide new insights into the physiological role of heme-binding proteins.

ACKNOWLEDGEMENTS

While collecting the material for compiling this review, the authors received financial support by The Kyoto University Foundation Fellowship Research Grant.

DISCLOSURE STATEMENT

All authors disclaim any form of conflicts of interest.

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FIGURE LEGENDS

Fig. (1). Multiple sequence alignment of mouse MAPR family. Cytochrome b5 heme-binding (Cyt-b5) domain is indicated by the grey box. Two conserved tyrosine residues, which are important for heme binding, are shown in white. The predicted transmembrane domains (PGRMC1 and PGRMC2) are single underlined and the predicted signal peptides (neudesin and neuferricin) are double underlined. Reported and speculated phosphorylation sites are shown in grey according to UniProtKB.

Figure 1

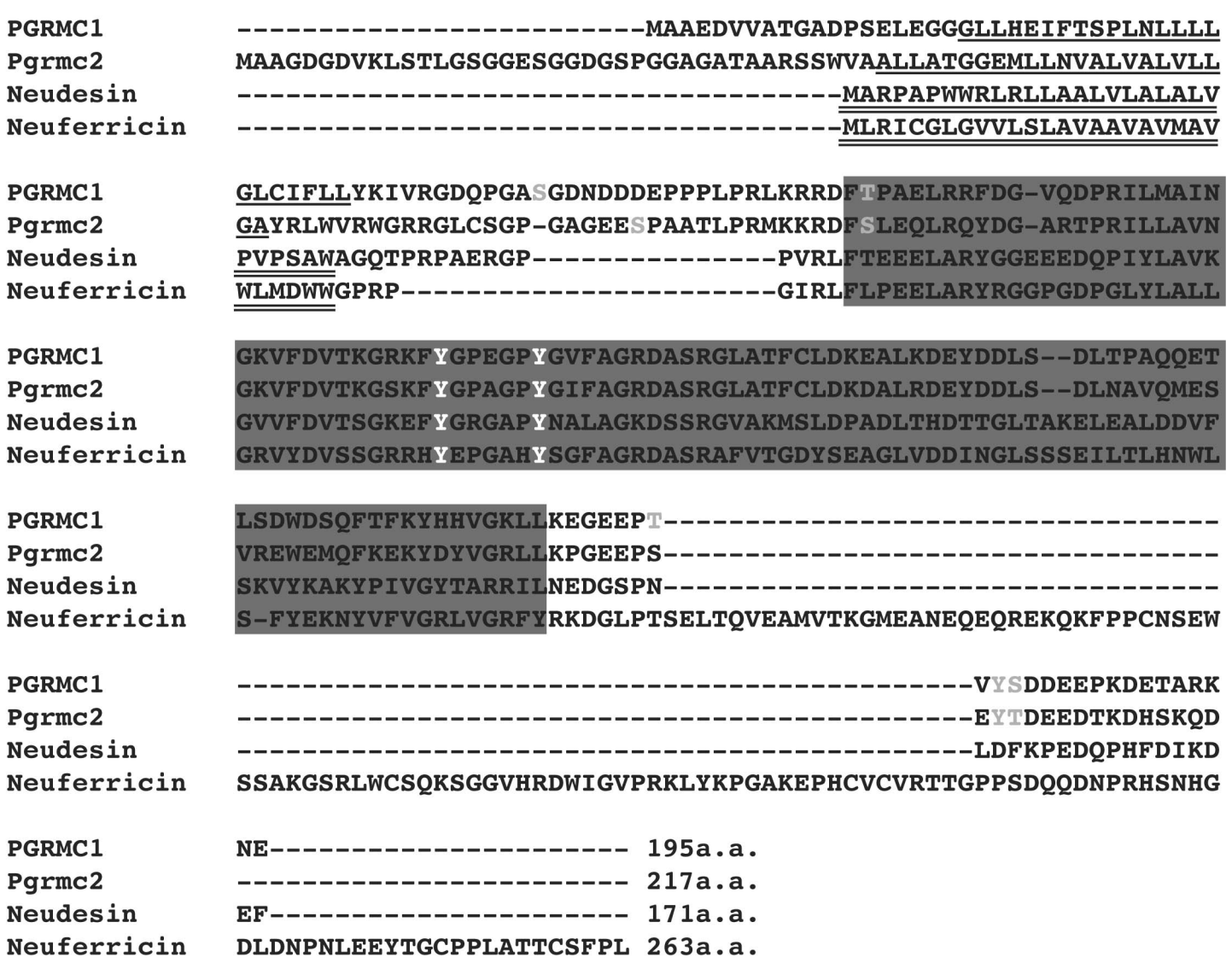
PGRMC1 ----------MAAEDVVATGADPSELEGGGLLHEIFTSPLNLLLL
Pgrmc2 MAAGDGDVKLSTLGSGGESGGDGSPGGAGATAARSSWVAALLATGGEMLLNVALVALVLL
Neudesin ----------MARPAPWWRLRLLAALVLALALV
Neuferricin ----------MLRICGLGVVLSLAVAAVAVMAV
PGRMC1 GLCIFLLYKIVRGDQPGASGDNDDDEPPPLPRLKRRDFTPAELRRFDG-VQDPRILMAIN
Pgrmc2 GAYRLWVRWGRRGLCSGP-GAGEESPAATLPRMKKRDFSLEQLRQYDG-ARTPRILLAVN
Neudesin PVPSAWAGQTPRPAERGP----------PVRLFTEEELARYGGEEEDQPIYLAVK
Neuferricin WLMDWWGPRP----------GIRLFLPEELARYRGGPGDPGLYLALL
PGRMC1 GKVFDVTKGRKFYGPEGPYGVFAGRDASRGLATFCLDKEALKDEYDDLS--DLTPAQQET
Pgrmc2 GKVFDVTKGSKFYGPAGPYGIFAGRDASRGLATFCLDKDALRDEYDDLS--DLNAVQMES
Neudesin GVVFDVTSGKEFYGRGAPYNALAGKDSSRGVAKMSLDPADLTHDTTGLTAKELEALDDVF
Neuferricin GRVYDVSSGRRHYEPGAHYSGFAGRDASRAFVTGDYSEAGLVDDINGLSSSEILTLHNWL
PGRMC1 LSDWDSQFTFKYHHVGKLLKEGEEPT----------
Pgrmc2 VREWEMQFKEKYDYVGRLLKPGEEPS----------
Neudesin SKVYKAKYPIVGYTARRILNEDGSPN----------
Neuferricin S-FYEKNYVFVGRLVGRFYRKDGLPTSELTQVEAMVTKGMEANEQEQREKQKFPPCNSEW
PGRMC1 ----------VYSDDEEPKDETARK
Pgrmc2 ----------EYTDEEDTKDHSKQD
Neudesin ----------LDFKPEDQPHFDIKD
Neuferricin SSAKGSRLWCSQKSGGVHRDWIGVPRKLYKPGAKEPHCVCVRTTGPPSDQQDNPRHSNHG
PGRMC1 NE---------- 195a.a.
Pgrmc2 ---------- 217a.a.
Neudesin EF---------- 171a.a.
Neuferricin DLDNPNLEEYTGCPPLATTCSFPL 263a.a.

Table 1. Homology among mouse cytb5 family.

Homology %	Cytb5	Cytb5b	Cytb5d1	Neuferricin	Neudesin	PGRMC1	PGRMC2
Cytb5		43.9 (54.6)	17.1 (22.2)	13.6 (19.4)	16.4 (23.8)	14.9 (20.5)	19.7 (18.5)
Cytb5b	43.9 (54.6)		16.4 (26.3)	17.0 (18.9)	21.3 (25.2)	21.6 (23.5)	22.0 (18.4)
Cytb5d1	17.1 (22.2)	16.4 (26.3)		18.5 (15.2)	18.4 (23.3)	18.3 (17.5)	16.3 (16.7)
Neuferricin	13.6 (19.4)	17.0 (18.9)	18.5 (15.2)		23.3 (39.6)	21.4 (39.3)	19.9 (36.4)
Neudesin	16.4 (23.8)	21.3 (25.2)	18.4 (23.3)	23.3 (39.6)		29.4 (39.8)	28.3 (38.2)
PGRMC1	14.9 (20.5)	21.6 (23.5)	18.3 (17.5)	21.4 (39.3)	29.4 (39.8)		49.3 (67.6)
PGRMC2	19.7 (18.5)	22.0 (18.4)	16.3 (16.7)	19.9 (36.4)	28.3 (38.2)	49.3 (67.6)	

full length
(heme binding domain)

Table 2. Functions of PGRMC1.

Cell Type	*Function*	*Experimental Method*
COS-7 cells (kidney cell line)	steroid synthesis and metabolism	overexpression
HEK293 cells (embryonic kidney cell line)	cholesterol regulation	overexpression
GnRH neurons	suppression of GnRH release by P4	inhibitor (AG-205)
SIGCs (ovarian granulosa cell line)	antiapoptotic action by P4	overexpression RNA interference blocking antibody
SKOV-3 cells (ovarian cancer cell line)	antiapoptotic action by P4	RNA interference
MDA-MB-231 cells (breast cancer cell line)	suppression of chemotherapeutic drug susceptibility	RNA interference
MCF-7 cells (breast cancer cell line)	promotion of cell death	overexpression
A549 cells (lung cancer cell line)	promotion of proliferation	RNA interference addition of recombinat protein
SCLC cells (lung cancer cell line)	promotion of cell survival	RNA interference inhibitor (AG-205) addition of recombinant protein

P4 : progesterone

Table 3. Functions of PGRMC2.

Cell Type	*Function*	*Experimental Method*
SKOV-3 cells (ovarian cancer cell line)	**inhibition of cell migration**	**overexpression**

Table 4. Functions of neudesin.

Cell Type	*Function*	*Experimental Method*
neurons	neurotrophic activity	addition of recombinant protein
neural precursor cells	promotion of neurogenesis	addition of recombinant protein
3T3-L1 cells (preadipocyte)	suppression of adipogenesis	addition of recombinant protein RNAi interference
MCF-7 cells (breast cancer cell line)	increase of invasiveness and tumorigenicity	overexpression

Table 5. Functions of neuferricin.

Cell Type	*Function*	*Experimental Method*
neural precursor cells	promotion of neurogenesis	addition of recombinant protein
Neuro2a cells (neuroblastoma cell line)	promotion of neurogenesis	RNA interference
HeLa cells (cervical cancer cell line)	protection from etoposide -induced cytotoxicity	overexpression

Table 6. Characteristics of MAPR family.

	PGRMC1	PGRMC2	Neudesin	Neuferricin
Structure	heme binding progesterone binding	putative heme binding profesterone binding	heme binding	heme binding
Localization	extracellular or endoplasmic reticulm	endoplasmic reticulm	extracellular	extracellular or endoplasmic reticulm
Expression	liver, kidney brain, lung broad expression in various tissues several tumors including breast tumor several cancer cells including breast cancer cell	placenta broad expression in various tissues breast tumor neural cell ovarian cancer cell	brain, heart, lung, kidney, white adipose broad expression in various tissues breast tumor breast cancer cell	brain, heart, kidney broad expression in various tissues
Physiological function	steroid synthesis and metabolism cholesterol regulation cell survival and proliferation	inhibition of cell migration	neurotrophic effect neurogenesis inhibition of late stage adipogenesis invasiveness and tumorigenicity	neurogenesis protection from etoposide-induced cytotoxicity