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Serranidae (*Coreoperca herzi*) 췌장 내분비세포에 대한 면역조직화학적 연구

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Immunohistochemical Study of the Endocrine Cells in the Pancreas of the Korean Aucha Perch, Serranidae (*Coreoperca herzi*)

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Abstract: The regional distribution and relative frequency of some endocrine cells in the pancreas of the Korean aucha perch, *Coreoperca herzi* Herzenstein belonging to the family Serranidae in order Perciformis, were observed using specific mammalian antisera against serotonin, insulin, glucagon, somatostatin and human pancreatic polypeptide (hPP) by peroxidase antiperoxidase (PAP) method. The pancreas was divided into four portions (principal and secondary islets, exocrine and pancreatic duct regions). In addition, the pancreatic islet regions were further subdivided into three regions (central, mantle and peripheral regions). Spherical to spindle or occasionally round to oval immunoreactive (IR) cells were demonstrated in the pancreatic islets and exocrine portions, but no cells were detected in the pancreatic duct portions. In the principal islets, serotonin-IR cells were not detected but most of insulin-IR cells were located in the central regions and they were also demonstrated in the mantle and peripheral regions in moderate and rare frequencies, respectively. Glucagon- and hPP-IR cells were mainly situated in the mantle regions but the cells were also demonstrated in the peripheral regions in relatively lower frequency. Somatostatin-IR cells were evenly distributed in the central and mantle regions in a few frequency and cells were also demonstrated in the peripheral regions in rare frequency. Cell clusters were consisted of hPP-IR cells that were situated in the peripheral to mantle regions. In the secondary islet portions, serotonin-IR cells were randomly distributed throughout the whole pancreatic islet regions but lower frequency was detected in the peripheral regions compared to that in central and mantle regions where cells were detected in a few frequency, respectively. Insulin-IR cells were restricted to the central regions in numerous frequency and glucagon-IR cells were evenly distributed in the mantle and peripheral regions in moderate frequencies, respectively. Somatostatin-IR cells were observed in the central and mantle regions in moderate and a few

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frequencies, respectively. In addition, hPP-IR cells showed similar distributional patterns to those of glucagon-IR cells except cells were also located in the central regions in rare frequency. In the exocrine portions, only glucagon- and hPP-IR cells were demonstrated in rare and a few frequencies, respectively. In conclusion, the regional distribution and relative frequency of pancreatic endocrine cells of the Korean aucha perch showed general patterns, which were observed in other teleost. However, some species-dependent different distributional patterns and/or relative frequencies were also demonstrated especially to serotonin-IR cells. In pancreas of the Korean aucha perch, insulin-IR cells were the most predominant cell type followed by glucagon-, somatostatin-, hPP- and serotonin-IR cells.

Key words: Korean aucha perch, teleost, pancreas, immunohistochemistry, immunoreactive cells, endocrine cells

Introduction

The stomach-containing teleost Korean aucha perch, *Coreoperch herzi*, belonging to the family Serranidae in order Perciformis is a fresh-water fish. This teleost has grayish brown back color, has pale belly, 7~8 black cross bars on sides and bluish green speckle like eyes on opercula. They are inhabited in clear water of upper streams with pebbly bottoms. The Korean aucha perch has been regarded as one of the most common Korean endemic species. However, the number and habitation of the Korean aucha perch have dramatically decreased because of pollution and immigration of other foreign species of teleost having similar feeding habits or predator.

It is generally known that pancreas of vertebrates is subdivided into two regions. One is an exocrine region where digestive enzymes are released and the other is an endocrine portion where regulatory hormones such as insulin, glucagon, somatostatin and pancreatic polypeptide (PP) are released into blood vessels. The appearance, regional distribution and relative frequency of these regulatory hormones secreted by endocrine cells in the pancreas are well recognized by Histochemistry [19], immunofluorescence method [25] and immunohistochemistry [33]. In addition to four regulatory hormones mentioned above, serotonin- [22], peptide YY-, neuropeptide Y- [2], and chromogranin family- [13, 27] immunoreactive (IR) cells were also demonstrated in the vertebrate pancreas. The pancreas has been treated as a valuable organ for endocrine studies and endocrine pancreas was extensively studied in association with diabetes [14]. Until now, investigations of gastroenteropancreatic (GEP) endocrine cells have been considered to be an important part of a phylogenetic study [6] and the distribution and relative frequency of these endocrine cells in the pancreas were varied with animal species and feeding habits. Recently most intensive studies have been done on the Pisces because some endocrine cells were demonstrated in the skin, gills and airways [38], and the alteration of regional distribution and relative frequency of these cells by heavy metal intoxication such as lead was also demonstrated [26]. In addition, the possibility of using the teleost fish endocrine tissues for treatment hormonal disorder such as diabetes was suggested [24]. The endocrine pancreas of teleost fish is mainly composed of two types of pancreatic islets: 1) one, two or even more multiple large islets, called principal pancreatic islets and 2) numerous, widely scattered small islets, called secondary pancreatic islets [8]. Until now, the appearance, regional distribution and relative frequency of numerous types of regulatory peptides have been demonstrated in the pancreas of the Pisces. Well corresponding to those of mammals, the regional distribution and relative frequency of endocrine cells within the pancreas, and the cell population seemed to be considerably variable among species and feeding habits, especially in case of occurrence in PP cells [37]. Namely, these IR cells that were generally demonstrated in teleost, were not detected in the pancreas of channel catfish and lungfish [11, 23]. In addition, it is also reported that somewhat different distributional patterns of pancreatic endocrine cells were found in two species of stomach- containing fresh water teleost having similar feeding habits [21]. Although many studies have elucidated regional distribution and relative frequency of endocrine cells in the pancreas of teleost, the localization of endocrine cells in the pancreas of the Korean aucha perch has not yet been reported.

In the present study, the regional distribution and relative frequency of some endocrine cells in the pancreas of stomach-containing fresh-water teleost, the Korean aucha perch, *Coreoperch herzi* (Serranidae), were observed using specific antisera against mammalian serotonin, insulin, glucagon, somatostatin and human PP (hPP) by peroxidase antiperoxidase (PAP) method.

Materials and Methods

Experimental animals

The five adult Korean aucha perches, *Coreoperch herzi*, belonging to the family Serranidae in order Perciformes (about 15~20cm in length) were purchased from a merchant around Daegu, Korea and used in this study without sexual distinction.

Histological procedures

After decapitation, samples from the pancreas were fixed in Bouin's solution. After paraffin embedding, 3~4 ㎛ sections were prepared. Representative sections of each tissue were stained with hematoxylin and eosin for light microscopic examination of the normal pancreatic architecture.

Immunohistochemical procedures

The each representative section was deparaffinized, rehydrated and immunostained with the peroxidase anti- peroxidase (PAP) method [34]. Blocking of nonspecific reaction was performed with normal goat serum prior to incubation with the specific antisera (Table 1). After rinsed in phosphate buffered saline (PBS; 0.01 M, pH 7.4), the sections were incubated in secondary antiserum. They were then washed in PBS buffer and finally the PAP complex was prepared. The peroxidase reaction was carried out in a solution 3,3'-diaminobenzidine tetrahydrochloride containing 0.01% H_2O_2 in Tris-HCl buffer (0.05 M, pH 7.6). After immunostained, the sections were lightly counterstained with Mayer's hematoxylin and the IR cells were observed under light microscope.

Table 1. Antisera used in this study

Antisera*	Code	Source	Dilution
Serotonin	BO68082C	BioGenex Lab, San Ramon, USA	1:20
Insulin	842613	Diasorin, Stillwater, USA	1:1,000
Glucagon	927604	Diasorin, Stillwater, USA	1:2,000
Somatostatin	917600	Diasorin, Stillwater, USA	1:600
hPP	A61P	DAKO Co., Carpenteria, USA	1:600

*All antisera were raised in rabbits except for insulin, which were raised in a guinea pig.
hPP, human pancreatic polypeptide.

Specificity of antiserum reaction

The specificity of each immunohistochemical reaction was determined as recommended by Sternberger [34], including the replacement of specific antiserum by the same antiserum, which had been preincubated with its corresponding antigen.

Category of relative frequency

The relative frequency of occurrence of each type of IR cell was placed into one of five categories, not detected ( ), rare (±), a few (+), moderate (++) and numerous (+++), according to their observed numbers as seen using light microscopy.

Classification of pancreatic regions

The distribution of IR cells was divided into four portions, 1) the principal and 2) secondary islets regions, 3) the exocrine regions and 4) pancreatic duct regions according to modified classifications of carp [20] which were classified by their histological profiles. In the principal islets, the regions were divided by the location of IR cells in the cluster of cell cord not the whole principal islet parenchyma. In addition, the regional distribution and relative frequency of endocrine cells in the pancreatic islets were further subdivided into three regions from centrally to marginally, central, mantle and peripheral regions according to types of cell composition.

Results

In the present study, all five kinds of the IR endocrine cells were detected using antisera against mammalian serotonin, insulin, glucagon, somatostatin and hPP in the pancreatic islets and exocrine portions. Different regional distributions and relative frequencies of these IR cells were observed in the different pancreatic portions, and these differences are shown in Tables 2 and 3. Spherical to spindle or occasionally oval to round immunoreactive cells were observed in this study.

Serotonin-IR cells

These IR cells were restricted to the secondary islet portions. In there, their cytoplasmic processes were intermingled with other IR cells and they were mainly located in the central and mantle regions with round to

spherical shapes, showing a few frequency. In addition, some cells showing rare frequency were also demonstrated in the peripheral regions (Fig. 1A and 1B). However, no serotonin-IR cells were found in the principal islet, exocrine and pancreatic duct portions (Tables 2 and 3).

Table 2. Regional distribution and relative frequency of the endocrine cells in the principal pancreatic islets of the Korean aucha perch

Hormones	Regions of Principal Pancreatic islets		
	Central	Mantle	Peripheral
Serotonin	–	–	–
Insulin	+++	++	±
Glucagon	–	++	±
Somatostatin	+	+	±
hPP	–	+	±

–, not detected; ±, rare; +, a few; ++, moderate; +++, numerous; hPP, human pancreatic polypeptide.

Table 3. Regional distribution and relative frequency of the endocrine cells in the secondary pancreatic islets, exocrine and pancreatic duct portions of the Korean aucha perch

Hormones	Regions of Principal Pancreatic islets			Exocrine	Pancreatic duct
	Central	Mantle	Peripheral		
Serotonin	+	+	±	–	–
Insulin	+++	–	–	–	–
Glucagon	–	++	++	±	–
Somatostatin	++	+	–	–	–
hPP	±	++	++	+	–

–, not detected; ±, rare; +, a few; ++, moderate; +++, numerous; hPP, human pancreatic polypeptide.

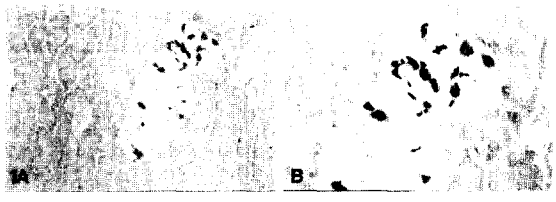

Fig. 1. Serotonin-IR cells in the secondary pancreatic islet of the Korean aucha perch. Note that these IR cells were evenly dispersed to the central and mantle regions and the cells were also demonstrated in the peripheral regions in lower frequency. They were restricted to the secondary islets only. A, ×150; B, ×300. PAP method.

Insulin-IR cells

In the principal pancreatic islets, spherical to spindle cells having cytoplasmic process were demonstrated in the central regions in numerous frequency and also cells were situated in the mantle regions in moderate frequency. In addition, cells were also found in the peripheral regions in rare frequencied. In there, especially their cytoplasmic processes were intermingled with other IR cells (Fig. 2A-D). In the secondary islet portions, they were mainly located in the central regions with similar shape compared to those in of principal islets, showing numerous frequencies. However, no cells were detected in the mantle and peripheral regions. In the pancreatic duct and exocrine portions, no insulin-IR cells were demonstrated in the present study (Table 2 and 3).

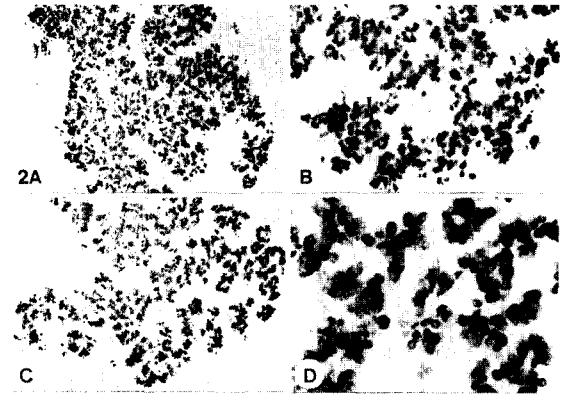

Fig. 2. Insulin-IR cells in the principal islets of the Korean aucha perch. Note that these IR cells were dispersed in the central regions of the principal islets and the cells, in lower frequency, were also demonstrated in the mantle and peripheral regions. A and C, ×75; B, ×150; D, ×300. PAP method.

Glucagon-IR cells

In the principal pancreatic islets, spherical to spindle cells having cytoplasmic process were demonstrated in the mantle regions in moderate frequency and some of these IR cells were situated in the peripheral regions in a few frequency. In there, their cytoplasmic processes were intermingled with other IR cells and relatively weakly and strongly reacting cells were intermingled with each other. The frequencies of weakly reacting cells were higher than those of strongly reacting cells (Fig. 3A and 3B). In the

secondary islet portions, glucagon-IR cells were mainly located in the mantle and peripheral regions with similar shape compared to those in principal islets, showing moderate frequencies. In there, their cytoplasmic processes were intermingled with other IR cells. Similar to those in principal islets, no glucagon-IR cells were demonstrated in the central regions of secondary islets (Fig. 3C and 3D). In the exocrine portions, spherical to spindle glucagon-IR cells were detected between acinar cells in rare frequency (Fig. 3E). In the pancreatic duct portions, no glucagon-IR cells were observed in the present study (Table 2 and 3).

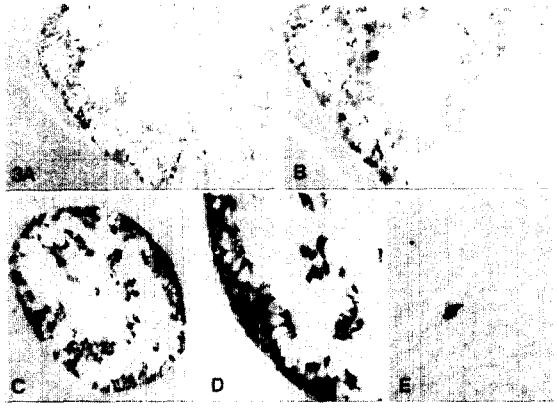

Fig. 3. Glucagon-IR cells in the pancreas of the Korean aucha perch. Note that most of glucagon-IR cells were restricted to the mantle regions of the principal islets and the cells in lower frequency were also demonstrated in the peripheral regions (A and B). The regional distribution of glucagon-IR cells in the secondary islets was quite similar to that in the principal islets (C and D) but stronger reactions were detected. Glucagon-IR cells were also observed in the inter-acinar regions of the exocrine portions (E). A, ×75; B and C, ×150; D and E, ×300. PAP method.

Somatostatin-IR cells

In the principal pancreatic islets, spherical to spindle cells having cytoplasmic process were dispersed throughout the central to peripheral regions in a few to rare frequencies, respectively. In there, their cytoplasmic processes were intermingled with other IR cells. The immunoreactivities to somatostatin were somewhat lower than those of other IR cells and those in secondary islets (Fig. 4A and 4B). In the secondary islet portions, somatostatin-IR cells were dispersed in the central regions with similar shape compared to those in principal islets, showing moderate frequency. In addition, the cells were also demonstrated in the mantle regions in a few frequency. In there, their cytoplasmic processes were intermingled with other endocrine cells similar to those in principal islets (Fig. 4C). In the exocrine and pancreatic duct portions, no somatostatin-IR cells were detected in the present study (Table 2 and 3).

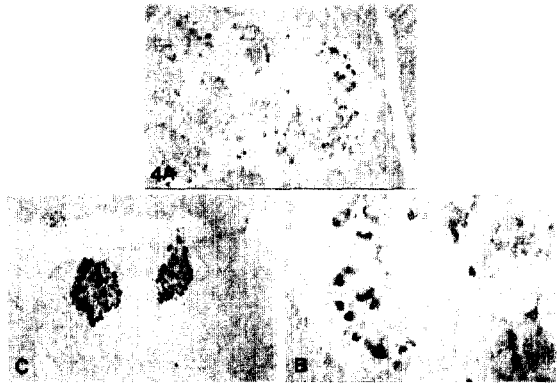

Fig. 4. Somatostatin-IR cells in the principal (A and B) and secondary (C) pancreatic islets of the Korean aucha perch. Note that these cells were dispersed in the central and mantle regions of the principal islets and the cells were also demonstrated in the peripheral regions in lower frequency (A and B). The regional distribution of somatostatin-IR cells in the secondary islets was quite similar to those in the principal islets (C). A, ×150; B, ×300; C, ×75. PAP method.

hPP-IR cells

In the principal pancreatic islets, spherical to spindle hPP-IR cells having cytoplasmic process were demonstrated in the peripheral to mantle regions in rare and a few frequencies, respectively. In there, their cytoplasmic processes were intermingled with other IR cells, especially with glucagon-IR cells. In addition, cell clusters consisted of 5 to 20 hPP-IR cells were located in the mantle to peripheral regions of principal islets (Fig. 5A-C). In the secondary islet portions, hPP-IR cells were mainly located in the mantle and peripheral regions with similar shape compared to those in principal islets, showing moderate frequencies, respectively. In addition, some cells were also demonstrated in the central regions in rare frequency. In there, their

cytoplasmic processes were intermingled with other endocrine cells, especially glucagon-IR cells (Fig. 5D and 5E). In the exocrine regions, clusters consisted of spherical to spindle hPP-IR cells were detected between acinar cells in a few frequency (Fig. 5F). However, no hPP-IR cells were demonstrated in the pancreatic duct portions in the present study.

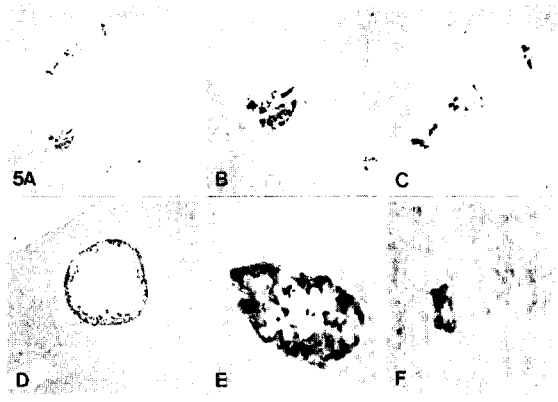

Fig. 5. hPP-IR cells in the principal (A-C), secondary (D and E) and exocrine (F) pancreas of the Korean aucha perch. Note that these cells were detected in the mantle regions of the principal islets and the cells in lower frequency were also demonstrated in the peripheral regions (A-C). In the secondary islets, hPP-IR cells were mainly located in the mantle to peripheral regions (D and E). Cell clusters were also demonstrated in the inter-acinar regions of the exocrine portions (F). A and D, ×75; B and C, × 150; E and F, ×300. PAP method.

Discussion

This study revealed the pancreatic endocrine cells of stomach-containing fresh-water teleost fish, the Korean aucha perch. In the present study, somewhat different distributional patterns of some endocrine cells were demonstrated according to region of pancreas and types of endocrine cells. In addition, species-dependent unique distributional patterns were also observed especially in hPP- and serotonin-IR cells.

Among numerous types of regulatory peptides detected in the gastroenteropancraetic (GEP) endocrine system, serotonin consisted of monoamines and widely distributed in nervous system and GEP endocrine cells [7]. El-Salhy *et al* [7] reported that these IR cells were found throughout the gastrointestinal tract of all species and established in the alimentary tract at the early stage of vertebrate evolution. In addition, the appearance of serotonin-IR cells was also demonstrated in the avian species [22] and mammals [5]. However, it is generally accepted that no serotonin-IR cells were located in the pancreas of teleost [1]. In the present study, serotonin-IR cells, restricted to the secondary islets, were dispersed throughout the pancreatic islet regions. These findings were quite different from those of previous report [1], and these differences were considered as a species specific characteristics of the Korean aucha perch.

Insulin is synthesized in the β cells of the pancreatic islets and regulates the serum glucose levels [12]. The regional distribution and relative frequency of the insulin-IR cells in the pancreas of Pisces including teleost have been reported in the carp [20], lungfish [11], flatfish [37], gilt-head sea bream [10], five species of osteoglossomorpha, an ancient teleostean group [3], *Protopterus annectens* [35], dipnoan fish [30], anglerfish and channel catfish [16]. From these previous reports, it seems to be a general rule in the pancreatic islets of Pisces that insulin-IR cells occur in central regions regardless of their types of pancreatic islets. Although somewhat relatively lower frequencies were demonstrated, compared to those in pancreatic islets, some insulin-IR cells were also located in the exocrine portions and pancreatic ducts. In the present study, well corresponded to those of previous reports [3, 10, 11, 16, 20, 30, 35, 37], although also demonstrated in the other regions of islets in lower frequency, most of insulin-IR cells were found in the central regions of the Korean aucha perch regardless of their types. However, somewhat different from other species of Pisces, no insulin-IR cells were demonstrated in the exocrine and pancreatic ducts, which was considered as a species- dependent characteristic of this species of stomach- containing fresh-water teleost, the Korean aucha perch.

Glucagon is synthesized in the α cells of the pancreas and regulates glucose levels in blood [12]. Morphologically similar cells are also observed in the digestive tract of the dog. The regional distribution and relative frequency of glucagon-IR cells in the pancreas of the Pisces have been reported in the carp [20], flatfish [37], *Barbus conchonius* [29], five species of osteoglossomorpha, an ancient teleostean group [3], gar [9], *Protopterus annectens* [35], dipnoan fish [30], anglerfish and channel catfish [16] and *Xiphophorus*

helleri [18]. It seems to be a general rule in the pancreatic islets of teleost that glucagon-IR cells occur in the peripheral regions and they form a small mantle zone or rim surrounding centrally located insulin-IR cells except for osteoglossomorpha [3] which shows scattered immunoreactivities throughout the central region of the islets besides the common peripheral regions regardless of principal and secondary types. Similar to those of insulin-IR cells, some cells were also demonstrated in the exocrine portions and pancreatic ducts in relatively lower frequencies compared to those in pancreatic islets. In the present study, quite similar to those of previous studies [3, 9, 16, 18, 20, 29, 30, 35, 37], most of glucagon-IR cells were located in the mantle to peripheral regions of the principal and secondary islets. In addition, some cells were also demonstrated in the exocrine portions. However, no glucagon-IR cells were demonstrated in the pancreatic duct portions of the Korean aucha perch, which was different from that of the carp [20].

Somatostatin, which consisted of 14 amino acids, was isolated from hypothalamus of sheep for the first time and it could be divided into straight and cyclic forms [4]. This substance suppresses the secretion of the gastrin, cholecystokinin, secretin, glucagon, insulin, motilin and gastric acid [17] and inhibits the absorption of amino acid, glucose and fatty acid in the gastrointestinal tract [4]. Somatostatin-IR cells of the teleost pancreas have been reported to be dispersed mainly in the central region, intermingled with insulin-IR cells [20, 28, 32]. However, Yoshida *et al* [37] suggested that somatostatin-IR cells occur in the peripheral regions of islets intermingled with insulin cells, besides the common central regions, and similar distributional patterns were also seen in *Protopterus annectens* [35]. In addition, Scheuermann *et al.* [30] reported that somatostatin-IR cells were scattered throughout the islets of dipnoan fish. Although somatostatin-IR cells were demonstrated in the mantle and peripheral regions of principal or secondary islets, more numerous IR cells were dispersed in the central regions of principal and secondary pancreatic islets of the Korean aucha perch used in this study similar to those of previous reports [20, 28, 32]. However, somewhat different from those of previous studies [20, 28, 30, 32, 35, 37], no cells were detected in the exocrine and/or pancreatic duct portions of the Korean aucha perch that was considered to have some characteristic distributional patterns in this species of stomach-containing teleost.

PP-IR cells as the fourth cell type were demonstrated first by Stefan *et al.* [31], and the appearance of PP-IR cells were detected by Van Noorden and Patent [36] in the pancreas of some teleost. Later, it has been revealed that PP-IR cells were conspicuously variable in distribution among species, although the cells, if they occur, were always located at the peripheral regions of the pancreatic islets. PP-IR cells were detected in the exocrine and endocrine pancreas of the carp [20], *Cottus scorpius* [32], *Barbus conchonius* [28], *Xiphophorus helleri* [18], anglerfish [16], flatfish [37], five species of osteoglossomorpha, an ancient teleostean group [3] and gar [9]. However, no PP-IR cells were found in the pancreas of the channel catfish [23] and lungfish [11]. Similar to those of previous reports [3, 9, 16, 18, 20, 28, 32, 37], although also demonstrated in the central regions in lower frequency, hPP-IR cells were mainly distributed in the mantle and peripheral regions of principal and secondary pancreatic islets of the Korean aucha perch except some cell clusters, consisted of hPP-IR cells, were demonstrated in the mantle to peripheral regions of the principal islets which were considered as a species-dependent characteristic of this stomach-containing freshwater teleost, the Korean aucha perch. However, differed from that of the carp [20], no cells were detected in the pancreatic duct portions.

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