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Short Communication

# Recently identified a novel neuropeptide manserin colocalize with the TUNEL-positive cells in the top villi of the rat duodenum

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**Abstract:** We recently isolated a novel 40 amino acid neuropeptide designated manserin from the rat brain. Manserin is derived from secretogranin II, a member of granin acidic secretory protein family by proteolytic processing, as previously reported secretoneurin and EM66. Manserin peptide are localized in the endocrine cells of the pituitary. In this study, we further investigated the manserin localization in the digestive system by immunohistochemical analysis using antimanserin antibody. In the duodenum, manserin immunostaining was exclusively observed in the nuclei of top villi instead of cytosol as observed in neurons in our previous study. Interestingly, manserin-positive cells in the duodenum are colocalized with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) positive cells, the cells whose DNA was damaged. Since the top villi of duodenum epithelial cells are known to undergo spontaneous apoptosis during epithelial cell turn over, and since other peptides such as secretoneurin and EM66 derived from SgII have been reported to be cancer-related, these results indicated that manserin peptide may have a role in apoptosis and/or cancer pathogenesis in the digestive organ. 

**Keywords:** neuropeptide; manserin; TUNEL; apoptosis

## INTRODUCTION

Secretogranin II (SgII) is an acidic secretory protein which was originally designated as chromogranin C [1,2]. SgII retains a number of pairs of basic residues within its amino acid sequence which are sites at which proteolytic processing could cleave smaller peptides from the precursor protein. Several peptides cleaved from SgII are reported to date, including secretoneurin and EM-66 [3,4]. We recently isolated a novel SgII-derived 40 amino acid neuropeptide designated manserin from the rat brain that showed interesting localization in the pituitary compared to its precursor protein SgII [5], suggesting its interesting role in the central nervous system. The roles of manserin in the gastrointestinal tract are not known to date. According to previous report, secretoneurin and EM66, a neighbor peptide derived from SgII are known to be cancer-related [6,7]. For example, serum concentration of EM66 is significantly different between benign and malignant pheochromocytomas [8]. Concentrations of secretoneurin in serum and urine are also useful for discriminating between benign and malignant neuroendocrine tumours [9]. The small intestine is not only one of the important organs for regulating metabolism via the digestion and absorption of food but also for physiological apoptosis [10–14]. These facts led us to investigate the relation of cancer or apoptosis with manserin.

## MATERIALS AND METHODS

All animal experiments were approved by the Community of Laboratory Animal Research Center in the University of Tsukuba which conforms to the National Institutes of Health (NIH) guide for the use of laboratory animals [15]. Rat antimanserin antibody was prepared as described previously [5]. The specificity of antimanserin antibody was already confirmed by immunoblot and by the fact that the reactions were specifically inhibited by addition of the corresponding antigen [5]. Rabbit anti-SgII antibody was from QED (San Diego, CA). Tissues were obtained from 6-week-old male Wister rats and fixed with 4% formaldehyde/0.1 M phosphate, pH 7.3 (0.1 M PB), freshly prepared from paraformaldehyde. Immunohistochemistry was performed as described previously [5]. Briefly, sections were first incubated in 0.3% $H_2O_2$/methanol for 20 min at room temperature, then were blocked with 5% normal goat serum (NGS, Invitrogen, Carlsbad, CA) in 0.1% Triton-X/PBS (T-PBS) for single immunostaining and with 5% normal horse serum (NHS, Vector Laboratories, Burlingame, CA) in T-PBS for

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double staining for 1 h at room temperature, prior to an incubation with the antibodies in T-PBS at 4 °C overnight. For terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay, we used *in situ* Cell Death Detection Kit, TMR red (Roche Applied Science, Penzberg, Germany). The secondary antibodies used were as follows: (i) biotinated goat antirabbit IgG (dilution 1 : 500 in T-PBS, Vector Laboratories), (ii) TSA plus Fluorescence Systems with FITC (dilution 1 : 50, NEN Life Science, Boston, MA), and (iii) horse antimouse conjugated with Texas red (dilution 1 : 500, Vector Laboratories). Antigen–antibody complex for single immunostaining was detected with either a Vectastatin ABC kit (Vector Laboratories) or an Envision + kit (Dako Cytomation, Kyoto, Japan) followed by a visualization with 0.02% 3,3′-diaminobenzidine solution with 0.06% ammonium nickel (II) sulfate hexahydrate and 0.005% $H_2O_2$. The slides were examined under microscopes (DM RBE and MZ 16, Leica) and FW 4000 or IM50 software (Leica) was used to obtain digital images for immnoperoxidase or iminofluorescent labeling.

## RESULTS

Immunolocalization of manserin in the intestine was of great contrast with SgII. Manserin mainly restricts its localization in the nuclei of the epithelial cells in the top villi (Figure 1(A)) in the duodenum cells, whereas the strongest SgII immunostaining was found mainly in the striated border of the epithelial cells restricted in the top villi (Figure 1(B)). These results suggest that manserin is engaged in nuclear transport for its specific role at the top villi of digestive organs.

Since the cells in the top villi are considered to be in the final stage of the turnover cycle, we next performed double fluorescent staining using the antimanserin antisera and the TUNEL staining. As also observed in Figure 1(A), immunofluorescent staining showed manserin-like staining in the epithelial cells in the top villi of the duodenum cells (Figure 2(A)). TUNEL experiment also revealed top villi-specific staining (Figure 2(B)) which was mostly overlapped with the manserin-positive cells (Figure 2(C)), suggesting its function in the programmed cell death.

## DISCUSSION

In this study, we have demonstrated that (i) manserin was nuclearly localized in the top villi of the duodenum and (ii) manserin was colocalized with TUNEL-positive cells. These results suggest that neuropeptide manserin might be related with some mechanism of programmed cell death.

Localization of manserin peptide in the top villi of the duodenum epithelium strongly suggests that manserin might be involved in the turnover mechanism of intestinal epithelium. Normally, a crypt stem cell proliferates and matures into an epithelial cell while it migrates upward along crypt/villus axis for approximately 2–3 days [10–13]. Given the existence of this dynamic system, cell proliferation has to be counterbalanced by cell death [16]. Regulation of epithelial cell growth is highly complex and is controlled by a variety of factors, including direct acting factors such as local nutrients and cell loss, and stimulants such as polyamines, or indirect acting factors such as gastrointestinal hormones and peptides [14,17,18]. We do not know how manserin exerts in that turnover mechanism, but the fact that manserin was localized especially in the upper third of the villi, in addition to its nuclear localization instead of cytosol, suggest that manserin might be involved in the programmed cell death occurring spontaneously [13,19] during the mechanism of intestinal turnover. Indeed, in our study, double staining revealed that TUNEL staining exclusively colocalized

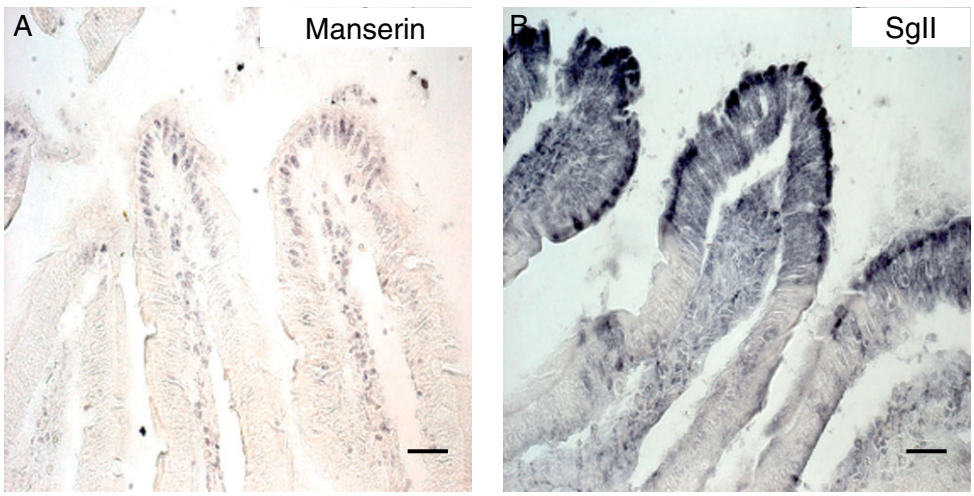


**Figure 1** Manserin (**A**) and SgII (**B**) immunostaining labeled with immunoperoxidase in the duodenal epithelial cells. **(A)** Immunostaining showed that manserin was specifically detected in nuclei of top villi in the duodenal. **(B)** In contrast, SgII was found mainly in the striated border of the epithelium cells of top villi. Scale bars: 20 μm.



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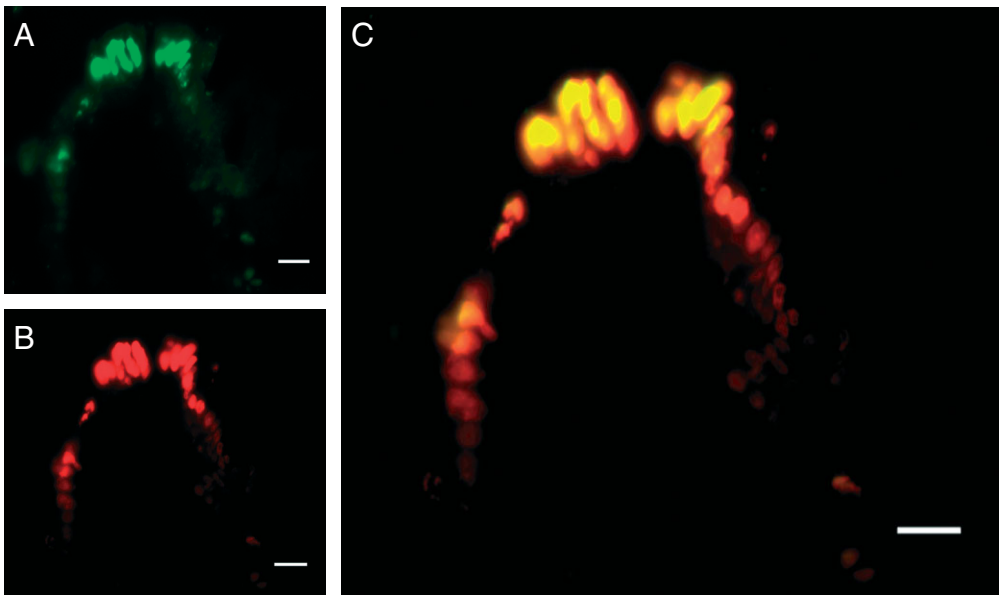


**Figure 2** Double fluorescent staining by antimanserin antiserum and TUNEL assay in the duodenum. Manserin (**A**, green) frequently existed in TUNEL positive apoptotic cells (**B**, red) of the duodenal villi (**C**, overlaid). Scale bars: 20 μm.

with manserin immunoreactivity. Many studies indicated that TUNEL staining labels damaged DNA in degenerative cells with high sensitivity [20]. It can detect apoptotic cells even when their concentration in the tissue is low. Although some people question its specificity of *in situ* detection of DNA fragmentation by TUNEL [21], these results strongly suggest that manserin is involved in some mechanism of apoptosis. Numerous studies about involvement of apoptosis in disease are reported [22–25].

Intestinal localizations in other SG-II-derived peptide and secretoneurin [3,4,26], have also been reported. However, instead of the top villi of intestinal epithelial cells, secretoneurin exists in nerve fibres of myenteric and submucous plexuses in all layers of the gut wall [26,27]. Physiological functions of secretoneurin in the intestine is not known to date. Schurmann *et al* and Trandaburu *et al* reported intestinal localization of granin proteins and secretoneurin [26,28]. Relations of secretoneurin in apoptosis is also known [6].

Possible relation to intestinal apoptosis of manserin peptide may lead to the interesting thought that manserin might be related to cancer pathogenesis. As a matter of fact, this thought is not, however, surprising because increased immunoreactivities of chromogranin/secretogranin are continuously reported in many kinds of tumours derived from endocrine cells, and some are used as diagnostic markers of such tumours [8,9,29]. Immunostaining of gastropancreatic neuroendocrine cells in neoplastic human tissue such as insulin-producing islet and duodenal carcinoid showed the cell specific reactions of granins [30]. Serum secretoneurin level is increased in patients of metastatic prostate cancer [31]. Thus, the presence of manserin exclusively in the apoptotic intestine nuclei suggests that this peptide has distinct function in programmed cell death system of the epithelium, and may be related to oncogenesis. However, we cannot exclude the possibility that manserin may be just one of the metabolic products of proteolytic cleavage in the pathways. Further studies should be necessary to elucidate the physiological role of this novel peptide, manserin.

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