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DISTRIBUTION OF TMEM100 IN THE MOUSE AND HUMAN GASTROINTESTINAL TRACT – A NOVEL MARKER OF ENTERIC NERVES

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Abstract—Identification of markers of enteric neurons has contributed substantially to our understanding of the development, normal physiology, and pathology of the gut. Previously identified markers of the enteric nervous system can be used to label all or most neuronal structures or for examining individual cells by labeling just the nucleus or cell body. Most of these markers are excellent but have some limitations. Transmembrane protein 100 (TMEM100) is a gene at locus 17q32 encoding a 134-amino acid protein with two hypothetical transmembrane domains. TMEM100 expression has not been reported in adult mammalian tissues but does appear in the ventral neural tube of embryonic mice and plays a role in signaling pathways associated with development of the enteric nervous system. We showed that TMEM100 messenger RNA is expressed in the gastrointestinal tract and demonstrated that TMEM100 is a membrane-associated protein. Furthermore TMEM100 immunoreactivity was restricted to enteric neurons and vascular tissue in the muscularis propria of all regions of the mouse and human gastrointestinal tract. TMEM100 immunoreactivity colocalized with labeling for the pan-neuronal marker protein gene product 9.5 (PGP9.5) but not with the glial marker S100ß or Kit, a marker of interstitial cells of Cajal. The signaling molecule, bone morphogenetic protein (BMP) 4, was also expressed in enteric neurons of the human colon and co-localized with TMEM100. TMEM100 is also expressed in neuronal cell bodies and fibers in the mouse brain and dorsal root ganglia. We conclude that TMEM100 is a novel, membrane-associated marker for enteric nerves and is as effective as PGP9.5 for identifying neuronal structures in the gastrointestinal tract. The expression of TMEM100 in the enteric nervous system may reflect a role in the development and differentiation of cells through a transforming growth factor β, BMP or related signaling pathway.

Key words: TMEM100, enteric nervous system, PGP9.5, TGFβ signaling, BMP4.

INTRODUCTION

Normal gastrointestinal function depends on many cell types including nerves (Furness, 2006). Enteric nerves are required for coordinated gastrointestinal motility and the identification of markers for neuronal structures has contributed substantially to our understanding of the development, normal physiology, and pathology of the gut. Markers reported to label all neuronal structures include neurofilaments (Bjorklund et al., 1984) neuron-specific enolase (NSE) (Bishop et al., 1985), protein gene product 9.5 (PGP9.5, also known as UchL1) (Krammer et al., 1993), Hu C/D (also known as ANNA-1) (Lin et al., 2002), cuprolinic blue (Heinicke et al., 1987) and FluoroGold (Powley and Berthoud, 1991). Each of these markers meet the criteria proposed by Karaosmanoglu et al. (1996) in that they are (i) neuron-specific, (ii) label all neurons, and (iii) are readily available. All have proven useful in the quantification of neuronal cell bodies (Hu C/D, cuprolinic blue and FluoroGold) and/or neuronal fibers (PGP9.5, NSE, neurofilaments) (Bjorklund et al., 1984; Heinicke et al., 1987; Powley and Berthoud, 1991; Krammer et al., 1993; Karaosmanoglu et al., 1996; Lin et al., 2002; Phillips et al., 2004; Ganns et al., 2006; Bernard et al., 2009).

There are limitations to the existing markers, including failure to label all neuronal structures as reported for PGP9.5 immunoreactivity in rat myenteric neurons (Eaker and Sallustio, 1994) and for Hu C/D in aged rat myenteric neurons (Phillips et al., 2004). In addition, in some conditions labeling is observed in non-neuronal structures of the gastrointestinal tract including Hu C/D expression in glia (Phillips et al., 2004) and cuprolinic blue labeling in mast cells (Heinicke et al., 1987) and fibroblast-like cells in the sub-mucosa (Holst and Powley, 1995). For the most part these limitations are surmountable and in some tissues absence of expression or ectopic expression can provide important information. For example, studies of NSE expression have revealed important information about the processes of neuronal maturation, because a switch in expression from non-neuronal enolase (αα isoenzyme)

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Abbreviations: ALK1, activin receptor-like kinase I; BMP, bone morphogenetic protein; EDTA, ethylenediaminetetraacetic acid; ENS, enteric nervous system; ICC, interstitial cells of Cajal; Kit, tyrosine protein kinase Kit (CD117); NSE, neuron-specific enolase; PBS, phosphate-buffered saline; PGP9.5, protein gene product 9.5; TGFβ, transforming growth factor beta; TIRF, total internal reflection fluorescence; TMEM100, transmembrane protein 100.

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to the neuron-specific isoenzyme (γγ, NSE) correlates with differentiation of precursor cells into mature neurons (Schmechel et al., 1980). The finding that HuC/D expression is high in small cell carcinomas and is immunogenic has led to the recognition of paraneoplastic enteropathies in which the enteric nervous system (ENS) is damaged by the anti-Hu or ANNA-1 antibodies (King et al., 1999).

Our work characterizes TMEM100 immunoreactivity as a novel marker for enteric nerves. TMEM100 expression has been reported outside of the nervous system. TMEM100 expression has been associated with clinical stage in lung adenocarcinomas (Frullanti et al., 2012) and may be part of the bone morphogenetic protein (BMP) signaling pathway (Somekawa et al., 2012), which is involved in development of the ENS (Chalazonitis et al., 2004). TMEM100 is a gene at locus 17q32 encoding a 134-amino acid protein with two hypothetical transmembrane domains (amino acids 53–75, 85–107) (Moon et al., 2010). TMEM100, first identified as a transcript from the mouse genome (FLJ10970, (Kawai et al., 2001), is well conserved in vertebrates and is not structurally related to any known family of proteins in any species. There appear to be several splice variants or alternative gene transcripts. Several expression profile studies have identified TMEM100 transcripts in the pre-tubular aggregate in the renal vesicle of the developing kidney (Georgas et al., 2009) and associated with transcripts for activin receptor-like kinase I (ALK1), the gene mutated in hereditary hemorrhagic telangiectasia (Moon et al., 2010). TMEM100 is down-regulated in mice with pulmonary arterial hypertension induced by over-expression of dominant-negative BMP receptor type II (GEO: GDS2147). In the lungs of ALK1-deficient mice, TMEM100 expression is down-regulated, implying its involvement in the ALK1/transforming growth factor beta (TGF-β) signaling pathway (Moon et al., 2010). Greater levels of TMEM100 are detected in the transition zone of the prostate when compared to the peripheral zone where the majority of aggressive tumors are detected (van der Heul-Nieuwenhuijsen et al., 2006). TMEM100 was also identified as a marker of apoptotic cell death after thapsigargin treatment of SH-SY5Y neuroblastoma cells. Co-treatment of the cells with FK506 (tacrolimus) prevented the transcription of TMEM100 in response to thapsigargin and decreased apoptosis of the cells (Yamazaki et al., 2011).

A genetically modified mouse strain has been generated that contains the lacZ reporter gene driven by the TMEM100 promoter. In embryonic mice, expression of the reporter was detected in the vascular endothelium, heart, mammary glands, notochord and the ventral region of the neural tube (Moon et al., 2010). TMEM100 knockout animals did not survive to birth (Moon et al., 2010).

These published reports on TMEM100 indicate that, while the function of TMEM100 is not known, this protein seems to play a role in cellular differentiation, particularly in the pathways activated by the TGFβ/BMP/activin family of signaling molecules. TMEM100 may have some function in the fate of neoplastic cells, however it is not known if TMEM100 is a membrane protein, and there are no reports on its cellular and sub-cellular distribution in adult tissues. With respect to the gastrointestinal tract, TMEM100 transcripts were expressed in the muscularis propria of mouse jejunum (Chen et al., 2007). Our aim was to determine if TMEM100 is a membrane protein and to report its distribution in the adult mouse and human gastrointestinal tract.

EXPERIMENTAL PROCEDURES

Immunohistochemistry

Mice were maintained and the experiments were performed with approval from the Institutional Animal Care and Use Committee of the Mayo Clinic. Six- to eight-week-old female BALB/c mice (Harlan Sprague-Dawley, Indianapolis, IN, USA) and C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME, USA) were killed by CO_2 inhalation. The use of human tissue for research was approved by the Institutional Review Board of Mayo Clinic. Normal human stomach ($N = 3$), jejunum ($N = 3$) and colon ($N = 3$) tissues were obtained from patients undergoing surgery for gastrointestinal cancers or having bariatric surgery (Mayo Clinic, Rochester, MN, USA).

Whole mounts. Whole mount preparations of the gastric body, jejunum, and proximal colon of BALB/c ($N = 3$) and C57BL/6J ($N = 3$) mice were immuno-labeled according to previously published techniques for fluorescence immuno-histochemistry in paraformaldehyde-fixed tissue (Tharayil et al., 2010).

Cryosections. Human tissue fixed by immersion in 4% paraformaldehyde was immunolabeled as 15-μm cryosections using standard techniques including all necessary controls as described previously (Bernard et al., 2009). To confirm specificity of tissue labeling, each TMEM100 primary antibody was incubated with the respective immunogens for 24 h prior to immunohistochemistry. In each experiment, samples were exposed to secondary antibody in the absence of primary antibody to test for non-specific staining. Secondary antibodies directed against IgG from a different species were used to control for the specificity of secondary antibodies in doubly labeled tissues to ensure that there was no cross reaction between secondaries targeting different species (see Table 1).

Mouse brain and dorsal root ganglia (Balb/c $N = 2$, C57BL/6J $N = 2$) were removed and fixed by immersion in 4% paraformaldehyde in 0.1 mol L^{-1} phosphate buffer (pH 7.2). The next day the tissue was washed 4×15 min with 1×0.1 mol L^{-1} phosphate-buffered saline (PBS, pH 7.2) then incubated in 30% sucrose in $1 \times$ PBS overnight before freezing and storage at −80 °C until needed. Fifteen micron tissue sections were cut and immunohistochemistry was performed with proper controls as described above. SlowFade® Gold Antifade Reagent with DAPI (Invitrogen, Carlsbad, CA, USA) was used as a counterstain to detect nuclei.

Reverse transcription PCR

The muscularis propria of the small intestine of adult BALB/c mice ($n = 3$) was removed from the mucosa by dissection. RNA was extracted, transcribed and amplified from each tissue separately. Extraction of mRNA was done using RNA-Bee reagent (AMS Biotechnology, Abingdon, UK). Complementary DNA was synthesized using SuperScript® VILO cDNA