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Review

# PNMA family: Protein interaction network and cell signalling pathways implicated in cancer and apoptosis

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A B S T R A C T

Paraneoplastic Ma Family (PNMA) comprises a growing number of family members which share relatively conserved protein sequences encoded by the human genome and is localized to several human chromosomes, including the X-chromosome. Based on sequence analysis, PNMA family members share sequence homology to the Gag protein of LTR retrotransposon, and several family members with aberrant protein expressions have been reported to be closely associated with the human Paraneoplastic Disorder (PND). In addition, gene mutations of specific members of PNMA family are known to be associated with human mental retardation or 3-M syndrome consisting of restrictive post-natal growth or dwarfism, and development of skeletal abnormalities. Other than sequence homology, the physiological function of many members in this family remains unclear. However, several members of this family have been characterized, including cell signalling events mediated by these proteins that are associated with apoptosis, and cancer in different cell types. Furthermore, while certain PNMA family members show restricted gene expression in the human brain and testis, other PNMA family members exhibit broader gene expression or preferential and selective protein interaction profiles, suggesting functional divergence within the family. Functional analysis of some members of this family have identified protein domains that are required for subcellular localization, protein-protein interactions, and cell signalling events which are the focus of this review paper.

## 1. Introduction

Paraneoplastic Ma Antigen (PNMA) family is represented by at least fifteen family members, and three of the family members, PNMA1–3, are known to be associated with the Paraneoplastic Disorder (PND) [1–8]. Recent human genome sequence analysis has identified additional members that belong to this family and a total of nineteen family members are currently known to be encoded by the human genome [9,10]. The patients of PND associated with PNMA1–3 were reported to exhibit syndrome consisting of paraneoplastic limbic and brain stem encephalitis, production of auto-antibodies specific to PNMA 1, 2 or 3 as well as cancer [1,3,11–16]. Molecular diagnostic methodologies are often used to diagnose PND, including detection of auto- antibodies against PNMA proteins as well as identification of aberrantly expressed PNMA 1, 2 or 3 in the tumor tissues [5,17,18]. Although the mechanism that contributes to development of PND is unclear, its likely to be associated with aberrant expressions of PNMA1, 2 or 3 in tumor cells originated from non-neuronal tissues, including lung, breast and testicular tumors, leading to the development of tumor immunity against PNMA1, 2, or 3 [19–24]. In normal individuals, PNMA family members are predominantly expressed in the human brain, and testis, except for MOAP-1 (PNMA4), and CCDC8 which are ubiquitously expressed in many human tissues and higher MOAP-1 expression is detected in the human brain and heart [5,8,25–27]. Among the PNMA family members, MOAP-1 is the most extensively studied protein in the family. MOAP-1 was reported to mediate apoptotic signalling by interacting with Bax, a pro-apoptotic member of Bcl-2 family [8,28–35]. Furthermore, MOAP-1 was shown to interact with RASSF1A tumor suppressor through TNF receptor mediated signalling upon activation of the TNF receptor by TNF ligand [28–31,36–39]. Other than PND, genetic mutations of two other PNMA family members are known to be associated with restrictive human growth and mental retardation [25,40]. This paper describes current information on some members of the PNMA family members that are relatively well characterized, including their roles in cancer and apoptotic signalling.

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**Table 1**
Chromosomal localization and gene structure of PNMA family members.[a]

| Protein | Protein name | Chromosome location (GRCh38.p10) and name of transcript | Exon | Intron | Number of amino acids | Protein coding in single exon | Homologues in other species, Chimpanzee (C) and Mouse (M), % amino acid identity |
|---|---|---|---|---|---|---|---|
| P1 | PNMA1 | 14: 73711783-73714372; PNMA1-201 | 1 | 0 | 353 | Yes | C (99%) M (76%) |
| P2 | PNMA2 | 8: 26504686-26514092; PNMA2-204 | 3 | 2 | 364 | Yes | C (100%) M (92%) |
| P3 | PNMA3 | X: 153057036-153058467; PNMA3-202 | 1 | 0 | 463 | Yes | C (99%) M (72%) |
| P4 | PNMA4 (MOAP1) | 14: 93,182,199-93,184,923 MOAP1-202 | 2 | 1 | 351 | Yes | C (99%) M (76%) |
| P5 | PNMA5 | X: 152988824-152992214; PNMA5-201 | 2 | 1 | 448 | Yes | C (98%) M (56%) |
| P6 | PNMA6A/PNMA6C | X: 153072482-153075018; PNMA6A-201 | 2 | 1 | 399 | Yes | C (99%) |
| P7 | PNMA 6B/ PNMA6D | X: 153075769-153076968; PNMA6B-201 | 1 | 0 | No protein; unprocessed pseudogene | N/A | N/A |
| P8 | PNMA6E | X: 153,396,906-153,401,420 PNMA6E-201 | 2 | 1 | 647 | Yes | M (64%) |
| P9 | PNMA6F | X: 153317681-153321822; PNMA6F-201 | 2 | 1 | 578 | Yes | C (99%) M (65%) |
| P10 | PNMA7A/ ZCCHC12/SIZN1 | X: 118823790-118826968; ZCCHC12-201 | 4 | 3 | 402 | Yes | C (99%) M (75%) |
| P11 | PNMA7B/ ZCCHC18 | X: 104,112,511-104,115,604 ZCCHC18-203 | 2 | 1 | 403 | Yes | C (92%) M (68%) |
| P12 | PNMA8A/PNMAL1 | 19: 46,466,498-46,471,563 PNMA8A-201 | 3 | 2 | 439 | No (2 exons) | C (99%) |
| P13 | PNMA8B/PNMAL2 | 19: 46,491,191-46,496,498; PNMA8B-202 | 1 | 0 | 635 | Yes | C (98%) |
| P14 | PNMA8C | 19: 46,424,697-46,428,951; PNMA8C-201 | 1 | 0 | 204 | Yes | C (99%) M (74%) |
| P15 | CCDC8 | 19: 46410372-46413584; CCDC8-201 | 1 | 0 | 538 | Yes | C (99%) M (62%) |

[a] Information derived from HGNC (HUGO Gene Nomenclature Committee), Uniprot, and Ensembl. Transcript name is based on information provided by Ensembl genome browser based on human genome assembly, GRCh38.p10.

## 2. Chromosomal localization and gene structure

PNMA family members are encoded by at least 15 genes localized to human chromosome 14 (PNMA1 and MOAP-1), chromosome 19 (PNMA8A-C, and CCDC8), chromosome 8 (PNMA2), and X chromosome (PNMA3, PNMA5, and PNMA6A, PNMA6B, PNMA6E, PNMA6F, PNMA7A, and PNMA7B, Table 1). In addition, three additional PNMA family members, PNMA6E, 6F, and 8C which are likely to be generated through gene duplication events, were identified after analysis of the human genome databases (GRCh37.p5, GRCh38.p10 [41]). In addition, extensive annotations of the human genome database (GRCh38.p10) has resulted in reclassification of some of the PNMA members, PNMA6C, and PNMA6D, as existing members of the PNMA family, PNMA6A, and PNMA6B, respectively (Table 1 [42,43]), as well as identification of pseudogenes that do not belong to the PNMA family (Table 2). Furthermore, among the PNMA gene family, PNMA6B is classified as unprocessed pseudogene without protein coding function [43]. It is interesting to know that although most PNMA family members are expressed from multiple exons, the coding sequences of PNMA family members are mostly encoded by a single exon (Table 1), raising the possibility that the expression of PNMA family members may be regulated through other cellular mechanisms. Indeed, MOAP-1 is known to be regulated by miRNAs as well as proteasome pathway [30,44,45].

## 3. Sequence homology

PNMA family members share high protein sequence homology with the highest sequence homology of 84% between PNMA7A and PNMA7B (Fig. 1). The lowest protein sequence homology of 9.5% is between PNMA7A and CCDC8 (Fig. 1). PNMA family members share high sequence homology at the N-terminal conserved domain (NCD), except PNMA7A, and PNMA7B (Fig. 2). The other conserved domain of the PNMA family members is the central conserved domain (CCD), except

**Table 2**
The genomic repositioning of earlier reported PNMAs.
Table 2 shows the new locations of 5 PNMA family members reported by Iwasaki et al. [41]. From sequence analysis, it was unearthed that hsPNMAs 7, 8 and 16, reported by the earlier group, are indeed, PNMAs. However, as can be seen from the table, they are already known by other aliases, namely 6F, 6E and 8C, respectively. The query length of the PNMAs covered while doing a BLAT analyses of the sequences located in GRCh37.p5 showed 100% match (identity) with those of the repositioned PNMAs, thereby validating that the genes encode additional PNMA family members which are likely to be generated through gene duplication events. However, the earlier reported hsPNMA 9 and 16, are found to be not belonging to the PNMA family. In fact, the earlier hsPNMA9 has been now found to be an unprocessed pseudogene AC243591.1. Similarly, hsPNMA16 reported earlier has now been mapped onto AC011551.1, an unprocessed pseudogene.

| hsPNMA No. | Genomic location in GRCh37.p5 | Genomic location in GRCh38.p10 | Overlapping genes | Orientation | Length | % ID |
|---|---|---|---|---|---|---|
| 7 | X: 152584221 – 152587591 | X: 153318763 – 153322133 | PNMA6F | Forward | 3371 | 100 |
| 8 | X: 152662364 – 152663269 | X: 153396906 – 153397811 | PNMA6E | Forward | 906 | 100 |
| 9 | X: 152197130 – 152200901 | X: 153028785 – 153032555 | AC243591.1 | Forward | 3772 | 99.68 |
| 15 | 19: 47036933 – 47037357 | 19: 46427925 – 46428338 | PNMA8C | Forward | 414 | 100 |
| 16 | 19: 46931182 – 46931595 | 19: 46533676 – 46534100 | AC011551.1 | Forward | 425 | 100 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | PNMA1 | PNMA1 |
| | | | | | | | | | | | PNMA2 | | PNMA2 |
| | | | | | | | | | | PNMA3 | | 47.99 | PNMA3 |
| | | | | | | | | | MOAP1 | | 47.37 | 48.43 | MOAP1 |
| | | | | | | | | PNMA5 | | 46.55 | 45.71 | 57.10 | PNMA5 |
| | | | | | | | PNMA6A | | 46.65 | 40.62 | 45.61 | 46.26 | PNMA6A |
| | | | | | | PNMA6E | | 39.33 | 41.54 | 42.28 | 42.86 | 43.73 | PNMA6E |
| | | | | | PNMA6F | | 45.84 | 31.03 | 36.86 | 31.53 | 37.47 | 37.22 | PNMA6F |
| | | | | PNMA7A | | 76.64 | 50.13 | 31.83 | 35.26 | 31.44 | 36.21 | 37.64 | PNMA7A |
| | | | PNMA7B | | 23.84 | 25.27 | 33.92 | 29.57 | 31.74 | 25.59 | 34.57 | 37.77 | PNMA7B |
| | | PNMA8A | | 83.79 | 23.71 | 24.32 | 34.15 | 30.61 | 31.74 | 27.19 | 36.63 | 38.63 | PNMA8A |
| | PNMA8B | | 15.33 | 16.33 | 22.06 | 23.00 | 25.69 | 24.26 | 27.78 | 22.03 | 27.58 | 30.18 | PNMA8B |
| PNMA8C | | 22.04 | 13.92 | 13.95 | 19.83 | 20.04 | 23.98 | 20.09 | 22.25 | 20.52 | 27.02 | 26.44 | PNMA8C |
| CCDC8 | 28.79 | 32.02 | 15.85 | 17.07 | 28.72 | 28.64 | 35.05 | 32.14 | 29.80 | 34.67 | 35.18 | 33.33 | CCDC8 |
| 28.03 | 14.32 | 15.03 | 10.97 | 9.46 | 13.54 | 12.09 | 18.79 | 15.75 | 17.44 | 18.27 | 20.75 | 22.30 | |

**Fig. 1.** Sequence comparisons. Sequence comparisons were carried out using MUSCLE sequence alignment software to identify sequence homology based on percent identity of amino acid sequences between members of PNMA family. Percent sequence homology greater than 40% are highlighted in bold.

PNMA8A-C, which show significant sequence divergence within this region (Fig. 2). Situated between NCD and CCD domains are unique protein sequence or domain (UPD) found in some members of PNMA including the BH3-like (BH3-L) domain of MOAP-1 and PNMA1 (Figs. 2–3). In addition, a region rich in Lysine and Arginine basic residues, and designated as KRs is found in the protein sequences of all the members of PNMA family, except PNMA8B, and PNMA8C. Furthermore, it is interesting to know that PNMA family members share sequence homology at a poly-glutamic acid rich region (PolyE) near the C-terminus, except PNMA6F, 8A, and 8C (Fig. 3A–B). Beyond the poly-glutamic acid rich region is the C-terminus with varying length and low sequence homology (variable C-terminal sequence, VCS) that can be identified in the protein sequences among the PNMA family members. Nuclear localization sequence (NLS) was identified in the VCS region of PNMA5, PNMA7A and PNMA7B (Fig. 3A–B). From the sequence analysis, PNMA family members are conserved in quite a number of species, including rat, mouse, monkey, and chimpanzee [4,41]. Further sequence analysis has shown that PNMA family members are conserved in chimpanzee and mouse with the lowest and highest amino acid sequence identity between 56% and 99% (Table 1), with the exception of PNMA6A, PNMA8A and PNMA8B which are found only in human and chimpanzee, but not in mouse, based on sequence analysis using the Blast tool of Ensembl [43]. Similarly, human PNMA6E shares significant amino acid sequence homology (64%) to mouse PNMA6E (Gm18336) and 77% of the sequence homology with chimpanzee PNMA6F. Although sharing high sequence homology, functional diversifications of PNMA family members have been reported [46,47]. It is likely that the functional diversifications stem from variation in amino acid sequences of the PNMA family members, particularly from the UPD and VCS regions, as evidenced by the presence of BH3-like domain of MOAP-1 in the UPD region, and nuclear localization signal of PNMA5, PNMA7A, and PNMA7B in the VCS region, respectively. In addition, based on sequence alignment, PNMA family members exhibit significant homology to Gypsy12_DR Gag protein of LTR retrotransposon, suggesting PNMA family members are derived from LTR retrotransposon with common origins in Eutherians and Marsupials [9,10,41].

## 4. Protein-protein interactions

### 4.1. Interaction with Bcl-2 family members

MOAP-1, also known as MAP-1, is the most extensively studied protein in the family. MOAP-1 is a 39.5 kDa novel molecule identified through interaction with Bax, a pro-apoptotic member of Bcl-2 family [8,32]. Deletion mapping and mutagenesis studies showed that MOAP-1 interacts with Bax through its BH3-like domain, which shows significant sequence homology to BH3 domains of pro-apoptotic molecules of the Bcl-2 family. The BH3-domain of Bcl-2 family plays a critical role in mediating apoptotic signalling as highlighted by the presence of BH3-only pro-apoptotic molecules which are involved in apoptosis signalling through interactions with multi-domain pro-apoptotic as well as pro-life molecules of the Bcl-2 family [48–57]. Structural information derived from X-ray crystallography and NMR studies of Bax in complex with the BH3 domain of pro-apoptotic molecules such as Bim and Bid have been determined and the data show the important role of BH3-domain of Bim and Bid in promoting activation of Bax through binding to Bax by inducing change in conformation of Bax, leading to exposures of the BH3 domain as well as C-terminal domain (α6–α8) of Bax [54,58–62]. Once exposed, Bax BH3 and the C-terminal domains engage neighbouring Bax monomer to generate Bax dimers, and oligomers [49,58,59,62]. Furthermore, change in the Bax conformation enables C-terminus of Bax (α9) to be associated with mitochondrial membrane, effectively anchoring Bax oligomers to mitochondria, leading to mitochondrial dysfunction and release of mitochondrial cytochrome C [58,59]. Although required for binding to Bax, deletion mapping studies have shown that the BH3-like domain of MOAP-1 is not required for MOAP-1 to interact with Bcl-XL, which interacts with the N- and C-terminal regions of MOAP-1 [8,32,34]. Due to its pro-apoptotic activity, Bax is known to be frequently mutated in cancer cells [63–66].

### 4.2. Interaction with transcription factors and growth factors

Other than MOAP-1, PNMA7A (ZCCHC12, SIZN1) which shares 32% amino acid sequence identity with MOAP-1 was reported to function as transcriptional co-activator and participate in Bone Morphogenic Protein (BMP) signalling by interacting with Smad1 and CBP [67]. Other than BMP cell signalling, PNMA7A was identified as transcriptional co-activator that interacts with c-Jun and activates AP-1 and CREB signalling [68]. Like many members of PNMA family, PNMA7A was found to be predominantly expressed in mouse brain, including cholinergic neurons as well as testis, pointing to the possibility that PNMA7A could play an important role in the developmental regulation or maintenance of neuronal cells [67,68]. In addition, mutations of PNMA7A were found to be associated with human mental retardation [40,69]. Currently, it's not clear whether PNMA7A plays a role in neuronal survival or apoptosis; however, recent studies showed that PNMA7A functions as an oncogene and the expression is elevated in thyroid cancer cells [70,71], suggesting that PNMA7A could function to promote neuronal and cancer cell growths. The coiled coil domain containing molecule, CCDC8, was one of the candidate genes identified to play a crucial role in human growth. Mutations of CCDC8 gene were

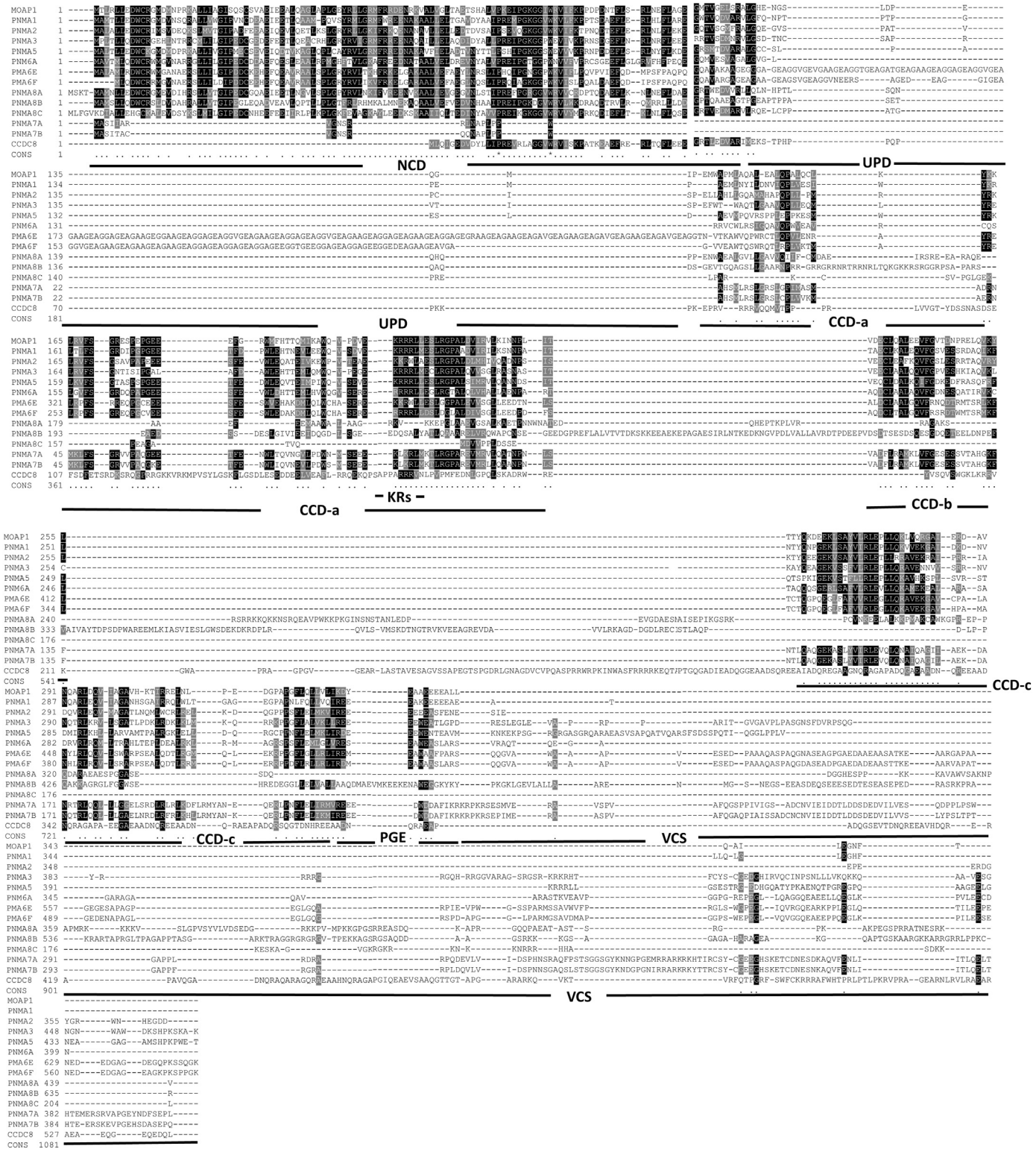


**Fig. 2.** Sequence Alignment of PNMA family members. Unitprot amino acid sequences of PNMA family members were aligned using T-Coffee and Clustal W sequence alignment programs, and conserved sequences were high-lighted in black using Box Shade software. Identity of unitprot protein sequences, Q96BY2 (MOAP1), Q8ND90 (PNMA1), Q9UL42 (PNMA2), Q9UL41 (PNMA3), Q96PV4 (PNMA5), P0CW24 (PNM6A), A0A0J9YXQ4 (PMA6E), A0A0J9YX94 (PMA6F), Q6PEW1 (ZCH12), P0CG32 (ZCC18), Q86V59 (PNM8A), Q9ULN7–5 (PNM8B), A0A1B0GUJ8 (PNM8C), Q9H0W5 (CCDC8).

associated with post-natal growth restriction, one of the well described features of 3M-syndrome [25,72]. Other than CCDC8 mutations in two other human genes, CUL7 and OBSL1, were also reported to be the important contributing factors of 3M-syndrome [72]. Interestingly, OBSL1 was found to be the common protein interacting partner of both CCDC8 and CUL7, suggesting that the molecules might participate in common cell signalling pathway that regulate human growth [25]. In fact, patients with 3-M syndrome were found to exhibit growth hormone deficiency or disordered growth factor signalling [73,74]. Furthermore, protein expression studies showed that CCDC8 protein level

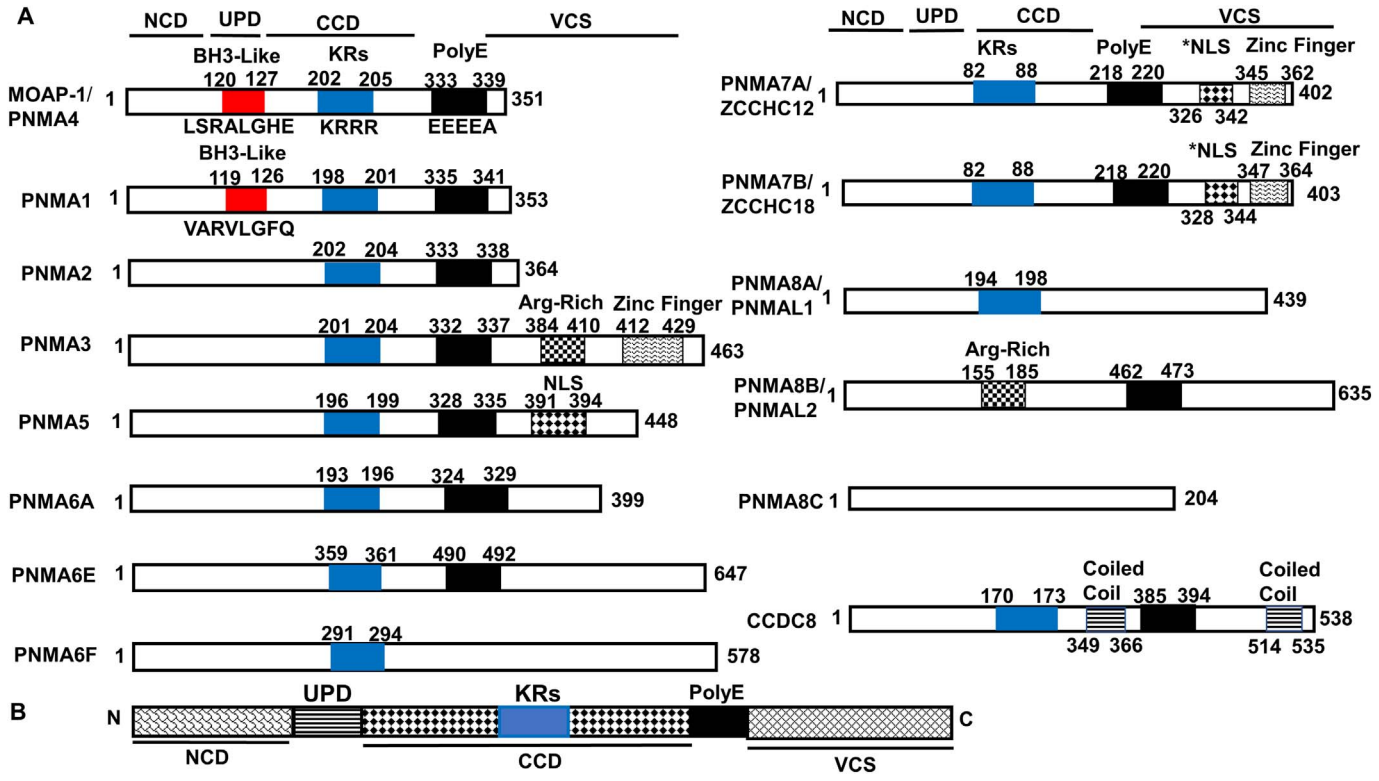


**Fig. 3.** Schematic representation and conserved domains of PNMA family members. A. Conserved domains identified in the protein sequences of PNMA family members are highlighted based on Unitprot amino acid sequences presented in Fig. 2. B. General structure of PNMA family member. NCD, N-terminal Conserved Domain; UPD, Unique Protein Domain; CCD, Conserved Central Domain (CCD-a, CCD-b, and CCD-c); KRs, KR-rich sequence, PolyE, Poly-Glutamic Acid Sequence; VCS, Variable C-terminal Sequence, NLS, nuclear localization sequence, *NLS, putative nuclear localization sequence.

is relative low in tumor cells, and over-expression of CCDC8 in cancer cells inhibits invasiveness and metastasis of cancer cells [75].

### *4.3. Interaction with death receptors associated signalling molecules and RASSF family members*

Other than interacting with Bcl-2 family members, MOAP-1 was reported to be associated with RASSF1A, and RASS6 [29,76–78]. RASSF1A is a tumor suppressor and its protein expression is frequently silenced in many cancer cells through hyper-methylation of its gene promoter [37,79–84]. Deletion and site directed mutagenesis studies showed that MOAP-1 interacts with poly-glutamic acid residues of RASSF1A [30,85,86]. In addition, MOAP-1 was shown to interact with RASSF1A, and TNF-R through its RASSF1A binding domain within the KRs region and poly-glutamic acid rich residues (polyE) of its C-terminus, respectively. Other than interacting with TNF-R1, MOAP-1 was reported to interact with TRAIL-R1 [38,86]. MOAP-1 was shown to be present in an inactive conformation through intramolecular interaction, and by interacting with RASSF1A, MOAP-1 is unfolded with its exposed BH3-like domain to interact and promote Bax activation, leading to transmigration of activated Bax to the mitochondria [38]. Other than RASSF1A, MOAP-1 was reported as MTCH2 interacting protein and plays an important role in Fas receptor mediated apoptosis signalling by promoting recruitment of tBid to the mitochondria during apoptosis [87].

### *4.4. Protein-protein interaction among the PNMA family members*

In addition, MOAP-1 was reported to interact with its family members, including PNMA2, and PNMA5 [46,47]. Interaction with PNMA2 leads to inhibition of pro-apoptotic function of MOAP-1. In contrast, interaction with PNMA5 enhances pro-apoptotic function of MOAP-1. Furthermore, although sharing relatively high sequence homology, interaction between MOAP-1 and PNMA1 was not detectable in co-immunoprecipitation studies [46]. In addition, it is likely that PNMA family members may interact with other cellular proteins that modulate its activity or promote activation of activity that leads to stimulation of cell growth such as the aberrantly expressed PNMA1–3 in cancer cells (Fig. 4).

## 5. Protein domains and cell signalling

### *5.1. Apoptosis signalling of PNMA family members*

Although MOAP-1 (PNMA4) was reported to mediate apoptosis by interacting with Bcl-2 family members, and RASSF protein isoforms, other PNMA family members are not known to be associated with these molecules, suggesting functional divergence among the PNMA family members [8,32]. In the absence of non-apoptotic stimuli, MOAP-1 is held in inactive conformation where the BH3-like domain of MOAP-1 is buried. This ‘closed’ confirmation is mediated via electrostatic interactions between the $^{178}$EEEF and $^{202}$KRRR motifs of MOAP-1 [34]. Similar to MOAP-1, Bid, a pro-apoptotic member of Bcl-2 family was reported to be held in inactive conformation through intramolecular interaction between the BH3 and BH3-like domains of Bid [55].

Activation of MOAP-1 requires death stimuli, RASSF1A or RASSF6, in the presence of K-RAS to promote or enhance apoptotic signalling through change of MOAP-1 conformation, leading to exposure of MOAP-1 BH3-like domain, which interacts with the pro-apoptotic Bax, resulting in activation of apoptotic cell death [30,39]. The pro-apoptotic Bid is similarly activated through cleavage of the N-terminus domain, leading to exposure of its BH3 domain that promotes apoptotic signalling, including interactions with pro-life and pro-death members of Bcl-2 family [55]. In addition, mouse xenograft experiments showed that expression of both MOAP-1 and MOAP-1 interacting molecule, RASSF1A, in cancer cells produce additive effect in tumor suppression

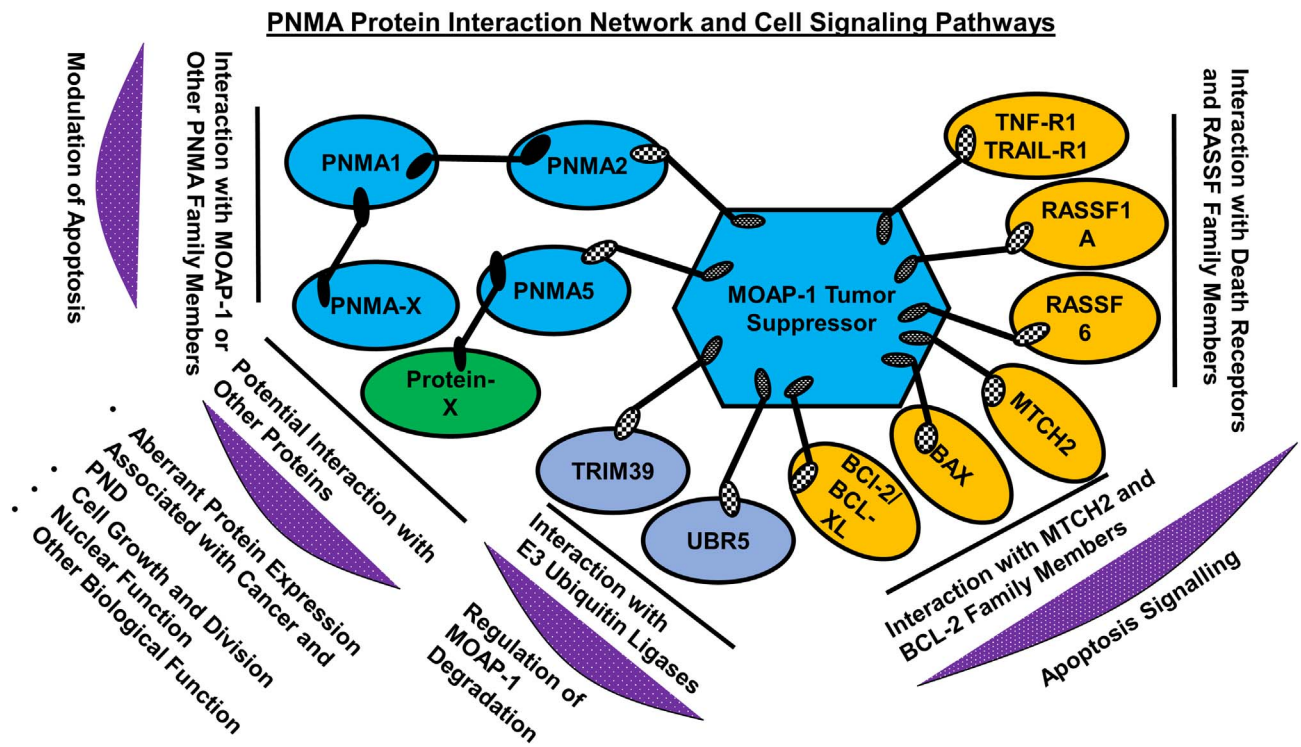


**Fig. 4.** Schematic representation of PNMA protein interaction network and cell signalling. Reported interactions of MOAP-1 were identified through protein-protein interaction assays, including co-immunoprecipitation, and GST-pull down assays. Cell signalling pathways mediated by the protein-protein interactions are highlighted.

better than individually expressed MOAP-1 or RASSF1A [29,88]. Furthermore, MOAP-1 knockout mouse model showed that MOAP-1 participates in Fas receptor mediated apoptosis by interacting with MTCH2 to facilitate recruitment of tBid to the mitochondria through two regions of MOAP-1 (aa 120–162 and 253–293), including the critical amino acid residues ($^{120}$L, and $^{125}$GHE) within the BH3-like domain of MOAP-1, which lends further support to the important role of MOAP-1 in apoptosis signalling through mitochondria signalling pathway [87].

Similar to MOAP-1, PNMA1 was reported to mediate apoptotic cell death of cerebellar granule neurons (CGN), which requires the N-terminal region of PNMA1 containing a BH3-like domain [6,89]. However, the BH3-like domain of PNMA1 is not known to be associated with pro-apoptotic Bax, and it is possible that it may interact with an un-identified member of the Bcl-2 family as apoptosis mediated by PNMA1 can be inhibited by over-expression of Bcl-2 in the neuronal cells even no direct interaction between PNMA1 and Bcl2 has been observed, suggesting that Bcl2 indirectly inhibits PNMA1 by sequestering the BH3-like domain-binding molecule of PNMA1 [89]. Further evidence shows that PNMA1 expression is elevated in the striatum of mouse model of Huntington's disease, suggesting that PNMA1 expression plays an important role in neuro-degeneration by inducing neuronal cell death in Huntington's disease model [89]. The nuclear localization signal (NLS) of PNMA7A plays an important role for protein translocation to nucleus where PNMA7A was reported to function as transcriptional activator by interacting with Smad1 and CBP in BMP mediated cell signalling [67]. In addition, PNMA7A was found to activate AP-1 and CREB, and interacts with C-Jun through its nuclear targeting function [68].

### 5.2. Anti-apoptosis cell signalling and cancer

PNMA2, an onconeuronal antigen, was reported to be aberrantly expressed in a number of tumors, and its over-expression is likely to be closely associated with tumorigenesis [5,14,15,46,90–100]. However, other than closely associated with paraneoplastic disease, the molecular function of PNMA2 remains unclear. In order to identify the potential function of PNMA2, over-expression studies were conducted which showed that unlike PNMA1 and MOAP-1, over-expressed PNMA2 failed to promote apoptotic signalling in transfected MCF-7 cells. However, PNMA2 was shown to interact with both PNMA1 and MOAP-1 in co-immunoprecipitation studies, as well as functionally antagonized the pro-apoptotic function of both MOAP-1 and PNMA1 [46]. The functional domain of PNMA2 involved in interaction with PNMA1 and MOAP-1 has not been identified. In contrast, the N-terminal domain (aa 1–315) of MOAP-1 was reported to be required to interact with PNMA5, and transient co-expression of MOAP-1 and PNMA5 synergistically enhanced apoptotic signalling in transfected cancer cells. The C-terminal domain of PNMA5 (aa 316–448) contains a NLS that is required for nuclear localization of PNMA5. Both the N-terminal and C-terminal domains of PNMA5 are required for apoptotic signalling in cancer cells [47]. Other than PNMA5, functional characterization of CCDC8 showed that over-expressed CCDC8 inhibited invasiveness of A549 cells while CCDC8 knock-downed cells promoted invasiveness of the cancer cells in cell migration assay [75], suggesting that CCDC8 functions as tumor suppressor in the cancer cells. In contrast, ZCCHC12 (PNMA7A) was found over-expressed in papillary thyroid cancer tumors and ZCCHC12 knocked downed thyroid cancer cell lines exhibited reduced number of colonies formed in the colony formation assay [70,71], providing further evidence to support that ZCCHC12 functioned as an oncogene in papillary thyroid cancer [71].

## 6. Regulation of PNMA family members

### 6.1. Regulation by ubiquitination and protein degradation

MOAP-1 has a short half-life of 25 min and the stability of the protein is regulated through ubiquitin proteasome pathway in which degradation of MOAP-1 occurred at G1, and S-G2 phases of cell cycle by the APC/C$^{Cdh1}$ and the UBR5 ubiquitin ligases, respectively [101–103]. In contrast, Trim39 ubiquitin ligase interacts with MOAP-1 and promotes stability of MOAP-1 by inhibiting the activity of APC/C$^{Cdh1}$-mediated ubiquitination and degradation [104,105]. Due to its short

half-life, the endogenous level of MOAP-1 in cancer cell lines was shown to be relatively low; however, MG-132, a proteasome inhibitor, could significantly enhance the stability and level of MOAP-1 protein in cancer cells [103].

### 6.2. Regulation by miRNA

Although the exact mechanism has not been identified, MOAP-1 protein level was enhanced or stabilized in the cancer cells when treated with chemo-drugs or apoptosis stimuli [103]. One possible explanation for stabilization of MOAP-1 during drug-induced apoptosis in cancer cells is the down-regulation of miR-1228 expression level during drug treatment [44]. miR-1228 negatively regulates MOAP-1 expression by binding to the miRNA target sequence of the MOAP-1 mRNA, and down-regulation of miR-1228 was shown to promote MOAP-1 stabilization [44]. Similarly, miR-25 has been reported to promote cell proliferation by promoting down-regulation of MOAP-1 expression in lung cancer [45].

### 6.3. Regulation by other mechanisms

In addition, MOAP-1 may be regulated by phosphorylation as several phosphorylation sites have been identified in MOAP-1 protein sequence [29]. Analysis of MOAP-1 gene led to the identification of CpG island located near the gene promoter of MOAP-1, pointing to the possibility that MOAP-1 might be regulated through hyper-methylation of its gene promoter [106]. Other than gene expression regulation, pro-apoptotic activity of MOAP-1 was reported to be regulated by members of PNMA family. Co-expression of MOAP-1 with PNMA2 resulted in inhibition of pro-apoptotic activity of MOAP-1 in transiently transfected cancer cells [46]. Interestingly, PNMA2 has been shown to antagonize both pro-apoptotic activities of MOAP-1 and PNMA1 through hetero-dimeric interaction with MOAP-1 and PNMA1 [46]. In contrast, co-expression of MOAP-1 with PNMA5 significantly enhanced pro-apoptotic activity of MOAP-1 in transfected cancer cells [47]. Similar to MOAP-1, CCDC8 was found to be hyper-methylated or epigenetically silenced in breast tumors that showed metastasis potential [107]. In addition, CCDC8 knock-downed cancer cells showed increase in migration potential when tested in cell migration assay [75]. Regulation of other PNMA family members is currently unknown.

## 7. Concluding remarks

PNMA family is represented by at least fifteen family members encoded by the human genome, and many family members share relatively conserved amino acid sequences near the N-terminal domain, whereas the C-terminal domain of some of the family members are functionally diverse, which includes nuclear localization signal and DNA binding domain. Although sharing relatively conserved amino acid sequence, some members of the PNMA family exhibit functional divergence, including members that are agonist or antagonist of apoptosis signalling when expressed in cancer cells. It's likely that the PNMA family represents a novel set of proteins that are involved in regulation of cell growth, apoptosis, or cell fate determination in different cell types, including brain, testis, and heart where PNMA family members have been shown to express at relatively high levels. Further investigations are required to determine functional relationship of other PNMA members, and their specific role in human cells which could lead to a better understanding of the functions of PNMA family.

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