Cloning, expression and localization of human BM88 shows that it maps to chromosome 11p15.5, a region implicated in Beckwith–Wiedemann syndrome and tumorigenesis

Maria GAITANOU*, Pasquale BUANNE†, Christina PAPPA*, Niki GEORGOPOULOU*, Avgi MAMALAKI*, Felice TIRONE† and Rebecca MATSAS*[1]

*Department of Biochemistry, Hellenic Pasteur Institute, 127 Vassilissis Sofias Avenue, 115 21 Athens, Greece, and †Istituto di Neurobiologia, Viale Marx 43, Rome 00137, Italy

Porcine BM88 is a neuron-specific protein that enhances neuroblastoma cell differentiation *in vitro* and may be involved in neuronal differentiation *in vivo*. Here we report the identification, by Western blotting, of homologous proteins in human and mouse brain and the isolation of their respective cDNAs. Several human and mouse clones were identified in the EST database using porcine BM88 cDNA as a query. A human and a mouse EST clone were chosen for sequencing and were found both to predict a protein of 149 amino acids, with 79.9% reciprocal identity, and 76.4% and 70.7% identities to the porcine protein, respectively. This indicated that the clones corresponded to the human and mouse BM88 homologues. *In vitro* expression in a cell-free system as well as transient expression in COS7 cells yielded polypeptide products that were recognized by anti-BM88 antibodies and were identical in size to the native BM88 protein. Northern-blot analysis showed a wide distribution of the gene in human brain whereas immunohistochemistry on human brain sections demonstrated that the expression of BM88 is confined to neurons. The initial mapping assignment of human BM88 to chromosome 11p15.5, a region implicated in Beckwith–Wiedemann syndrome and tumorigenesis, was retrieved from the UniGene database maintained at the National Centre for Biotechnology Information (NCBI, Bethesda, MD, U.S.A.). We confirmed this localization by performing fluorescence *in situ* hybridization on BM88-positive cosmid clones isolated from a human genomic library. These results suggest that *BM88* may be a candidate gene for genetic disorders associated with alterations at 11p15.5.

Key words: mouse BM88, neuroblast proliferation, neuron-specific protein, neuronal differentiation, neuronal lineage.

INTRODUCTION

The generation of the nervous system requires several active gene-regulated processes, including cell proliferation of progenitor cells, subsequent withdrawal from the cell cycle and differentiation to distinctive neuronal phenotypes. During these complex processes, a large number of genes are expressed in a predetermined and co-ordinated manner [1,2]. In the mammalian central nervous system, neurogenesis occurs largely in ventricular zones [3] and the external germinal zone of the cerebellar cortex [4,5], where neural precursor cells initially undergo rapid proliferation and, subsequently, give rise to post-mitotic cells that differentiate into neurons [6,7]. At this point, neural precursor cells commit to specific differentiation pathways and show a tightly regulated inverse relationship between cell proliferation and differentiation [8]. The molecular mechanisms that control these two inter-related but opposing processes are beginning to be delineated and the identification of molecules involved in such developmental events has emphasized the important inverse relationship between neuronal differentiation and tumorigenesis [9–12].

We have previously identified BM88, a neuron-specific molecule, which enhances neuroblastoma cell differentiation *in vitro* [13] and appears to be involved in neuronal differentiation *in vivo* [14]. BM88 protein, first identified by means of a monoclonal antibody in porcine brain [15,16], is expressed widely in the nervous systems of the pig and the rat [15,17]. It is an integral membrane protein, composed of two identical polypeptide chains, of 22–23 kDa depending on the species tested. These polypeptide chains are apparently not glycosylated and are linked together by disulphide bridges. Electron-microscopic observations in the adult rat brain have shown that BM88 is associated mainly with the limiting membrane of a number of intracellular organelles, such as the endoplasmic reticulum, small electron-lucent vesicles and the mitochondrial outer membrane, but is also present at the plasma membrane, especially at the level of synaptic densities [17].

Developmental studies have demonstrated that the molecule is detected at the onset of neurogenesis in the rat brain, and that it is retained in the adult. In the embryonic brain, BM88 is expressed by both proliferating neuroblasts and early differentiating neurons [17,18], a fact that renders it an early marker of the neuronal lineage and implies a possible regulatory role in the processes of neuronal specification and differentiation. A first confirmation of this hypothesis came from the cDNA cloning of the molecule from porcine brain and expression studies, which revealed that BM88 slows down the proliferation and enhances the differentiation of mouse neuroblastoma cells *in vitro* [13]. In particular, stably transfected Neuro 2a cells overexpressing BM88 exhibit a significant change in morphology reflected by enhanced process outgrowth and a slower rate of division. Moreover, these cells show different sensitivity, when compared with the parental Neuro 2a, to polypeptide growth factors, such as basic fibroblast growth factor and glial-cell-line-derived neurotrophic factor

Abbreviations used: BWS, Beckwith–Wiedemann syndrome; GDNF, glial-cell-line-derived neurotrophic factor.

[1] To whom correspondence should be addressed (e-mail rmatsa@mail.pasteur.gr).

[2] Sequence data for the human and mouse BM88 cDNAs have been deposited in the EMBL/GenBank Nucleotide Sequence Databases under accession numbers AF235030 and AF243130, respectively.

(GDNF), which are known to play pivotal roles during brain development *in vivo* [19]. Furthermore, in the presence of differentiation agents such as sucrose or retinoic acid, an accelerated morphological and molecular differentiation of the transfected cells was observed [13]. Thus overexpression of the molecule in a cell line that is inherently capable of extending neurites given the appropriate conditions unveiled an ability of BM88 to influence cell proliferation and differentiation.

Here we have sought to identify homologous proteins in, and clone their cDNAs from, human and mouse brains to facilitate further research on the physiological role of this molecule in the nervous system. By making use of our knowledge of porcine BM88 and searching through EST databases, we have now cloned and sequenced the homologous human and mouse BM88 cDNA molecules and we have investigated their expression in brain by Northern- and Western-blot analyses and immunohistochemistry. Moreover, we have mapped the human *BM88* gene to chromosome 11p15.5, a region associated with the overgrowth genetic disorder Beckwith–Wiedemann syndrome (BWS) and several types of embryonal, childhood and adult cancers [20–22]. Given the previously characterized functional properties of BM88 in arresting cell growth and enhancing differentiation, our results suggest that *BM88* may be a useful candidate gene for genetic disorders associated with alterations at 11p15.5.

EXPERIMENTAL

Sequence analysis and computer-assisted search of databases

The human (clone identity, IMAGE 34819) and mouse (IMAGE 317735) BM88 cDNA clones were obtained from the U.K. Human Genome Mapping Project Resource Centre (Hinxton, Cambridge, U.K.). They were cloned into Lafmid BA and pT7T3D vectors, respectively. Sequence analysis was carried out on both strands by the dideoxy nucleotide method [23], using synthetic oligonucleotides and Sequenase version 2.0 (United States Biochemical Corporation), as described by the manufacturer. For sequence analysis and similarity searches PCGENE software and BlastN, BlastP and FASTA algorithms were used against the GenBank and EMBL databases. Analysis of the deduced amino acid sequence for identification of functional domains was performed by the ProfileScan of PROSITE (http://www.isrec.isb-sib.ch).

Northern-blot analysis

Total RNA was isolated from HeLa cells, adult mouse brain or from post-mortem cerebellum of a 50-year-old man by the guanidinium isothiocyanate method [24], separated by electrophoresis on agarose gel and transferred on to Zeta-probe nylon membrane (BioRad). Human BM88 cDNA and/or a 330 bp-long PCR-generated fragment corresponding to nucleotides 310–640 of the coding region of the human BM88 cDNA molecule were ^{32}P-labelled with a random-priming kit (Amersham) and used as probes for hybridization, as described in [25]. For regional distribution of BM88 in human brain, a Northern-blot membrane was used from Clontech (MTN human brain II blot 7755-1) loaded with 2 μg/lane poly(A)$^+$ RNA. The Northern blots were then stripped and probed with human β-actin cDNA as a control for loaded mRNA.

Western-blot analysis

Preparation of human, porcine, rat and mouse brain membrane fractions for antigen identification by immunoblotting were obtained as described previously [15]. Samples (30 μg of pig and 100 μg of rat, mouse or human brain membranes) were run on SDS/PAGE (12% gels) and transferred on to nitrocellulose sheets. Immunoblotting was carried out as described previously [17], using specific mono- and poly-clonal antibodies against BM88 followed by peroxidase-conjugated secondary antibodies (Amersham). The production, characterization and specificity of monoclonal and affinity-purified polyclonal anti-BM88 antibodies, which were raised against the porcine BM88 protein, have been described previously [15,17].

In vitro transcription and translation

The human BM88 cDNA was excised from Lafmid BA vector and was subcloned into *Hin*dIII and *Not*I restriction sites of the pBluescript KS II vector (Stratagene). RNAs were transcribed *in vitro* using T3 and T7 RNA polymerases (Promega) according to the manufacturer's protocols. For sense or antisense RNAs, the plasmid DNA was linearized in the *Not*I or *Hin*dIII sites, respectively. *In vitro* translation was carried out with the Reticulocyte Lysate System (Promega) in the presence of ^{35}S-labelled methionine. For immunoprecipitation with the affinity-purified polyclonal anti-BM88 antibody, translation products were diluted 10-fold in 10 mM Tris/HCl, 500 mM NaCl and 0.1% Triton X-100, pH 7.5, containing 0.3 TIU (trypsin-inhibitor units) aprotinin/ml (1 TIU will decrease the activity of 2 trypsin units by 50%, where 1 trypsin unit releases 1 μmol of *N*-benzoyl-D,L-arginine *p*-nitroanilide/min at pH 7.8 and 25 °C). The mixture was then absorbed for 2 h at 4 °C with BSA coupled to Sepharose, followed by overnight incubation at 4 °C with 50 μl of the polyclonal anti-BM88 antibody. The antigen–antibody complex was then precipitated by incubation at room temperature for 2 h with 20 μl of Protein A–Sepharose. Immune complexes were collected by centrifugation and washed. Proteins were recovered by boiling in SDS loading buffer and were analysed by SDS/PAGE (15% gels) and autoradiography.

Expression in COS7 cells and immunofluorescence

For expression studies, the entire human, mouse and porcine BM88 cDNAs were subcloned into the pcDNA 3 vector (Invitrogen) at *Hin*dIII and *Not*I sites (human), *Eco*RI and *Not*I sites (mouse) or *Hin*dIII and *Xba*I sites (pig). COS7 cells (ATCC) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum and antibiotics. For transfections, cells were plated on to poly-L-lysine-coated sterile coverslips placed in six-well plates at a density of 1×10^5 cells/well and were allowed to reach 70% confluence. Transfections were performed for 5 h with 1 μg of plasmid DNA and LipofectAMINE reagent in accordance with the manufacturer's instructions (Gibco). The transfection medium was then removed and cells were fed with complete growth medium for 48 h. For immunofluorescence labelling, cells were fixed in 4% paraformaldehyde and incubated overnight at 4 °C with BM88 polyclonal antibody followed by 2 h of incubation with FITC-conjugated anti-rabbit secondary antibody [13]. Immunofluorescence microscopy was carried out using a Zeiss Axiophot photomicroscope.

Immunohistochemistry on tissue sections

Post-mortem human cerebellum of a 45-year-old male individual was obtained from the University of Athens Medical School. Small pieces of tissue were fixed by overnight immersion at 4 °C in 4% paraformaldeyde in PBS. After fixation, tissues were embedded in paraffin (Paraplast, BDH) and sections, 8 μm thick,

were cut and collected on glass slides coated with TESPA (3-aminopropyltriethoxy-silane, Sigma). Paraffin sections were immunostained with the monoclonal or affinity-purified polyclonal anti-BM88 antibodies [15,17] followed by peroxidase-conjugated secondary antibodies (Amersham). The reaction was developed in DAB (3,3′-diaminobenzidine hydrochloride) with nickel enhancement as described previously [17]. In addition, a mouse monoclonal antibody against neurofilament protein was used [26].

Human *BM88* gene cloning and chromosomal localization

A human genomic cosmid library (SuperCos1, Stratagene) was screened using ^{32}P-labelled human BM88 cDNA as a probe. Several positive genomic clones were identified and were further confirmed by Southern hybridization. One of these, containing the whole *BM88* gene (O. Papadodima, A. Mamalaki and R. Matsas, unpublished work), was used for physical mapping on human chromosomes. Chromosomal localization of the human *BM88* gene was performed by Genome Systems (St. Louis, MO, U.S.A.). The cosmid clone containing the *BM88* gene was labelled with digoxigenin dUTP by nick translation. Labelled probe was combined with sheared human DNA and hybridized to metaphase chromosomes derived from phytohaemagglutinin-stimulated peripheral blood lymphocytes in a solution containing 50 % formamide, 10 % dextran sulphate and 2 × SSC (where 1 × SSC is 0.15 M NaCl/0.015 M sodium citrate). Specific hybridization signals were detected by incubating the hybridized slides in fluorescein-conjugated anti-digoxigenin antibodies followed by counterstaining with DAPI (4,6-diamidino-2-phenylindole).

RESULTS

Detection of BM88 protein homologues in human and mouse brains

To identify the BM88 proteins in human and mouse, we performed Western-blot experiments using the monoclonal and polyclonal anti-BM88 antibodies. As we have shown previously to occur in immunoblots of rat brain membranes [17], the monoclonal anti-BM88 does not cross-react with any polypeptide band on human or mouse brain membrane preparations although it efficiently recognizes the porcine protein (Figure 1A). In contrast, the polyclonal anti-porcine BM88 recognizes polypeptides in the rat, human and mouse brains with a slightly higher molecular mass (23 kDa) than that of the corresponding porcine protein (22 kDa; Figure 1B). It is noteworthy that the 44 kDa homodimer that we have identified previously is always visible in the porcine molecule, even under reducing SDS/PAGE conditions, while it is hardly detectable in the other species. However, in immunoblots obtained after non-reducing SDS/PAGE, the polyclonal anti-BM88 bound to a polypeptide of approx. 46 kDa in human and mouse preparations (results not shown). These results indicate that, as is the case with the porcine and rat proteins [13,15,17], the human and mouse BM88

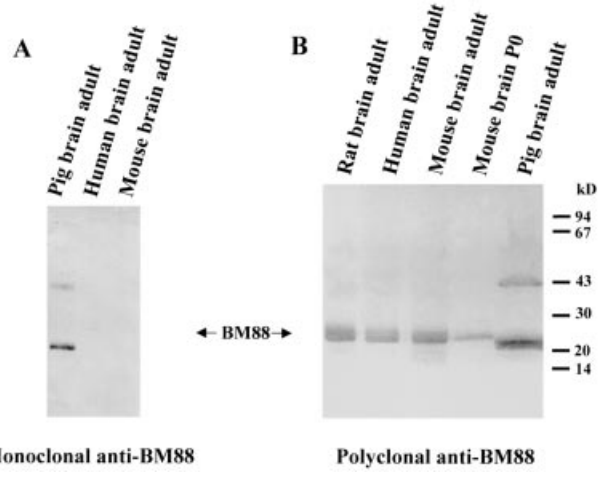

Figure 1 Western-blot analysis of brain membrane preparations from pig, human, rat and mouse

Immunoblotting was performed using the monoclonal (**A**) and polyclonal (**B**) anti-porcine BM88 antibodies. Both antibodies detect a 22 kDa polypeptide band in the pig while a 23 kDa band is detected in all other species with the polyclonal antibody. The S–S linked 44 kDa homodimeric polypeptide is also visible in the pig. P0, day of birth.

```
ACGAGGCGCCCATAGAATGCCCAGGGGCGACAAACTGTCCTGAGCCCCTCTGGTGCAGCC 60
ACCTGCCTGTCCCATACGCCCCGCCCACCATGGAGTCCAGAGGGAAGTCAGCCAGCAGCC 120
                              M  E  S  R  G  K  S  A  S  S  10
                                                          o

CCAAGCCCGACACCAAGGTGCCCCAGGTCACCACCGAGGCCAAGGTACCCCCGGCAGCCG 180
P  K  P  D  T  K  V  P  Q  V  T  T  E  A  K  V  P  P  A  A  30

ATGGGAAAGCCCCCTTGACCAAGCCCTCGAAGAAGGAGGCCCCGGCCGAGAAGCAGCAGC 240
D  G  K  A  P  L  T  K  P  S  K  K  E  A  P  A  E  K  Q  Q  50
                              o  •

CGCCAGCAGCCCCCACCACGGCACCTGCCAAGAAGACCTCGGCCAAGGCCGACCCTGCCC 300
P  P  A  A  P  T  T  A  P  A  K  K  T  S  A  K  A  D  P  A  70
                                 ϒ        o

TTCTCAACAACCACAGCAACCTGAAGCCAGCCCCCACGGTCCCCAGCAGTCCCGATGCAA 360
L  L  N  N  H  S  N  L  K  P  A  P  T  V  P  S  S  P  D  A  90
      □                                    •

CCCCGGAGCCCAAGGGTCCTGGGGACGGGGCGGAGGAAGATGAGGCTGCCAGTGGGGGGC 420
T  P  E  P  K  G  P  G  D  G  A  E  E  D  E  A  A  S  G  G  110

CTGGGGGCCGAGGTTCCTGGTCCTGTGAGAACTTCAACCCCCTGCTGGTGGCTGGGGGTG 480
P  G  G  R  G  S  W  S  C  E  N  F  N  P  L  L  V  A  G  G  130
   ∇        ∇                                              ∇

TGGCCGTGGCAGCCATAGCCCTGATTCTCGGTGTGGCCTTCCTGGTCCGGAAAAAATAAT 540
V  A  V  A  A  I  A  L  I  L  G  V  A  F  L  V  R  K  K  *  149

ACCTGGGGGCCAGGCGGGGGGCACGGAGCCACTTCCTGTACAGACCCGAGGAAGCCAGTG 600
CATGCAGAGTTCACCCTTACCTATTCGTACACACGCACATTCATTACACACCTACATACG 660
CCCCCAACACACGCGCACGGTGAAGAGGACGCCCGAGCCCACCCCTGCTGACCCAGGACT 720
TCCCCAACCTCCAGGGCAGAAAGAGCCCAGGCTCCGGGGTCCACAGCACAGGATGTGGGG 780
CGAGGGACAGCTGGGGAACGGCAAGAAAGGAATGGACCCTGTGTGTGGCCCCCCCCACCC 840
TGGCGGCTGGGTGATCCTGGGCCCCCAGGGCTGGTCTGAGTGCAGGTGGGGGTGCCGGGA 900
TGGGTTGGGCCTGGGCCCGGCCCTCGTGGGGACATTAAAGGGCCCGGTGGCTCCACTCGC 960
CCCATTTCTGCTCTTTGCGTACCCCCCGGGGGTCTGCCTGGGCGAATGCAGTGGGACGGA 1020
CCTGGGGTGGGCACTCACCTCAGCAGAGCCTCAATGCCACCTCCCCACACCACCCAAGCC 1080
TAGCTGGGTGGGAAGACGGAGCCCTGAAGCCTGCGTGTGGAGGGGGTGCTGCAAGGGTCG 1140
CCTTCCCCAGCTTGTGCTCCAGGGGAGCCCCGACGCCGGCGGGGAGGCAGGGATGTCGAG 1200
GGCCCCTCACAGCTGTGCCCGCCCTGCCCCGCTAGACTGCCCCTCCAGCTTGCACCCAGC 1260
ACCACCTGAGTCTAACCAGCGTATAATGCAATAACAGGTAGAGTAGAACTGCTTTTGGCG 1320
GCGACCGTCACACACTGTCCTCAGCCCACTGCACCTGGGGAAGCCCCTCGTGCAGCCCCT 1380
CCTTAGCACATCCAGTCCCAGCGTGGCCCTTGGGCGGCAGCAGCAGGCGAACTCCCAGAA 1440
GGTGGTGGTGGGCACTGTGAGAACGTGGCCTGCCCTCACTGCCAGTGGCCACAGGAACCC 1500
CGGGCCTGAGTGGAGGCTGAGTCTGAAATAAACTCTGTCGTCTGAAAAAAAAAAAAAAAA 1560
AAAAAAAAA                                                    1570
```

Figure 2 Complete nucleotide sequence of the human BM88 cDNA and the deduced protein sequence

The translated amino acid sequence is shown below the nucleotide sequence; both are numbered on the right. Start and stop codons and polyadenylation signal are in bold letters. The putative transmembrane region is underlined and the asterisk denotes a stop codon. Symbols indicate potential sites as follows: □, N-linked glycosylation; ○, protein kinase C phosphorylation; ●, casein kinase phosphorylation; ϒ, cAMP- and cGMP-dependent protein kinase phosphorylation; ∇, myristoylation site.

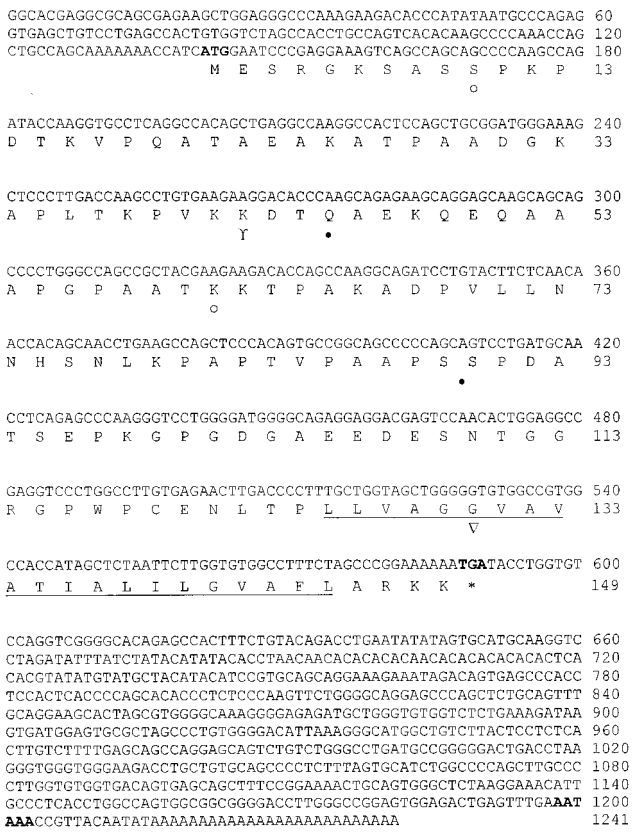

Figure 3 Complete nucleotide sequence of the mouse BM88 cDNA and the deduced protein sequence

The translated amino acid sequence is shown below the nucleotide sequence. See the Figure 2 legend for details.

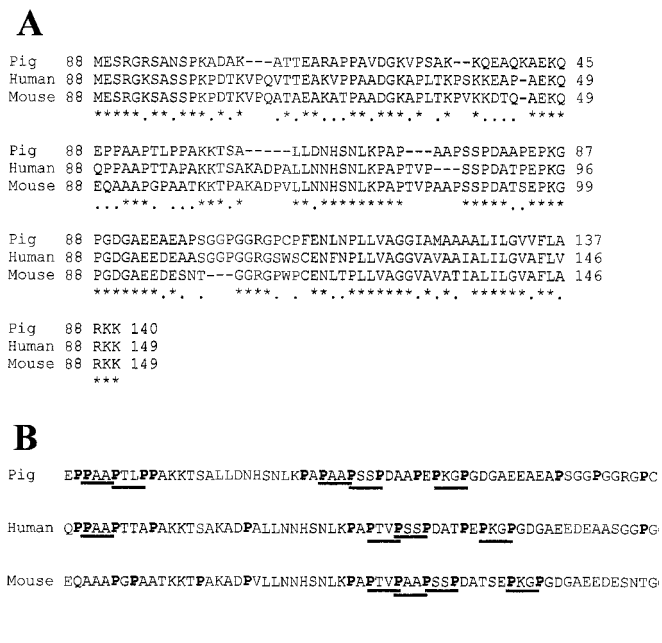

Figure 4 Comparison of the deduced amino acid sequence of pig, human and mouse BM88

(**A**) Dots and asterisks indicate, respectively, identity or similarity of the proteins between species. (**B**) Comparison of the predicted BM88 amino acid sequence of the proline-rich domains containing the PXXP motifs from pig (residues 46–109), human (residues 50–118) and mouse (residues 50–118). Proline residues are indicated in bold and PXXP motifs, some of which are overlapping, are underlined.

molecules also consist of two identical polypeptide chains linked together by disulphide bridges.

Sequencing of human and mouse BM88 cDNAs

Our protein analyses suggested the presence of a unique BM88 protein product of similar size, in mouse, human and pig. Therefore, we proceeded to identify and isolate in these species the cDNAs encoding the BM88 protein. To this end, the porcine BM88 cDNA sequence [13] was compared with EST databases (dbEST) [27] using the BlastN algorithm [28]. We detected nine human ESTs from Soares' infant brain cDNA library and one mouse EST from Soares' fetal (19.5 days post-coitus) mouse brain cDNA library that shared significant homology with the query sequence. Sequence analysis was then carried out on human cDNA clone 34819, corresponding to human EST 189881, and on mouse cDNA clone 317735, corresponding to mouse EST 519998.

The putative human BM88 cDNA clone has an insert of 1570 bp and contains the entire coding region. The first in-frame ATG, located 90 bp from the 5′ end of the clone, fulfils Kozak's criteria for an initiation codon [29] and is followed by a 447 nucleotide open reading frame and a termination codon at position 536. The coding region is followed by a long 3′-untranslated sequence containing a poly(A)$^+$ tail preceded by a polyadenylation signal at position 1527 (Figure 2). The open reading frame encodes for a polypeptide of 149 amino acids with a predicted molecular mass of 14.94 kDa.

The putative mouse BM88 cDNA clone has an insert of 1241 bp and also contains the entire coding region. The initiation codon ATG is located 141 bp from the 5′ end of the clone and is followed by a 447 nucleotide open reading frame and a termination codon at position 587. The coding region is again followed by a long 3′-untranslated sequence containing a poly(A)$^+$ tail preceded by a polyadenylation signal at position 1198 (Figure 3). As with the human cDNA, the mouse open reading frame encodes for a polypeptide of 149 amino acids with predicted molecular mass of 14.98 kDa. Potential functional sites for the human and mouse proteins were identified by searching the ProfileScan program of PROSITE and are shown in Figures 2 and 3. In accordance with the membrane association of the BM88 protein in brain [13,17], a putative transmembrane domain was identified in both proteins predicted from the human and mouse cDNAs (Figures 2 and 3).

The overall deduced amino acid identities of BM88 between human, mouse and pig species are as follows: human/mouse, 79.9%; human/pig, 76.4%, and mouse/pig, 70.7% (Figure 4A). Further analysis of the deduced amino acid sequence of the porcine, human and mouse BM88 utilizing ProfileScan of PROSITE revealed a proline-rich region (PROSITE pattern PS50099) located between amino acid residues 47 and 109 in the porcine molecule and 11 and 97 in the human molecule. This putative signalling region [30] respectively contains five (porcine BM88) and four (human BM88) repeats of the amino acid sequence PXXP (Figure 4B), which have been shown previously to represent binding sites for SH3 (Src homology 3) domains [30,31]. Although a corresponding proline-rich region was not

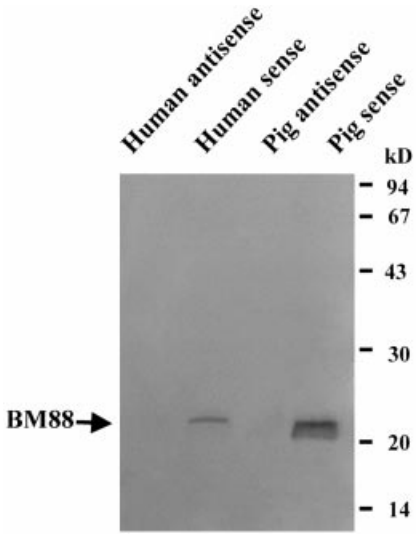

Figure 5 ***In vitro*** **expression of human and porcine BM88 cDNAs**

Sense and antisense RNAs were produced by *in vitro* transcription and translation and the resulting polypeptides were immunoprecipitated with polyclonal anti-BM88 and separated by SDS/PAGE (15% gels). A 22 kDa and a 23 kDa polypeptide were produced from the respective porcine and human sense RNA molecules whereas no polypeptide was produced from the respective antisense RNAs.

identified in the mouse BM88 protein using the same analysis software, it is clear that four PXXP motifs are also found in the mouse protein (Figure 4B).

Expression of human and mouse BM88 cDNA clones

The *in vitro* expression of cloned human BM88 cDNA was carried out in a cell-free protein-synthesizing system. For this purpose, we subcloned the human BM88 cDNA in pBluescript KS vector (Stratagene). Sense and antisense RNAs were produced by *in vitro* transcription and translation using the appropriate enzymes and T3 and T7 RNA polymerases. As a positive control, porcine BM88 sense RNA was used [13]. A 23 kDa polypeptide was produced (Figure 5). This polypeptide was immunoprecipitable with polyclonal anti-BM88 and, as seen by reducing SDS/PAGE and autoradiography, had a size identical to the native human protein (Figure 5). As a control in the same experiment, the previously reported 22 kDa porcine polypeptide was also obtained whereas no polypeptide was detected using the RNA transcribed from the antisense strand.

Human, mouse and porcine BM88 cDNAs were also expressed by transient transfection of COS7 cells. In all three cases, the expressed proteins were visualized by immunofluorescence microscopy using the anti-BM88 polyclonal antibody (Figure 6). A punctate staining was obtained for the recombinant proteins in accordance with the mitochondrial and vesicular localization of

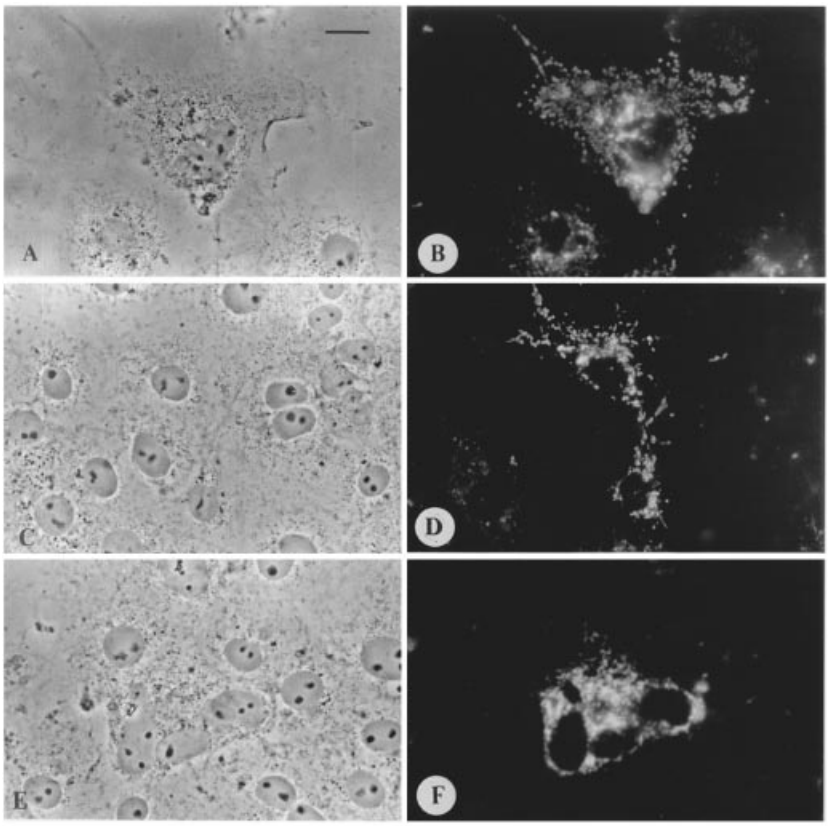

Figure 6 Immunofluorescence localization of BM88 in transfected COS7 cells

COS7 cells were transfected with the pcDNA3 vectors containing the entire human (**A**, **B**), mouse (**C**, **D**) or porcine (**E**, **F**) BM88 cDNAs and were grown for 48 h before fixation and immunofluorescence staining with the anti-BM88 polyclonal antibody. Left-hand panels, phase-contrast; right-hand panels, immunofluorescence. Labelling was visualized with fluorescein optics. Scale bar, 20 μm.

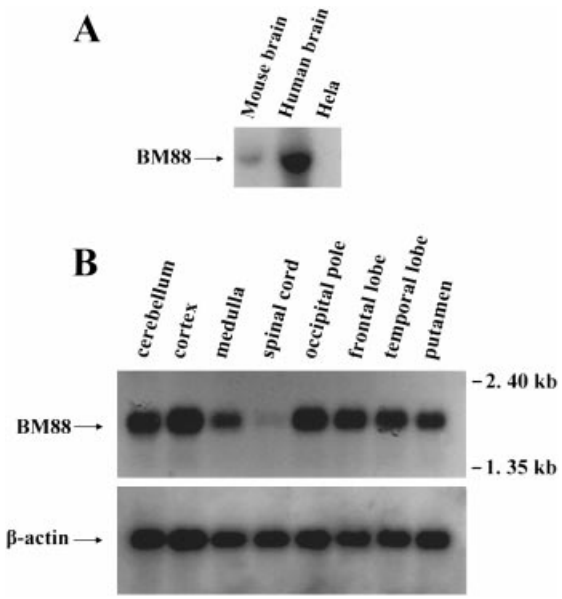

Figure 7 Expression of BM88 mRNA in adult human and mouse brains

Northern-blot analysis was performed with total (**A**) or poly(A)$^+$ (**B**) RNA as described in the Experimental section, using a human BM88 probe (**A** and top panel of **B**). A human β-actin probe was used (lower panel in **B**) as a control for loaded RNA.

the native BM88 protein present in nerve cells [17]. Western-blot analysis of the expressed proteins in COS7 cells resulted in the detection of polypeptide bands with molecular sizes corresponding to 22 kDa for the porcine protein or 23 kDa for the human and mouse proteins (results not shown).

Expression of human and mouse BM88 mRNAs

Northern-blot analysis was performed using total RNA from human and mouse brains and revealed in both species one transcript (Figure 7A). Human and mouse transcripts had a size of approx. 1.8 kb. As expected, no transcript was detected in RNA from Hela cells, used as a negative control (Figure 7A). In addition, the distribution of BM88 mRNA was examined in various regions of human brain and spinal cord. BM88 mRNA was detected in abundance in all the regions of the central nervous system analysed except the spinal cord, where its abundance was significantly lower (Figure 7B). Control hybridization with a β-actin cDNA probe indicated the presence of approximately equal amounts of mRNA from all regions.

Immunohistochemical detection of BM88 in human cerebellum

We have reported previously that expression of the BM88 protein and mRNA is confined to neurons in the porcine and rat brains [13,15,17]. Here the expression of the human BM88 protein was examined by immunohistochemistry in paraffin-embedded sections of adult human cerebellum using the affinity-purified polyclonal anti-BM88 antibody in conjunction with peroxidase-conjugated secondary antibodies. Strong immunoreactivity was seen in the molecular layer, the Purkinje cell layer and the granule neurons of the internal granular layer, while the white matter remained largely unstained (Figures 8A and 8B). These observations are in good agreement with the previously reported expression pattern of BM88 in the rat cerebellum [17] and

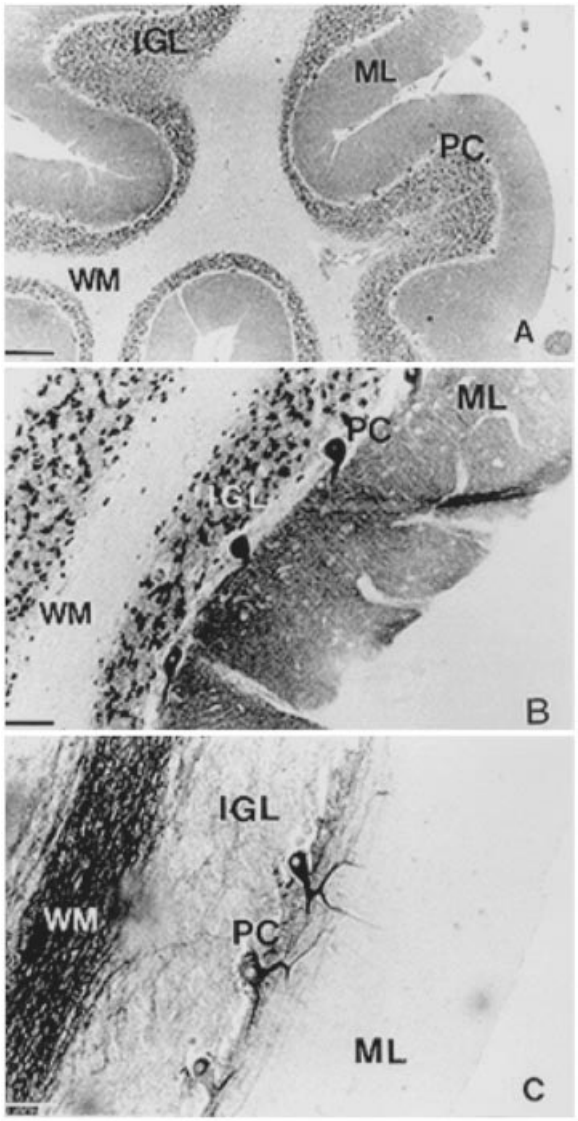

Figure 8 Immunocytochemical localization of BM88 in the adult human cerebellum

Staining was performed with the polyclonal anti-BM88 antibody (**A**, **B**). Labelling for neurofilament protein is seen in (**C**). ML, molecular layer; PC, Purkinje cell layer; IGL, internal granular layer; WM, white matter.

confirm its neuronal localization in human brain. For comparison, in Figure 8(C) is shown the labelling of another section for neurofilament protein, which elegantly decorates the cell bodies and proximal dendrites of the Purkinje cells as well as the neurofilament-positive axons transversing the white matter. It is noteworthy that the cell bodies and proximal dendrites of the Purkinje cells are also BM88-positive, whereas the BM88-positive elaborate dendritic tree of these cells extending throughout the molecular layer is neurofilament-protein-negative.

BM88 localizes to human chromosome 11p15.5

Computer searches of available sequences of the human genome in the UniGene database revealed that human EST clone 34819 belongs to a cluster of EST sequences that correspond to stSG4431 (Sanger Centre, Hinxton, Cambridge, U.K.), located

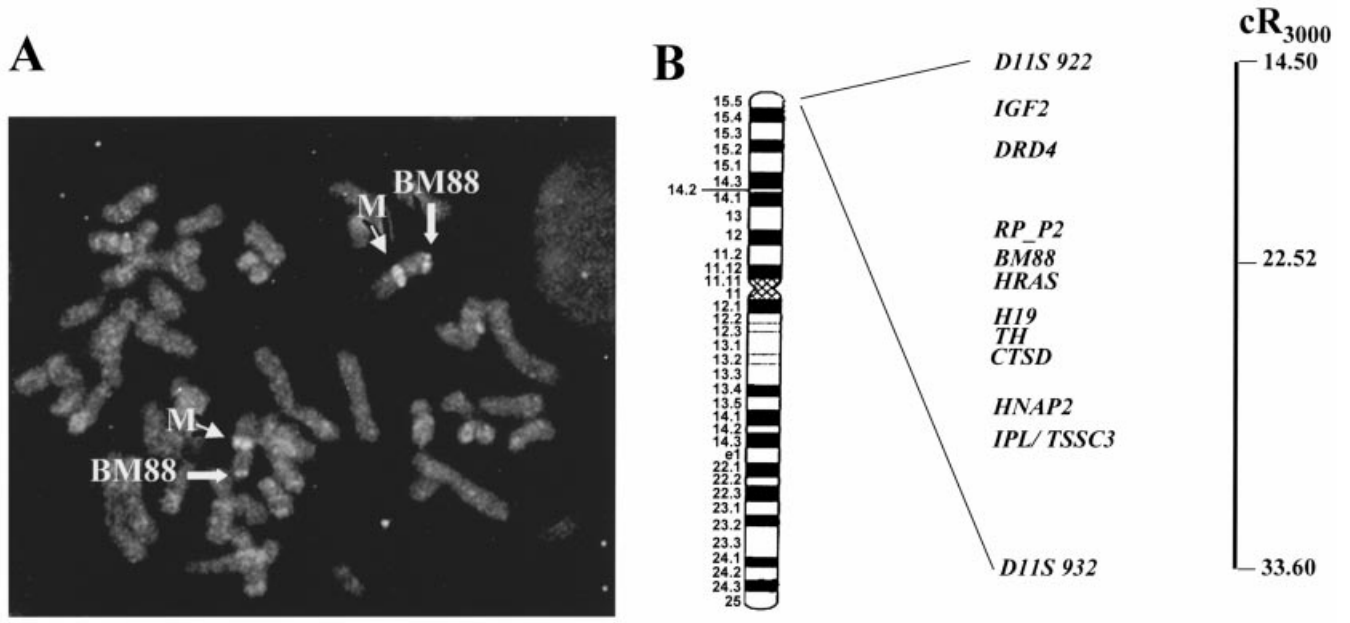

Figure 9 Human *BM88* localizes at chromosome 11p15.5

(**A**) Metaphase chromosomal spreads of peripheral blood leucocytes were double labelled for *BM88* and a specific marker (M) for chromosome 11. The signal on the telomeric region of chromosome 11 at band p15.5 corresponds to *BM88* labelling while the signal on the long arm of chromosome 11 is due to the marker for the region 11q13. (**B**) Computer-assisted mapping of human *BM88* using data from the UniGene database maintained at the National Centre for Biotechnology Information. The locus assigned to *BM88* lies between the framework markers *D11S922* and *D11S932*. The distance of *BM88* from these two markers is shown on the right in centirays (cR). The approximate position of *BM88* relative to insulin growth factor II (*IGF2*), dopamine receptor 4 (*DRD4*), ribosomal protein, large P2 (*RP_P2*), the oncogene H-Ras (*HRAS*), the putative growth suppressor gene *H19*, tyrosine hydroxylase (*TH*), cathepsin D (*CTSD*), nucleosome assembly protein 2 (*HNAP2*) and the apoptosis-related gene *TSSC3* was determined based on the GeneMap '99 profile of the International Radiation Hybrid Mapping Consortium at the National Centre for Biotechnology Information.

at chromosome 11p15.5. To confirm the localization of the human *BM88* gene, we isolated several cosmid clones from a human genomic library using as a probe the human BM88 cDNA. One of our cosmid clones, containing the *BM88* gene, was used to perform fluorescence *in situ* hybridization (or FISH) analysis on human chromosomes. Initially, specific labelling was seen in the short arm of a chromosome belonging to chromosomal group C, and classified as chromosome 11 on the basis of size, morphology and banding pattern. Subsequently, double labelling was performed for BM88 and a specific marker for the region 11q13. This experiment resulted in specific labelling of the long and short arms of chromosome 11 (Figure 9A). Observation of specifically labelled chromosomes 11 demonstrated that BM88 is located at the terminus of the short arm, an area that corresponds to band 11p15.5. A total of 80 metaphase cells were analysed, with 72 exhibiting specific labelling. We thus conclude that BM88 maps to 11p15.5.

After submission of the human BM88 nucleotide sequence to the EMBL/GenBank databases, BM88 was assigned with the locus number LOC51286 by the International Radiation Hybrid Mapping Consortium at the National Centre for Biotechnology Information (NCBI, Bethesda, MD, U.S.A.) and its position relative to framework marker sequences is shown in Figure 9(B). The assigned BM88 locus is 8.02 and 11.08 cR_{3000} from the marker sequences *D11S922* and *D11S932*, respectively, on chromosome 11p at band 15.5 (Figure 9B). Using these distances BM88 was placed relative to genes mapped previously between these marker sequences. Genes localizing to this region include the oncogene H-*Ras*, cathepsin D, dopamine receptor 4 and nucleosome assembly protein 2, and an imprinted region containing insulin-like growth factor 2, *H19* and the apoptosis-related gene *TSSC3*.

A more recent search in the EMBL/GenBank databases revealed the existence of a highly homologous sequence on chromosome 13 corresponding to the 38–943 nucleotide sequence of human BM88 cDNA. The chromosome 13 sequence, although extending much further for approx. 5.5 kb, does not have any homology with the rest of the 3′-untranslated region of the BM88 cDNA. This raises the possibility that, additionally to chromosome 11, a homologous gene exists on chromosome 13.

DISCUSSION

Genes involved in the regulation of the exit from the cell cycle and the initiation of differentiation in the embryonic nervous system are fundamental for normal development of neuronal cells [32]. De-regulation of these processes results in abnormalities in brain development and is associated with tumorigenesis. Although some of the genes that control cell-cycle progression have been identified, such as the cyclins, cyclin-dependent kinases and their inhibitors (see [33] for reviews; and [34]), their spatiotemporal and functional relationships with differentiation-promoting genes are just beginning to emerge. We have previously identified BM88, a novel neuron-specific protein present in porcine brain, which appears to slow down the proliferation and enhance the differentiation of neuroblastoma cells *in vitro*. Here we report the identification of homologous proteins in human and mouse brain, the isolation of their respective cDNA molecules and the localization of the human *BM88* gene to the 15.5 region of human chromosome 11.

Our cDNA sequence data predict that human and murine proteins have 149 amino acids, with 79.9% identity between them and 76.4% and 70.7% identities with the porcine protein, respectively. The human and mouse proteins are both slightly larger than the predicted porcine protein, which is 140 amino acids long [13]. This small difference in size is also reflected in the molecular mass of the native proteins, as determined by Western-blot analysis of brain membrane preparations, whereby the porcine protein is identified as 22 kDa while the human and mouse proteins appear as 23 kDa polypeptides. Another potential issue of discrepancy is the predicted molecular size of the proteins versus their estimated size in SDS/PAGE. However, as we have shown previously for the porcine cDNA [13], transcription and translation of the human cDNA in a cell-free system yielded a 23 kDa polypeptide, identical in size with the native protein. Furthermore, transient transfection of COS7 cells with all three cDNAs resulted in the immunocytochemical detection of the expressed proteins and a similar estimation of their molecular masses by Western-blot analysis.

Analysis of the deduced amino acid sequence of BM88 utilizing the ProfileScan of PROSITE revealed a putative proline-rich signalling domain in the porcine and human molecules that may be involved in protein–protein interactions (for reviews see [30,35]). This proline-rich domain in porcine and human BM88 contains several PXXP repeats, also found in the mouse molecule (Figure 4B), which have been detected in a variety of proteins comprising diverse signal-transduction pathways [36]. Apart from representing putative SH3-binding sites, the PXXP domains may also account for the anomalous electrophoretic behaviour of BM88 in SDS/polyacrylamide gels. Previous studies using deletion mutants of murine p53 demonstrated that the proline-rich part of the molecule containing the PXXP motifs is responsible for the anomalous migration of p53 in SDS gels and, particularly, for its retardation relative to the size predicted from the nucleotide sequence [37]. Detailed structural studies have shown that PXXP residues form a left-handed polyproline type II helix [36], which not only creates a binding site for SH3 domains but also forms a relatively rigid region that may retain a certain degree of structure in SDS/PAGE and hence contribute to the anomalous migration.

Northern-blot analysis demonstrated that human BM88 mRNA is widely distributed throughout the human brain with lower levels found in the spinal cord, whereas immunocytochemical experiments revealed that human BM88 is confined to neurons. A similar wide distribution of the BM88 protein was seen previously in neurons of the porcine and rat brains [15,17]. However, some variations in the levels of expression of the BM88 protein have also been noted: for example, low amounts of BM88 are expressed in the cerebellar Purkinje neurons of the adult rat as compared with other brain regions, such as the hippocampus, the striatum and the thalamus [17]. Moreover, in the adult mouse BM88 immunoreactivity is most prominent in the larger neurons of the dorsal root ganglia whereas the medium-sized and small neurons express significantly lower amounts of the protein (E. Boutou and R. Matsas, unpublished work). It is noteworthy that the dorsal root ganglion BM88-poor neurons are those in which c-Ret, the functional receptor for the GDNF, is most abundantly found [38]. We have shown previously that stably transfected Neuro 2a cells overexpressing the BM88 protein respond differentially to growth factors when compared with the wild-type cells [19]. In particular, they show decreased sensitivity to GDNF [19] and express lower levels of its functional receptor c-Ret (E. Boutou and R. Matsas, unpublished work). These *in vitro* data together with the *in vivo* restricted expression of BM88 in dorsal root ganglion neurons and especially the negative correlation between BM88 and c-Ret expression raises the intriguing possibility that BM88 may be associated with the majority of, but not all, neuronal phenotypes. Therefore the reduction in BM88 mRNA levels in spinal cord noted in this study may reflect a similar situation and may be associated with the known high levels of c-Ret expression in spinal cord motor neurons [39].

Physical mapping and computer-assisted analysis demonstrated that the *BM88* gene maps to human chromosome 11p15.5, although according to recent data the possibility exists that, additionally to chromosome 11, a homologous gene exists on chromosome 13. 11p15.5 is one of the most heavily studied genomic regions because of its association with human disease [20,22,40–43]. Chromosome 11p15.5 contains an important tumour-suppression locus and is characterized by frequent loss of heterozygosity in several childhood and adult cancers, including Wilm's tumour [44,45], rhabdomyosarcoma [46], hepatoblastoma [47], and breast [48,49], ovarian [50,51] and lung cancer [52,53]. This region is also involved in recurrent translocations t7;11 (p15;p15) in acute myeloid leukaemia and myeloproliferative disorders [54]. In addition, 11p15.5 has been shown by linkage analysis to harbour the susceptibility gene(s) for BWS [21], a genetic overgrowth disease characterized by prenatal and neonatal multi-organ developmental abnormalities and predisposition to cancer, especially Wilm's nephroblastoma [44]. Further investigation of 11p15.5 revealed that it also contains a cluster of growth-related genes that are genomically imprinted and that loss of imprinting or gain of imprinting in this region can also contribute to tumorigenesis [47,55]. Among the genes at 11p15.5 that are implicated in BWS and cancer are insulin growth factor II (*IGF2* [43]), the cyclin-dependent kinase inhibitor gene $p57^{KIP2}$ [56], the voltage-gated potassium channel K_vLQT1 [57], the *H19* gene that encodes a non-translatable RNA and the apoptosis-related gene *TSSC3* [58]. Of particular interest, the assigned locus of *BM88* places this gene within the genomic region corresponding to the second Wilm's tumour locus (from *IGF2* to *CTSD* [59,60]) and within the gene cluster associated with BWS (*IGF2*, *H19*, *HNAP2* and *IPL/TSSC3*). Additionally, the *BM88* gene is flanked by several imprinted genes at 11p15.5, including *IGF2*, *H19* and *TSSC3*. In this respect, it will be important to investigate the precise linkage of *BM88* to other genes contained at 11p15.5 and to determine whether *BM88* is subject to genomic imprinting.

Apart from genetic studies, some of the 11p15.5 genes have been linked to the tumorigenic process by functional studies. Thus it has been reported previously that transfection of *H19* into G401 cells resulted in suppression of the tumorigenic phenotype [61], whereas transfer of subchromosomal fragments of the 11p15.5 region resulted in either tumour suppression or growth arrest *in vitro* [62,63]. We have shown previously that transfection of mouse neuroblastoma cells with the porcine BM88 cDNA slows down the proliferation and enhances the differentiation of these cells into a phenotype resembling that of early differentiating neurons [13,19]. Therefore the functional properties of BM88 in arresting cell growth and enhancing differentiation render this molecule a good candidate as a target gene for BWS and cancer. In addition, it appears that BM88 is an early marker *in vivo* for proliferating neuroblasts and early differentiating neuronal cells in the embryonic brain [17,18]. This observation reinforces our view that BM88 is associated with the molecular mechanisms that control exit from the cell cycle and the initiation of differentiation. It is well understood that neuroblast growth arrest and differentiation constitute major developmental events for the proper formation of neuronal cells. Moreover, these processes are tightly, but inversely, linked to

tumorigenesis. Consequently, it may well prove beneficial to examine BWS and cancer associated with 11p15.5 for alterations in BM88. Towards this goal studies are in progress in our laboratory for the analysis of the human and mouse *BM88* genes.

This work was supported by the Greek General Secretariat of Research and Technology Programme Grants EKVAN and PENED '99 (to R.M.) and EU grant QRLT-2000-00072.

REFERENCES

1 Jessell, T. M. (1988) Adhesion molecules and the hierarchy of neural development. Neuron **1**, 3–13
2 Matsas, R. (1997) Genes controlling neural fate and differentiation. Adv. Exp. Med. Biol. **429**, 3–17
3 McKay, R. D. (1989) The origins of cellular diversity in the mammalian central nervous system. Cell **58**, 815–821
4 Altman, J. (1972) Postnatal development of the cerebellar cortex in the rat. I. The external germinal layer and the transitional molecular layer. J. Comp. Neurol. **145**, 353–397
5 Altman, J. (1972) Postnatal development of the cerebellar cortex in the rat. II. Phases in the maturation of Purkinje cells and of the molecular layer. J. Comp. Neurol. **145**, 399–463
6 McKay, R. (1997) Stem cells in the central nervous system. Science **276**, 66–71
7 Sidman, R. L. and Rakic, P. (1973) Neuronal migration, with special reference to developing human brain: a review. Brain Res. **62**, 1–35
8 Cattaneo, E. and McKay, R. (1991) Identifying and manipulating neuronal stem cells. Trends Neurosci. **14**, 338–340
9 Hedrick, L., Cho, K. R., Fearon, E. R., Wu, T. C., Kinzler, K. W. and Vogelstein, B. (1994) The DCC gene product in cellular differentiation and colorectal tumorigenesis. Genes Dev. **8**, 1174–1183
10 Kanno, H., Saljooque, F., Yamamoto, I., Hattori, S., Yao, M., Shuin, T. and U, H. S. (2000) Role of the von Hippel-Lindau tumor suppressor protein during neuronal differentiation. Cancer Res. **60**, 2820–2824
11 Kumar, S., Matsuzaki, T., Yoshida, Y. and Noda, M. (1994) Molecular cloning and biological activity of a novel developmentally regulated gene encoding a protein with beta-transducin-like structure. J. Biol. Chem. **269**, 11318–11326
12 Reale, M. A., Reyes-Mugica, M., Pierceall, W. E., Rubinstein, M. C., Hedrick, L., Cohn, S. L., Nakagawara, A., Brodeur, G. M. and Fearon, E. R. (1996) Loss of DCC expression in neuroblastoma is associated with disease dissemination. Clin. Cancer Res. **2**, 1097–1102
13 Mamalaki, A., Boutou, E., Hurel, C., Patsavoudi, E., Tzartos, S. and Matsas, R. (1995) The BM88 antigen, a novel neuron-specific molecule, enhances the differentiation of mouse neuroblastoma cells. J. Biol. Chem. **270**, 14201–14208
14 Boutou, E., Hurel, C. and Matsas, R. (2000) Early expression of the BM88 antigen during neuronal differentiation of P19 embryonal carcinoma cells. Int. J. Dev. Neurosci. **18**, 321–328
15 Patsavoudi, E., Hurel, C. and Matsas, R. (1989) Neuron- and myelin-specific monoclonal antibodies recognizing cell-surface antigens of the central and peripheral nervous system. Neuroscience **30**, 463–478
16 Patsavoudi, E., Hurel, C. and Matsas, R. (1991) Purification and characterization of neuron-specific surface antigen defined by monoclonal antibody BM88. J. Neurochem. **56**, 782–788
17 Patsavoudi, E., Merkouri, E., Thomaidou, D., Sandillon, F., Alonso, G. and Matsas, R. (1995) Characterization and localization of the BM88 antigen in the developing and adult rat brain. J. Neurosci. Res. **40**, 506–518
18 Thomaidou, D., Koutmani, Y., Patsavoudi, E. and Matsas, R. (2000) BM88 is a marker for proliferating neuroblasts and early differentiating neuronal cells. Soc. Neurosci. Abstracts **26(2)**, 1854
19 Gomez, J., Boutou, E., Hurel, C., Mamalaki, A., Kentroti, S., Vernadakis, A. and Matsas, R. (1998) Overexpression of the neuron-specific molecule BM88 in mouse neuroblastoma cells: altered responsiveness to growth factors. J. Neurosci. Res. **51**, 119–128
20 Li, L., Li, X., Francke, U. and Cohen, S. N. (1997) The TSG101 tumor susceptibility gene is located in chromosome 11 band p15 and is mutated in human breast cancer. Cell **88**, 143–154
21 Ping, A. J., Reeve, A. E., Law, D. J., Young, M. R., Boehnke, M. and Feinberg, A. P. (1989) Genetic linkage of Beckwith-Wiedemann syndrome to 11p15. Am. J. Hum. Genet. **44**, 720–723
22 Richard, C. W., Boehnke, M., Berg, D. J., Lichy, J. H., Meeker, T. C., Hauser, E., Myers, R. M. and Cox, D. R. (1993) A radiation hybrid map of the distal short arm of human chromosome 11, containing the Beckwith-Wiedemann and associated embryonal tumor disease loci. Am. J. Hum. Genet. **52**, 915–921
23 Sanger, F., Nicklen, S. and Coulson, A. R. (1992) DNA sequencing with chain-terminating inhibitors. Biotechnology **24**, 104–108
24 Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162**, 156–159
25 Church, G. M. and Gilbert, W. (1984) Genomic sequencing. Proc. Natl. Acad. Sci. U.S.A. **81**, 1991–1995
26 Wood, J. N. and Anderton, B. H. (1981) Monoclonal antibodies to mammalian neurofilaments. Biosci. Rep. **1**, 263–268
27 Boguski, M. S., Tolstoshev, C. M. and Bassett, D. E. J. (1994) Gene discovery in dbEST. Science **265**, 1993–1994
28 Altschul, S. F., Gish, W., Miller, W., Myers, E. W. and Lipman, D. J. (1990) Basic local alignment search tool. J. Mol. Biol. **215**, 403–410
29 Kozak, M. (1984) Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. Nucleic Acids Res. **12**, 857–872
30 Pawson, T. (1995) Protein modules and signalling networks. Nature (London) **373**, 573–580
31 Walker, K. K. and Levine, A. J. (1996) Identification of a novel p53 functional domain that is necessary for efficient growth suppression. Proc. Natl. Acad. Sci. U.S.A. **93**, 15335–15340
32 Ross, M. E., Carter, M. L. and Lee, J. H. (1996) MN20, a D2 cyclin, is transiently expressed in selected neural populations during embryogenesis. J. Neurosci. **16**, 210–219
33 O'Connor, L., Huang, D. C., O'Reilly, L. A. and Strasser, A. (2000) Apoptosis and cell division. Curr. Opin. Cell Biol. **12**, 257–263
34 Sherr, C. J. and Roberts, J. M. (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev. **13**, 1501–1512
35 Cohen, G. B., Ren, R. and Baltimore, D. (1995) Modular binding domains in signal transduction proteins. Cell **80**, 237–248
36 Yu, H., Chen, J. K., Feng, S., Dalgarno, D. C., Brauer, A. W. and Schreiber, S. L. (1994) Structural basis for the binding of proline-rich peptides to SH3 domains. Cell **76**, 933–945
37 Hansen, S., Lane, D. P. and Midgley, C. A. (1998) The N terminus of the murine p53 tumour suppressor is an independent regulatory domain affecting activation and thermostability. J. Mol. Biol. **275**, 575–588
38 Honda, T., Takahashi, M. and Sugiura, Y. (1999) Co-localization of the glial cell-line derived neurotrophic factor and its functional receptor c-RET in a subpopulation of rat dorsal root ganglion neurons. Neurosci. Lett. **275**, 45–48
39 Oppenheim, R. W., Houenou, L. J., Parsadanian, A. S., Prevette, D., Snider, W. D. and Shen, L. (2000) Glial cell line-derived neurotrophic factor and developing mammalian motoneurons: regulation of programmed cell death among motoneuron subtypes. J. Neurosci. **20**, 5001–5011
40 Bischoff, F. Z., Feldman, G. L., McCaskill, C., Subramanian, S., Hughes, M. R. and Shaffer, L. G. (1995) Single cell analysis demonstrating somatic mosaicism involving 11p in a patient with paternal isodisomy and Beckwith-Wiedemann syndrome. Hum. Mol. Genet. **4**, 395–399
41 DeChiara, T. M., Robertson, E. J. and Efstratiadis, A. (1991) Parental imprinting of the mouse insulin-like growth factor II gene. Cell **64**, 849–859
42 Giannoukakis, N., Deal, C., Paquette, J., Goodyer, C. G. and Polychronakos, C. (1993) Parental genomic imprinting of the human IGF2 gene. Nat. Genet. **4**, 98–101
43 Weksberg, R., Shen, D. R., Fei, Y. L., Song, Q. L. and Squire, J. (1993) Disruption of insulin-like growth factor 2 imprinting in Beckwith-Wiedemann syndrome. Nat. Genet. **5**, 143–150
44 Koufos, A., Grundy, P., Morgan, K., Aleck, K. A., Hadro, T., Lampkin, B. C., Kalbakji, A. and Cavenee, W. K. (1989) Familial Wiedemann-Beckwith syndrome and a second Wilms tumor locus both map to 11p15.5. Am. J. Hum. Genet. **44**, 711–719
45 Reeve, A. E., Sih, S. A., Raizis, A. M. and Feinberg, A. P. (1989) Loss of allelic heterozygosity at a second locus on chromosome 11 in sporadic Wilms' tumor cells. Mol. Cell Biol. **9**, 1799–1803
46 Sait, S. N., Nowak, N. J., Singh-Kahlon, P., Weksberg, R., Squire, J., Shows, T. B. and Higgins, M. J. (1994) Localization of Beckwith-Wiedemann and rhabdoid tumor chromosome rearrangements to a defined interval in chromosome band 11p15.5. Genes Chrom. Cancer **11**, 97–105
47 Schwienbacher, C., Gramantieri, L., Scelfo, R., Veronese, A., Calin, G. A., Bolondi, L., Croce, C. M., Barbanti-Brodano, G. and Negrini, M. (2000) Gain of imprinting at chromosome 11p15: a pathogenetic mechanism identified in human hepatocarcinomas. Proc. Natl. Acad. Sci. U.S.A. **97**, 5445–5449
48 Ali, I. U., Lidereau, R., Theillet, C. and Callahan, R. (1987) Reduction to homozygosity of genes on chromosome 11 in human breast neoplasia. Science **238**, 185–188
49 Negrini, M., Rasio, D., Hampton, G. M., Sabbioni, S., Rattan, S., Carter, S. L., Rosenberg, A. L., Schwartz, G. F., Shiloh, Y. and Cavenee, W. K. (1995) Definition and refinement of chromosome 11 regions of loss of heterozygosity in breast cancer: identification of a new region at 11q23.3. Cancer Res. **55**, 3003–3007
50 Lee, J. H., Kavanagh, J. J., Wharton, J. T., Wildrick, D. M. and Blick, M. (1989) Allele loss at the c-Ha-ras1 locus in human ovarian cancer. Cancer Res. **49**, 1220–1222

51 Viel, A., Giannini, F., Tumiotto, L., Sopracordevole, F., Visentin, M. C. and Boiocchi, M. (1992) Chromosomal localisation of two putative 11p oncosuppressor genes involved in human ovarian tumours. Br. J. Cancer **66**, 1030–1036
52 Bepler, G. and Garcia-Blanco, M. A. (1994) Three tumor-suppressor regions on chromosome 11p identified by high-resolution deletion mapping in human non-small-cell lung cancer. Proc. Natl. Acad. Sci. U.S.A. **91**, 5513–5517
53 Weston, A., Willey, J. C., Modali, R., Sugimura, H., McDowell, E. M., Resau, J., Light, B., Haugen, A., Mann, D. L. and Trump, B. F. (1989) Differential DNA sequence deletions from chromosomes 3, 11, 13, and 17 in squamous-cell carcinoma, large-cell carcinoma, and adenocarcinoma of the human lung. Proc. Natl. Acad. Sci. U.S.A. **86**, 5099–5103
54 Trent, J. M., Kaneko, Y. and Mitelman, F. (1989) Report of the committee on structural chromosome changes in neoplasia. Cytogenet. Cell Genet. **51**, 533–562
55 Feinberg, A. P. (1999) Imprinting of a genomic domain of 11p15 and loss of imprinting in cancer: an introduction. Cancer Res. **59**, 1743–1746
56 Matsuoka, S., Thompson, J. S., Edwards, M. C., Bartletta, J. M., Grundy, P., Kalikin, L. M., Harper, J. W., Elledge, S. J. and Feinberg, A. P. (1996) Imprinting of the gene encoding a human cyclin-dependent kinase inhibitor, p57KIP2, on chromosome 11p15. Proc. Natl. Acad. Sci. U.S.A. **93**, 3026–3030
57 Jiang, S., Hemann, M. A., Lee, M. P. and Feinberg, A. P. (1998) Strain-dependent developmental relaxation of imprinting of an endogenous mouse gene, Kvlqt1. Genomics **53**, 395–399
58 Lee, M. P. and Feinberg, A. P. (1998) Genomic imprinting of a human apoptosis gene homologue, TSSC3. Cancer Res. **58**, 1052–1056
59 Bepler, G., O'briant, K. C., Kim, Y. C., Schreiber, G. and Pitterle, D. M. (1999) A 1.4-Mb high-resolution physical map and contig of chromosome segment 11p15.5 and genes in the LOH11A metastasis suppressor region. Genomics **55**, 164–175
60 McKusick, V. A. and Amberger, J. S. (1993) The morbid anatomy of the human genome: chromosomal location of mutations causing disease. J. Med. Genet. **30**, 1–26
61 Hao, Y., Crenshaw, T., Moulton, T., Newcomb, E. and Tycko, B. (1993) Tumour-suppressor activity of H19 RNA. Nature (London) **365**, 764–767
62 Dowdy, S. F., Fasching, C. L., Araujo, D., Lai, K. M., Livanos, E., Weissman, B. E. and Stanbridge, E. J. (1991) Suppression of tumorigenicity in Wilms tumor by the p15.5-p14 region of chromosome 11. Science **254**, 293–295
63 Reid, L. H., West, A., Gioeli, D. G., Phillips, K. K., Kelleher, K. F., Araujo, D., Stanbridge, E. J., Dowdy, S. F., Gerhard, D. S. and Weissman, B. E. (1996) Localization of a tumor suppressor gene in 11p15.5 using the G401 Wilms' tumor assay. Hum. Mol. Genet. **5**, 239–247

Received 11 September 2000/5 January 2001; accepted 15 February 2001