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Expression pattern of BM88 in the developing nervous system of the chick and mouse embryo

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Abstract

We isolated a chick homologue of BM88 (cBM88), a cell-intrinsic nervous system-specific protein and examined the expression of BM88 mRNA and protein in the developing brain, spinal cord and peripheral nervous system of the chick embryo by *in situ* hybridization and immunohistochemistry. cBM88 is widely expressed in the developing central nervous system, both in the ventricular and mantle zones where precursor and differentiated cells lie, respectively. In the spinal cord, particularly strong cBM88 expression is detected ventrally in the motor neuron area. cBM88 is also expressed in the dorsal root ganglia and sympathetic ganglia. In the early neural tube, cBM88 is first detected at HH stage 15 and its expression increases with embryonic age. At early stages, cBM88 expression is weaker in the ventricular zone (VZ) and higher in the mantle zone. At later stages, when gliogenesis persists instead of neurogenesis, BM88 expression is abolished in the VZ and cBM88 is restricted in the neuron-containing mantle zone of the neural tube. Association of cBM88 expression with cells of the neuronal lineage in the chick spinal cord was demonstrated using a combination of markers characteristic of neuronal or glial precursors, as well as markers of differentiated neuronal, oligodendroglial and astroglial cells. In addition to the spinal cord, cBM88 is expressed in the HH stage 45 (embryonic day 19) brain, including the telencephalon, diencephalon, mesencephalon, optic tectum and cerebellum. BM88 is also widely expressed in the mouse embryonic CNS and PNS, in both nestin-positive neuroepithelial cells and post-mitotic βIII-tubulin positive neurons.

Keywords: Neuroepithelial cells; Neuronal precursors; Chick; Mouse; Embryo; Brain; Differentiation; Proliferation; Ventricular zone; Mantle zone; Spinal cord; Motor neuron; Dorsal root ganglia; Sympathetic ganglia; Telencephalon; Diencephalon; Mesencephalon; Optic tectum; Cerebellum; Tectum; Ganglionic eminence; Lateral; Medial; Notch1; Nkx6.1; Nkx2.2; Olig2; BrdU; O4; PLP; GFAP; SCG10; βIII-Tubulin; Islet1; Nestin; Primary cultures; Oligodendrocytes; Astrocytes

1. Results and discussion

BM88 (Cend1, for cell cycle exit and neuronal differentiation 1 – NCBI nomenclature www.ncbi.nlm.nih.gov) is a neuronal protein implicated in mechanisms of cell cycle exit and neuronal differentiation. It was first identified as a protein widely expressed in terminally differentiated neurons of the adult mammalian central (CNS) and peripheral (PNS) nervous systems (Patsavoudi et al., 1989, 1995). BM88 cloned from porcine, mouse and human brain (Gaitanou et al., 2001; Mamalaki et al., 1995) is an integral membrane protein composed of two 22–23 kDa polypeptide chains linked together by disulphide bridges. It is anchored to the membrane of intracellular organelles, including the outer membrane of mitochondria, the endoplasmic reticulum and other electrolucent vesicles, via a transmembrane domain so that the bulk of the protein faces towards the cytoplasm (Mamalaki et al., 1995; Patsavoudi et al., 1995). Gain-of-function approaches have shown that BM88 is sufficient to drive mouse neuroblastoma Neuro 2a cells towards exit from the cell cycle and differentiation into a neuronal phenotype in the absence of external stimuli (Mamalaki et al., 1995). Further,

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it has been recently demonstrated that BM88 is not only sufficient, but also required for proper synchronization of withdrawal from the cell cycle and differentiation of neuroblastoma cells (Georgopoulou et al., 2006). In the developing nervous system control of cell cycle progression plays an essential role in the generation of the appropriate number of neurons and the formation of functional neuronal circuits. Therefore, there is intense interest in delineating the molecular mechanisms underlying the timing of cell cycle exit and synchronization with the initiation of differentiation.

The human *BM88* gene is localized in chromosome 11p15.5, a region associated with human disease while the mouse gene is mapped at the corresponding region of the syntenic chromosome 7 (Gaitanou et al., 2001). The human 11p15.5 region contains an important tumour-suppression locus implicated in several childhood and adult cancers (Reeve et al., 1989; Trent et al., 1989; Weksberg et al., 2001) and it is also associated with the overgrowth genetic disorder Beckwith–Wiedemann syndrome (BWS), (Arima et al., 2005; Bischoff et al., 1995; Murrell et al., 2004; Richard et al., 1993; Sparago et al., 2004; Weksberg et al., 1993, 2003). Recently, the human *BM88* promoter was characterized and an 88 bp proximal promoter fragment was identified which is sufficient to confer specific transcriptional activity in primary neurons (Papadodima et al., 2005). The *BM88* promoter lies within a GpC island, it lacks TATA box and initiator element and is activated by the transcription factor Sp1. Importantly, transcriptional deregulation of Sp1-dependent pathways in neurons has emerged as a pathogenic mechanism in neurodegenerative disorders (Dunah et al., 2002; Zhai et al., 2005).

In vertebrates BM88 expression has been previously described only in the adult rat brain and in the developing rat forebrain. BM88 protein was detected in progenitor cells before terminal mitosis and in their neuronal progeny during the neurogenic interval (Koutmani et al., 2004). To study the functional properties of BM88 *in vivo*, we planned to use the developing neural tube of the chick embryo which is a convenient model system for *in vivo* functional studies and easily amenable to genetic manipulations for loss- or gain-of-function approaches. Here, we report cloning and expression of a chick homologue of *BM88* that was isolated as a first step in understanding the possible functions of this gene in embryonic development. We also present the expression of this molecule in the developing mouse CNS and PNS.

1.1. Cloning of a chick homologue of BM88

The chick homologue of *BM88* was obtained by an RT-PCR approach. We first screened cryosections of embryonic HH stage 31 (Hamburger and Hamilton, 1951; corresponding to embryonic day E7) chick embryos for BM88 expression using a specific, affinity purified polyclonal anti-BM88 antibody (Patsavoudi et al., 1995). Strong expression of BM88 was detected in the sympathetic ganglia (not shown). To isolate the chick *BM88* gene (*cBM88*), total RNA from HH stage 31 chick sympathetic ganglia was prepared and used in RT-PCR experiments. The products of these RT-PCR experiments were cloned and sequenced. Blast analysis of the produced sequences in the NCBI chicken genome database demonstrated the presence of *cBM88* in the Gallus gallus chromosome 5 genomic contig NW_060374.1. Comparison with the known amino acid sequences of the human, mouse and porcine homologues (Fig. 1) revealed regions of striking similarity (red lines in Fig. 1). Chick and human BM88 proteins showed 42.3% identity and 72.3% similarity. The most extended homologous region between BM88 proteins from all four species is observed in the hydrophobic transmembrane domain in the carboxy terminal region of the protein (Fig. 1), via which BM88 is anchored to the membrane of intracellular organelles, including the outer membrane of mitochondria, the endoplasmic reticulum (ER) and other electrolucent vesicles (Mamalaki et al., 1995; Patsavoudi et al., 1995). Interestingly, all four homologues contain the same C-terminus tail composed of three positively charged amino acids (RKK), adjacent to the transmembrane domain. Such

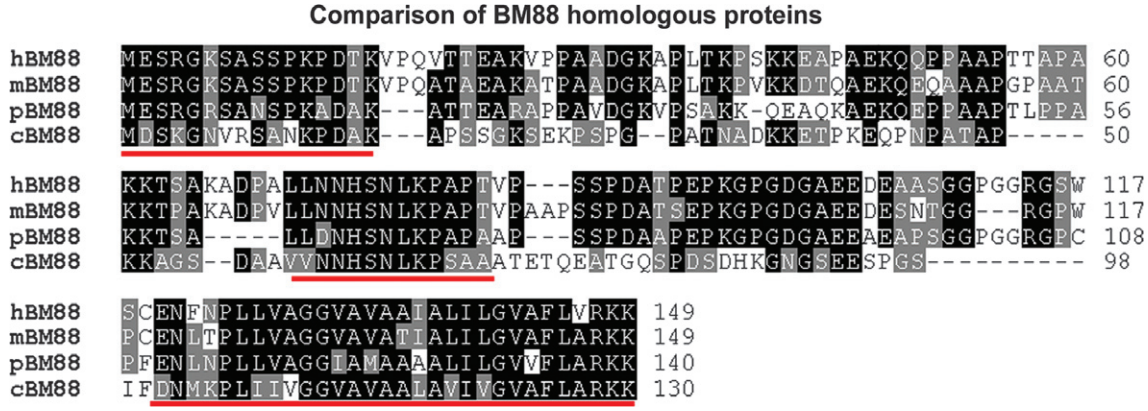

Fig. 1. Comparison of the predicted cBM88 amino acid sequence with cloned mammalian BM88 homologues. The predicted amino acid sequence of cBM88 protein is aligned with that of the human, mouse and porcine proteins. Black boxes indicate identical residues and grey boxes similar residues. Regions of high homology are underlined (red lines).