Journal of Neuroimmunology 169 (2005) 172 – 176

Journal of Neuroimmunology

www.elsevier.com/locate/jneuroim

Short communication

The human PNMA family: Novel neuronal proteins implicated in paraneoplastic neurological disease

Martina Schüller [a], Dieter Jenne [b], Raymond Voltz [a,c,*]

[a] *Department of Clinical Neuroimmunology, Klinikum Grosshadern,Ludwig-Maximilians-University, Marchioninistr. 15, 81377 Munich, Germany*
[b] *Department of Neuroimmunology, Max-Planck-Institute of Neurobiology, Am Klopferspitz 18a, 82152 Martinsried, Germany*
[c] *Department of Palliative Medicine, University Hospital, 50924 Cologne, Germany*

Received 18 March 2005; received in revised form 25 July 2005; accepted 1 August 2005

Abstract

Using sera from patients with paraneoplastic neurological syndromes, several novel neuronal autoantigens such as the paraneoplastic Ma antigens (PNMA) have been identified. Here, we report the correction and completion of the previously published prototype member PNMA1, the brain and testis restricted expression of a third member (PNMA3) and the sequences for further partially uncharacterized members of this novel neuronal protein family. Murine and rat orthologs exist for this protein family. By analogy to the pro-apoptotic MOAP1, similar functional interactions may exist between members of the PNMA family and the bcl-2 family.

Keywords: Paraneoplastic; PNMA protein family; Neuronal antigens

1. Introduction

Since identification of the Hu antibody in 1985, an increasing number of clinically relevant antineuronal antibodies have been described as markers of a paraneoplastic etiology of a given neurological syndrome (Graus et al., 1985; Voltz, 2002; Darnell and Posner, 2003). Besides their clinical relevance, many of these antibody reactivities have been used to identify novel neuronal proteins such as HuD, NOVA, cdr2 or CRMP-5 (Darnell, 1996; Yu et al., 2001). Under physiological circumstances, these proteins (onconeuronal proteins) are expressed in immune privileged sites. Once they become "ectopically" expressed in systemic tumors, this may be one factor to trigger an autoimmune response eventually leading to the paraneoplastic neurological syndrome (Voltz, 2002; Darnell and Posner, 2003). Besides the highly specific antibody response, T cells, especially of cytotoxic phenotype, are involved in the pathogenesis (Voltz et al., 1998; Pellkofer et al., 2004).

Recently, two novel antibody reactivities (anti-Ma and anti-Ta/Ma2) were described, and these sera were used to clone the paraneoplastic Ma (PNMA) proteins PNMA1, PNMA2 and PNMA3 (Dalmau et al., 1999; Voltz et al., 1999; Rosenfeld et al., 2001). Here, we report the correction of the previously published sequence for PNMA1 protein and use this sequence for identifying several members of this novel neuronal protein family in humans and other species. Using Northern blot, we complete the expression analysis of PNMA proteins. Considering the homology between PNMA proteins and an apoptosis regulating protein, we hypothesize an involvement of PNMA proteins in apoptosis.

2. Materials and methods

2.1. Sequencing

Plasmid pBSMa1 (Dalmau et al., 1999) was prepared fom *E. coli* using the Perfectprep Plasmid Mini Kit

* Corresponding author. Current address: Department of Palliative Medicine, University Hospital, D-50924 Köln, Germany. Tel.: +49 221 478 3361; fax: +49 221 478 87579.
E-mail address: raymond.voltz@uk-koeln.de (R. Voltz).

0165-5728/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2005.08.019

(Eppendorf, Hamburg) and was sequenced from both strands by Primer walking (TopLab, Martinsried, Germany).

2.2. Sequence analysis

Used internet databases: http://www.ncbi.nlm.nih.gov, http://www.ebi.ac.uk.

2.3. Northern blot

A PNMA3-specific cDNA probe (GenBank AF083116, bp1040–1416) was generated by PCR from plasmid pBSMA5, gel purified (QIAquick Gel Extraction Kit, Qiagen, Hilden) and used to hybridize Human Multiple Tissue Northern blot and Human Multiple Tissue Northern blot II (Clontech, Heidelberg). PNMA3 primers were F: CCACTTTAGGTCCAGATA and R: CTTGGCCTTGG-ACTTG. As a positive control, a β-actin probe (Clontech, Heidelberg) was used. Probes were random prime labelled (50×10^6 dpm) using the Rediprime System (Amersham Pharmacia Biotech, Freiburg) and Redivue [^{32}P]dCTP. Unincorporated labelled nucleotides were removed through gravity-flow chromatography using a Sephadex G-50 column (Nick Column, Amersham Pharmacia Biotech, Freiburg). Hybridization was carried out overnight at 68 °C in ExpressHyb Hybridization Solution (Clontech, Heidelberg). Blots were washed at room temperature with 2× SSC, 0.05% SDS and exposed to Kodak film at −80 °C.

3. Results

3.1. Human PNMA1 sequence

The human PNMA1 sequence had to be corrected (Fig. 1, GenBank accession AF320308). In comparison with the original publication (Dalmau et al., 1999), the A at position 770 was deleted and an additional C inserted after position 798 (numbering of the original sequence entry AF037364, version AF037364.1 GI: 4104633) resulting in a new amino acid (aa) sequence. In addition, G and C at positions 1223 and 1272 were not confirmed, instead an A after position 1274 was inserted leading to two frameshifts with the consequence of a new stop codon at position 1333–1335 and an open reading frame coding for 353 instead of 330 aa.

3.2. Expression pattern of human PNMA3

Northern blot analysis of the expression pattern of PNMA3 demonstrated a major (4.4 kb) and a smaller (3.5 kb) transcript in brain (Fig. 2). In testis, one major signal (5.5 kb) with two weaker signals at about 4.4 and 3.5 kb was found. With longer exposure time, an approximately 4.4 kb transcript in kidney and a weak signal in ovary of about 5 kb was obtained, perhaps due to neuronal contamination.

3.3. The human PNMA protein family

Using the putative PNMA1 protein sequence, we searched databases for other human homologous proteins (up to September 2004) of more than 40% aa identity (Fig. 3, Table 1).

The second member of this family is PNMA2 (XP_376764, Q9UL42, AAG28165, AAH62301). MM2 (AAD02098), Ma3 (AAF05625) and Ma4 (AAF05626) all represent different but overlapping sections of the PNMA2 protein with nearly 100% identity to it. BAA74906 has 10 additional aa due to an unidentified start codon.

NP_037496 and AAF05627 represent the third member of this family, PNMA3, also known as Ma5. A further clone, CAB66812, derived from testis is identical to PNMA3 between aa 1 and 335 and then diverges. This may represent a splice variant (see below).

A fourth member of the PNMA family, modulator of apoptosis-1 (MOAP1) alias PNMA4 (AAH15044, NP_071434, AAG31786, Q96BY2) was identified (Tan et al., 2001). Clone BAB14788 represents the C-terminus of this protein.

PNMA1, 2, 3 and 4 are expressed in human adult brain as demonstrated by Northern blot analysis (this paper, see Section 3.2 and Dalmau et al., 1999; Voltz et al., 1999; Rosenfeld et al., 2001) and immunohistochemistry for PNMA1 and PNMA2 (Dalmau et al., 1999; Voltz et al., 1999). PNMA-1, -2, and -3 were found through screening of expression libraries using sera from patients with paraneoplastic syndromes, the MOAP1/PNMA4 protein was identified through a yeast-two-hybrid screen for Bax-associating proteins.

Further homologous protein sequences for which no experimental data exist so far can be found in the databases. A putative fifth member is PNMA5 alias KIAA1934 or BJ-HCC-25 tumor antigen (BAB67827, NP_443158, AAM82754). Differences in the predicted number of aa are due to an unclear start codon. The sequence of this hypothetical protein is derived from a tumor antigen mRNA, that was investigated during analysis of differentially expressed genes in carcinomas (GenBank AAM82754). The homology to human PNMA1 is 45%, to human PNMA2 43%, to human PNMA3 40% and to human MOAP1/PNMA4 44%. Homologous sequences in mice and rat could be found indicating a conserved protein.

A further putative member of this protein family is called PNMA6A (AAH07631, NP_116271, BAB70902). The homology to human PNMA1 is 44%, to PNMA2 41%, to PNMA3 45%, to MOAP1 40% and to PNMA5 40%. PNMA6A proteins in other species were not identified yet.

Further hypothetical proteins and not clearly identified homologous sequences in other species are found. Whether these sequences are coding for functional proteins remains to be seen.

```
   1 atggcgatgacactgttggaagactggtgccgggggatggatgtg
     M  A  M  T  L  L  E  D  W  C  R  G  M  D  V
  46 aactcccagagagctctgttagtctggggcatcccagtgaactgt
     N  S  Q  R  A  L  L  V  W  G  I  P  V  N  C
  91 gatgaggctgaaatcgaagagaccctccaggctgcgatgccccag
     D  E  A  E  I  E  E  T  L  Q  A  A  M  P  Q
 136 gtctcctaccgaatgcttgggagaatgttctggagggaagaaaat
     V  S  Y  R  M  L  G  R  M  F  W  R  E  E  N
 181 gcgaaagcagccttattagagctcactggcgctgtagattacgcc
     A  K  A  A  L  L  E  L  T  G  A  V  D  Y  A
 226 gcgatccccagggagatgccgggcaaaggaggggtctggaaagtg
     A  I  P  R  E  M  P  G  K  G  G  V  W  K  V
 271 ttatttaagcccccaacttctgatgctgaatttttagaaagattg
     L  F  K  P  P  T  S  D  A  E  F  L  E  R  L
 316 cacctcttcctagctagagaggggtggaccgtgcaagatgttgcc
     H  L  F  L  A  R  E  G  W  T  V  Q  D  V  A
 361 cgtgtccttgggtttcagaaccctactccgaccccgggcccagag
     R  V  L  G  F  Q  N  P  T  P  T  P  G  P  E
 406 atgccagcagagatgctaaactatattttggataatgttattcag
     M  P  A  E  M  L  N  Y  I  L  D  N  V  I  Q
 451 cctcttgttgagtccatatggtacaagaggctgacacttttctcg
     P  L  V  E  S  I  W  Y  K  R  L  T  L  F  S
 496 gggagggacatcccagggcctggagaggaaaccttgatccctgg
     G  R  D  I  P  G  P  G  E  E  T  F  D  P  W
 541 ctggagcacactaatgaggtcctagaggagtggcaggtgtccgat
     L  E  H  T  N  E  V  L  E  E  W  Q  V  S  D
 586 gtagaaaagaggcggcggttgatggagagtcttagaggccccgcc
     V  E  K  R  R  R  L  M  E  S  L  R  G  P  A
 631 gctgatgttattcgcatccttaagtccaacaaccccgcgataacc
     A  D  V  I  R  I  L  K  S  N  N  P  A  I  T
 676 actgccgaatgcctgaaggcgcttgagcaggtgtttgggagcgtt
     T  A  E  C  L  K  A  L  E  Q  V  F  G  S  V
 721 gagagctctagggatgcccagatcaaatttctgaacacttatcag
     E  S  S  R  D  A  Q  I  K  F  L  N  T  Y  Q
 766 aacccgggagaaaaattgtctgcttatgtcattcgtctggagcct
     N  P  G  E  K  L  S  A  Y  V  I  R  L  E  P
 811 ctgctacagaaggtggtagagaaggggccattgataaagataat
     L  L  Q  K  V  V  E  K  G  A  I  D  K  D  N
 856 gtgaaccaggcccgcctagagcaggtcattgccggggccaaccac
     V  N  Q  A  R  L  E  Q  V  I  A  G  A  N  H
 901 agcggggccatccgaaggcagctgtggcttaccggggctggggaa
     S  G  A  I  R  R  Q  L  W  L  T  G  A  G  E
 946 gggccagccccaaacctctttcagttgctggtgcagatccgtgag
     G  P  A  P  N  L  F  Q  L  L  V  Q  I  R  E
 991 gaggaagccaaggaggaggaggaggaggctgaggccacccttctg
     E  E  A  K  E  E  E  E  E  A  E  A  T  L  L
1036 cagttaggcctggaagggcacttctga 1062
     Q  L  G  L  E  G  H  F  *
```

Fig. 1. The corrected PNMA1 sequence. Nucleotide sequence of the coding region and the corresponding amino acid sequence are shown.

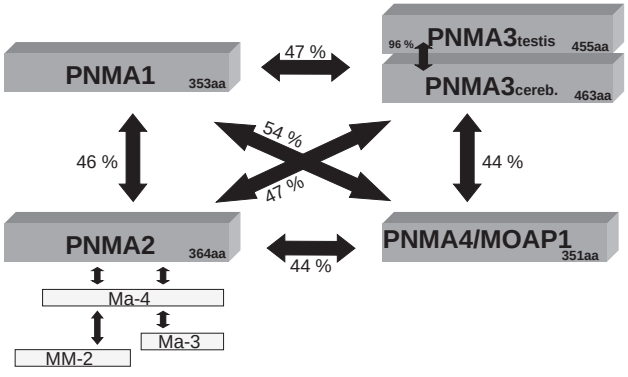

Fig. 2. Homology of different members of the human PNMA protein family. Only proteins with experimental evidence for protein expression are shown. Identity of the proteins on amino acid level is given in percent. PNMA1 and MOAP1 share greatest identity (54%). MM2, Ma3 and Ma4 represent different sections of PNMA2. The putative splice variants of PNMA3 are named according to the tissues they were cloned from.

3.4. PNMA proteins in other species

PNMA proteins can also be found in mice and rat (Table 2). The homology between the corresponding proteins is greater between species than within one species.

4. Discussion

We corrected the PNMA1 sequence and identified new members of a novel neuronal protein family. Furthermore, we show mRNA expression of PNMA3 mainly in brain and testis.

Identifying the correct protein sequence of all PNMA proteins is essential for studies on the immunology of these proteins. Indeed, T cells are involved in the pathogenesis of the paraneoplastic disorders associated with an immune

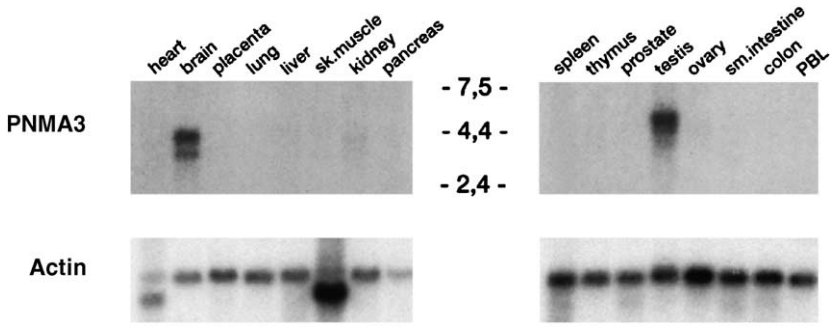

Fig. 3. Northern blot analysis of PNMA3. Poly A$^+$RNA blots of normal human tissues (Clontech) were hybridized with a ^{32}P-labelled specific cDNA probe for PNMA3 and a β-actin probe.

reaction against PNMA proteins (Voltz et al., 1998; Pellkofer et al., 2004). Differential recognition of PNMA proteins has been demonstrated for PNMA1 and PNMA2 on the B-cell level, as all patients' sera recognise PNMA2, but only some PNMA1 or PNMA3 (Dalmau et al., 1999, 2004; Voltz et al., 1999; Rosenfeld et al., 2001; Hoffmann et al., 2004). Preabsorbing patient sera with recombinant PNMA1 block the reactivity against PNMA1, but not at all against PNMA2 showing that there is no cross-reactivity of antibodies between these two proteins (Dalmau et al., 1999; Voltz et al., 1999).

Examination of PNMA1-related protein sequences indicated that at least four genes exist that code for this protein family. These genes are expressed in the brain. PNMA1

Table 1
Accession numbers of proteins of PNMA family members in human, mouse and rats

	Human	Mouse	Rat
PNMA1	Q8ND90 353 aa AAN05100 353 aa AAH39577 353 aa NP_006020 353 aa AAD13810 353 aa	XP_127024 353 aa BAB30762 353 aa Q8C1C8 353 aa BAC25885 353 aa 91%[a]	NP_570833 353 aa Q8VHZ4 353 aa AAL73196 353 aa 93%
PNMA2	XP_376764 364 aa Q9UL42 364 aa AAG28165 364 aa AAH62301 364 aa BAA74906[b] 373 aa	NP_780707 365 aa Q8BHK0 365 aa BAC31626 365 aa BAC31700 365 aa AAH65116 365 aa 78%	XP_224322 364 aa 79%
PNMA3	NP_037496 463 aa AAF05627 463 aa CAB66812b 455 aa	NP_694809 466 aa AAH36726 466 aa 74%	XP_219736 465 aa 74%
PNMA4/MOAP1	NP_071434 351 aa Q96BY2 351 aa AAH15044 351 aa AAG31786 351 aa	AAH55374 352 aa NP_071718 352 aa Q9ERH6 352 aa AAG31787 352 aa BAB31810 352 aa AAH14715 352 aa 77%	XP_234474 352 aa XP_225513b 345 aa 76%
PNMA5	BAB67827 452aa NP_443158b 448 aa AAM82754 448 aa	XP_358214 711 aa XP_359410b 618 aa 53%	XP_219735 436 aa 56%
PNMA6	AAH07631 399 aa BAB70902 399 aa NP_116271 399 aa		

[a] % homology to corresponding human aa sequence is given for mice and rat sequences.
[b] Sequence differences may be due to sequencing errors, splice variants or different transcriptional starts used for prediction.

Table 2
Homology of PNMA proteins among different species

Mouse		Human		Rat
91%	←	PNMA1	→	93%
78%	←	PNMA2	→	79%
74%	←	PNMA3	→	74%
77%	←	PNMA4/MOAP1	→	76%
53%	←	PNMA5	→	56%
n.y.i.	←	PNMA6	→	n.y.i.

n.y.i. not yet identified.

and PNMA3 are also expressed in adult testis and PNMA3 is transcribed in some more tissues (this paper, Section 3.2; Dalmau et al., 1999; Voltz et al., 1999). MOAP1/PNMA4 is expressed in brain and heart, and at lower levels in various tissues (Tan et al., 2001).

Using RT-PCR, PNMA3 transcripts have been found in brain, testis, trachea, kidney and milder expression in heart (Rosenfeld et al., 2001). This is consistent with our Northern blot data showing different PNMA3 transcripts in brain and testis and with less intensity in kidney and ovary. The second 5.5 kb transcript may have a longer 3′UTR and/or 5′UTR, may be the result of a second tissue-specific promoter or a second noncanonical poly (A) signal. Transcript length differences between different tissues are not unusual. For example, mRNA of BAP-1, a potential tumor suppressor gene, is longer in testis than in all other tissues (Jensen et al., 1998).

PNMA genes are intronless along their entire region of homology except for PNMA3 from testis. Most of the polypeptide chain of PNMA3 from testis shared with all family members is encoded by a single exon but the C-terminal extension is encoded by an additional exon.

PNMA1 and PNMA2 are expressed in tumors of patients suffering from a paraneoplastic neurological syndrome, but PNMA3 and MOAP1/PNMA4 have not yet been studied (Dalmau et al., 1999; Voltz et al., 1999).

MOAP1/PNMA4 was identified as a Bax-associating protein that induced apoptosis in cultured mammalian cells (Tan et al., 2001). The great homology between MOAP1 and the other PNMA proteins suggests a role in apoptosis for PNMA proteins.

Acknowledgements

This work was supported by Deutsche Krebshilfe (10-1607-Vo1), the Wilhelm-Sander Stiftung (Az. 2002.121.1), the DFG (SFB571, projects A4 and D7) and the Hermann and Lilly Schilling Foundation.

References

Dalmau, J., Gultekin, S.H., Voltz, R., Hoard, R., DesChamps, T., Balmaceda, C., Batchelor, T., Gerstner, E., Eichen, J., Frennier, J., Posner, J.B., Rosenfeld, M.R., 1999. Ma1, a novel neuron- and testis-specific protein, is recognized by the serum of patients with paraneoplastic neurological disorders. Brain 122, 27–39.

Dalmau, J., Graus, F., Villarejo, A., Posner, J.B., Blumenthal, D., Thiessen, B., Saiz, A., Meneses, P., Rosenfeld, M.R., 2004. Clinical analysis of anti-Ma2-associated encephalitis. Brain 127, 1831–1844.

Darnell, R.B., 1996. Onconeural antigens and the paraneoplastic neurologic disorders: at the intersection of cancer, immunity, and the brain. Proc. Natl. Acad. Sci. U. S. A. 93, 4529–4536.

Darnell, R.B., Posner, J.B., 2003. Paraneoplastic syndromes involving the nervous system. N. Engl. J. Med. 16 (349), 1543–1554.

Graus, F., Cordon-Cardo, C., Posner, J.B., 1985. Neuronal antinuclear antibody in sensory neuronopathy from lung cancer. Neurology 35, 538–543.

Hoffmann, L., Ciszewski, R., Voltz, R., 2004. Anti-Ma and anti-Ta positive paraneoplastic neurological syndromes: an update on 14 newly diagnosed cases. J. Neurol. III (10) (abstract).

Jensen, D.E., Proctor, M., Marquis, S.T., Gardner, H.P., Ha, S.I., Chodosh, L.A., Ishov, A.M., Tommerup, N., Vissing, H., Sekido, Y., Minna, J., Borodovsky, A., Schultz, D.C., Wilkinson, K.D., Maul, G.G., Barlev, N., Berger, S.L., Prendergast, G.C., Rauscher, F.J. III, 1998. BAP1: a novel ubiquitin hydrolase which binds to the BRCA1 RING finger and enhances BRCA1-mediated cell growth suppression. Oncogene 16, 1097–1112.

Pellkofer, H., Schubart, A.S., Höftberger, R., Schutze, N., Pagany, M., Schüller, M., Lassmann, H., Hohlfeld, R., Voltz, R., Linington, C., 2004. Modeling paraneoplastic disease: onconeuronal antigen specific T cells mediate autoimmune encephalomyelitis in the rat. Brain 127 (8), 1822–1830.

Rosenfeld, M.R., Eichen, J.G., Wade, D.F., Posner, J.B., Dalmau, J., 2001. Molecular and clinical diversity in paraneoplastic immunity to Ma proteins. Ann. Neurol. 50, 339–348.

Tan, K.O., Tan, K.M., Chan, S.L., Yee, K.S., Bevort, M., Ang, K.C., Yu, V.C., 2001. MAP-1: a novel pro-apoptotic protein containing a BH3-like motif that associates with Bax through its Bcl-2 homology domains. J. Biol. Chem. 276, 2802–2807.

Voltz, R., 2002. Paraneoplastic neurological syndromes: an update on diagnosis, pathogenesis and therapy. Lancet Neurol. 1, 294–305.

Voltz, R., Dalmau, J., Posner, J.B., Rosenfeld, M.R., 1998. T-cell receptor analysis in anti-Hu associated paraneoplastic encephalomyelitis. Neurology 51, 1146–1150.

Voltz, R., Gultekin, S.H., Rosenfeld, M.R., Gerstner, E., Eichen, J., Posner, J.B., Dalmau, J., 1999. A serologic marker of paraneoplastic limbic and brain-stem encephalitis in patients with testicular cancer. N. Engl. J. Med. 340, 1788–1795.

Yu, Z., Kryzer, T.J., Griesmann, G.E., Kim, K., Benarroch, E.E., Lennon, V.A., 2001. CRMP-5 neuronal autoantibody: marker of lung cancer and thymoma-related autoimmunity. Ann. Neurol. 49, 146–154.