Immunohistochemical Distribution of Intestinal 15 kDa Protein in Human Tissues

Kazuo WATANABE¹, Nobuo HOSHI¹, Yukio TSUURA¹, Tatsuo KANDA², Michiyo FUJITA², Hiroshi FUJII², Teruo ONO² and Toshimitsu SUZUKI¹

Department of Pathology¹, Fukushima Medical College, Fukushima; and Department of Biochemistry², Niigata University School of Medicine, Niigata, Japan

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Summary. The distribution of human intestinal 15 kDa protein (I-15P), a new fatty acid-binding protein (FABP), was observed in normal tissues using immunohistochemical techniques. The antiserum against human I-15P intensely reacted with the villous epithelium of the terminal ileum but not with the enterocytes of the crypts. Although the surface epithelium of the stomach and villous epithelium of the duodenum showed weak reactivities, the epithelial cells of the jejunum, proximal ileum, colon and rectum, and also glandular epithelia with intestinal metaplasia of the stomach were not immunostained. The other human tissues examined were negative for anti-human I-15P antibody. Human I-15P thus represents a distinctive, confined tissue distribution different from the other FABPs and is expected to serve as a useful cellular marker of terminal ileal enterocytes.

Fatty acid-binding proteins (FABPs) belong to a large group of low-molecular, 14-15 kDa, soluble proteins and are abundantly distributed in many organs of humans, rats, and other animals (OCKNER et al., 1982; TAKAHASHI et al., 1983; MATARESE et al., 1990; VEERKAMP et al., 1991). To date, several FABPs with different chemical structure and tissue distribution designated as liver (hepatic) FABP (CHAN et al., 1985), heart FABP (OFFNER et al., 1988), intestinal FABP (ALPERS et al., 1984), adipocyte P2 protein (MATARESE et al., 1990), myelin P2 protein (SUZUKI et al., 1982), and gastrotropin (WALZ et al., 1988) have been reported. FABPs are present in the cytosole and are thought to play an important role in the transport and metabolism of fatty acids (TIPPING and KETTERER, 1981; COOPER et al., 1987).

Recently, KANDA et al. (1991) purified a 15 kDa protein (rat I-15P) from the rat intestinal epithelium. Rat I-15P is thought to be a new member of the FABP family, based on its structural feature and amino acid sequence similar to porcine gastrotropin (KANDA et al., 1991). Immunohistochemistry using polyclonal antibody against rat I-15P revealed the characteristic expression of rat I-15P in the gastro-intestinal epithelium, corpus luteum cells and adrenocortical cell (ISEKI et al., 1993). The ligands and role in vivo of rat I-15P have, however, remained unclear. We have more recently cloned a cDNA for the human homologue of rat I-15P from a human ileum library (unpublished data, manuscript in preparation). The present study reports on the organ-specific distribution of I-15P in human tissues using specific antiserum against human I-15P.

MATERIALS AND METHODS

Preparation of antiserum specific for human I-15P

The human I-15P cDNA was obtained by screening the human ileum library, probed with a ³²P-labeled full length rat I-15P cDNA (FUJITA et al., manuscript in preparation). The cloned cDNA was subcloned into the expression vector, pET3a. The recombinant human I-15P bacterially expressed was purified to homogeneity and used as the antigen for immunization (unpublished data).

The antiserum against recombinant human I-15P was raised in rabbits by intracutaneous and intramuscular injections on the back at multiple sites with an emulsion of 150µg I-15P in a mixture of 1 ml Tris-HCl (pH 8.0) and 1 ml of Freund’s complete adjuvant (Difco, Michigan). For booster injections, 100 µg of antigen with Freund’s incomplete adjuvant (Difco, Michigan) was given twice at intervals of four and three weeks, respectively.

Western blot analysis was performed to confirm the specificity of the antiserum. The recombinant human...
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I-15P, rat I-15P, 105,000 g supernatant from human ileum, and rat intestinal and liver FABPs were electrophoresed on polyacrylamide gel. The proteins were electrically transferred to a nitrocellulose membrane. The membrane was incubated with the antiserum, diluted at 1:1,500, and then with horseradish peroxidase-conjugated anti-rabbit IgG (Gibco BRL, New York), at a dilution of 1:3,000. The antiserum specifically reacted with human I-15P, rat I-15P, and the supernatant human ileum, but not with rat intestinal and liver FABPs.

**Tissue**

The normal human tissues prepared were as follows: the cerebrum, cerebellum, midbrain, pons, medulla oblongata, tongue, salivary gland, trachea, lung, esophagus, gastro-intestinal tract, including cardia, fundus and antrum of stomach, duodenum, jejunum, ileum, ascending, transverse and descending colon, sigmoid colon, and rectum, liver, gallbladder, pancreas, spleen, lymph node, kidney, pituitary gland, thyroid gland, uterus, prostate, and testis. The gastro-intestinal tract and female reproductive system were obtained from surgical specimens; the other tissues were sampled at the autopsy of a 25-year-old male succumbing from traumatic injury. The small intestine of a 44-year-old male having died of thymic cancer was serially sampled from the Treitz’ ligament to the terminal ileum at about one meter intervals to investigate the difference in expression of human I-15P by the site. In addition, seven surgically resected stomachs with intestinal metaplasia were prepared. All specimens obtained were fixed in 10% neutral formalin and embedded in paraffin.

**Immunohistochemistry**

Sections cut at 3-4 μm were deparaffinized, and pretreated with 0.3% hydrogen peroxide in methanol to block endogenous peroxidase activity for 20 min. After blocking the non-specific adsorption of antiserum using 5% skimmed milk (Yukijirushi, Sapporo), the sections were incubated in the antiserum diluted 1:500 at 4°C overnight. For immunohistochemistry, the streptavidin-biotin-peroxidase complex method (SAB kit, Nichirei, Tokyo) was used. The reaction products were visualized by incubation with Graham-
Karnovsky solution (GRAHAM and KARNOVSKY, 1966) containing 65 mg/100 ml sodium azide (Sigma, St. Louis) to again block the endogenous peroxidase activity. The slides were counterstained by 1% methyl green for nuclei.

For negative controls, the sections were either incubated in the antiserum absorbed with 100 µg/ml of purified human I-15P or preimmunized normal serum obtained from same rabbit. Immunostaining by anti-chromogranin A (Dako, Kyoto; 1:200) was applied to tissues of the gastrointestinal tract to confirm the endocrine cells.

RESULTS

Intensive immunopositivity for human I-15P was observed in the ileal epithelium, the so-called absorptive cells (Fig. 1a). The reactivity was strong in about the upper half of villi, whereas enterocytes in the portion of the crypts were not immunostained (Fig. 1a). The cytoplasm of goblet cells was also immunolabelled only in the periphery around the mucus (Fig. 1b). Immunoreactive enterocytes were localized in about two meters length of the terminal ileum. The epithelial cells in the proximal portion of the ileum, jejunum and seven stomachs with intestinal metaplasia did not show positivity. In control slides incubated in antiserum preabsorbed with human I-15P, these immunoreactivities either disappeared or were close to it.

A weak positivity was seen in the surface epithelium of normal gastric mucosa and also in the villous epithelium of the duodenal mucosa. A few enterocytes were sporadically immunolabelled in the stomach, small and large intestine. Their distribution however, did not correspond to that of chromogranin A-positive endocrine cells (Figure not shown). The collecting ductal cells of the salivary gland, a few adrenocortical and medullary cells, and the luteinizing cells of the ovary, showed weak reactivity; they, however, were thought to be non-specific because absorption tests revealed the same reactivity. Other tissues, including the cerebrum, cerebellum, midbrain, pons, medulla oblongata, tongue, trachea, lung, esophagus, liver, gallbladder, pancreas, spleen, lymph node, kidney, pituitary gland, thyroid gland, uterus, prostate and testis were not immunolabelled. Control slides reacted with normal rabbit serum gave negative results.

DISCUSSION

In the FABPs and related proteins, at least four types of structurally distinct proteins including liver FABP (SUZUKI and ONO, 1988), intestinal FABP (SHIELD et al., 1986), cellular retinol-binding protein II (CROW and ONG, 1985) and porcine gastrotropin (WALZ et al., 1988) have been reported to be expressed in the intestinal epithelium. Human I-15P is distinctive in the view of its extremely localized expression in the enterocytes of the terminal ileum. For instance, liver FABP is extensively distributed in the liver and intestinal tract (SUZUKI and ONO, 1988), and intestinal FABP is expressed in enterocytes of the small intestine over its entire length (SHIELD et al., 1986). Only porcine gastrotropin is known to show a distribution analogous with human I-15P (WALZ et al., 1988).

Porcine gastrotropin (GT) is a member of the FABPs with a similar amino acid sequence to liver FABP. GT was first isolated from pig ileal mucosa and named porcine ileal polypeptid (PIP) (WIDER et al., 1984). Immunohistochemistry using antiserum for PIP revealed a localized expression of PIP exclusively in the terminal ileum (BORGSTROM et al., 1986). PIP was subsequently redesignated porcine gastrotropin because of its characteristic hormonal functions such as the stimulation of gastric acid secretion, in vivo and in vitro (WALZ et al., 1988), and of acid and pepsinogen secretion from guinea pig parietal and chief cells respectively, in vitro (TSUNODA and WIDER, 1987). Since rat I-15P shows both tissue distribution and an amino acid sequence analogous with GT, it has been controversial whether these two proteins are identical. There are, however, biochemical differences between the two proteins. Firstly, the identical amino acid residues of the total residues are not more than 71% between rat I-15P and GT (KANDA et al., 1991). Secondly, GT binds palmitate and oleate and also binds chenodeoxycholate, a major component of bile acid, while the ligand of rat I-15P has so far not been determined (KANDA et al., 1991). In addition, the hormonal function such as the stimulation of gastric acid secretion has not been demonstrated in rat I-15P (KANDA et al., 1991). We now consider rat I-15P a distinct protein, but further investigation is necessary concerning the function of the protein, in vivo and in vitro.

Because FABPs are a family widely expressed in many organs of mammals, each of the FABPs can be a useful marker of cells, cellular maturation and some diseases including neoplasm (SUZUKI and ONO, 1988; SUZUKI et al., 1990; WATANABE et al., 1993). Human I-15P is also expected to be a useful tissue marker of the terminal ileum.
REFERENCES


Dr. Kazuo WATANABE
Department of Pathology
Fukushima Medical College
1 Hikariga-oka, Fukushima
960-12 Japan