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Expression of carbonic anhydrase-related protein VIII, X and XI in the enteric autonomic nervous system

Isao Nishimori[a], Tamotsu Takeuchi[b], Kaori Morimoto[a], Keisuke Taniuchi[a], Nobuto Okamoto[a], Saburo Onishi[a] , and Yuji Ohtsuki[b]

[a]First Department of Internal Medicine and [b]Department of Pathology, Kochi Medical School, Nankoku, Kochi 783-8505, Japan

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Abstract

Three isoforms of carbonic anhydrase-related protein (CA-RP), CA-RPs VIII, X, and XI, belong to the CA gene family but exhibit no CA catalytic activity. Although the biological function of CA-RPs is uncertain, among a panel of vital organs, the strongest mRNA expression is consistently observed in the brain. Immunohistochemical study showed that the neural cells express all three CA-RPs and that the glial cells express only CA-RP X. To study CA-RP expression in the autonomic nervous system, immunohistochemical analysis by using monoclonal antibodies to all three CA-RPs was performed in tissue sections from gastrointestinal tracts. The results showed CA-RP VIII and X expression in the neural ganglionic cells constituting both myenteric (Auerbach's) and mucosal (Meissner's) plexuses, but that CA-RP XI was not expressed in both plexuses. Although the exact function of these CA-RPs is still unknown, CA-RP plays an important role in not only the central nervous system but also the enteric nervous system.

Introduction

The carbonic anhydrases (CA) (EC 4.2.1.1) constitute a family of zinc metalloenzymes that catalyze the reversible hydration of bicarbonate in the reaction: H2O + CO2 <-> HCO3- + H+ [1]. The human CA gene family includes 11 isozymes that exhibit the characteristic catalytic activity of CA (CA I-IV, VA, VB, VI, VII, IX, XII, and XIV) [2] and three isoforms that lack CA activity because of absence of one or more histidine residues required to bind the zinc ion, which is essential for CO2 hydration activity. Thus, the latter have been designated CA-related proteins (CA-RP) VIII, X, and XI [3]. In addition, two receptor-type protein tyrosine phosphatases (RPTP) β (=ζ) and γ are known to contain N-terminal CA-RP sequences [4,5], which also have been believed to be "acatalytic" isoforms.

We have been working with cDNA cloning and mRNA expression of the human and murine CA gene families including CA-RPs X and XI [6-8]. Screening analyses of mRNA expression in a panel of human vital organs consistently showed the strongest expression of CA-RPs VIII, X, and XI in the brain [6,7,9]. We produced monoclonal antibodies to all three CA-RPs and further studied their regional and cellular distribution in the human and murine brains by immunohistochemical analysis [8, 10]. The result showed that CA-RPs VIII and XI were expressed in the neural cell body and neurite of both the human and murine brains. In the human brain, a weak signal for CA-RP X expression was observed in only Purkinje cells and neurons in the olivary nuclei, while, in most parts of the murine brain, it was expressed in the neural cell body. Interestingly, CA-RP X was also expressed in the myelin sheath of both the human and murine brains, which was reconfirmed by using tissue sections from a patient with acute disseminated encephalomyelitis and a shiverer demyelinated model mouse [8,10]. These findings suggest that CA-RPs play an important role in various biological processes of the central nervous system (CNS). However, CA-RP expression in the peripheral nervous

cells have never been studied. In the present study, expressions of all three CA-RPs in the autonomic nervous system, especially in the enteric nervous system, were examined by immunohistochemical analysis.

Materials and Methods

Gastrointestinal tissue

Archival pathological specimens of gastrointestinal tissues were used in the present study. Non-tumorous regions of the esophagus (n=10), stomach (n=10), duodenum (n=10), small intestine (n=10), and colon (n=50) in the surgically resected margins of various carcinomas were immunohistochemically stained. All specimens were fixed in 10% buffered formalin and paraffin-embedded.

Monoclonal antibodies

We previously produced mouse monoclonal antibodies to human CA-RPs VIII, X, and XI by means of an immunohistochemical screening analysis in tissue sections of the human brain [10]. All antibodies obtained belonged to an IgM subclass [10] and can also recognize mouse homologues of each CA-RP [8]. The specificity of each antibody was fully confirmed by absorption of the positive staining by preincubation with the synthetic peptide including the epitope.

Immunohistochemical analysis

Immunohistochemical analyses were performed by means of the streptoavidine-biotin peroxidase method without any pretreatment procedures [11]. The tissue sections were deparaffinized and incubated with fetal bovine serum, and then incubated with each monoclonal antibody at 4□ overnight. After washing with PBS, immunodetection was performed using biotinylated second antibody followed by peroxidase-labeled streptavidin (Dako LSAB kit; Dako, Glostrup, Denmark). Finally, the immunohistochemical reactants were developed with 3, 3'-diaminobenzidine. Counter-staining was performed using methyl green or hematoxylin.

Results

The results of immunohistochemical analyses of the neural ganglionic cells forming the enteric autonomic nervous system are summarized in Table 1. CA-RPs VIII and X were distinctly expressed in the neural cell body constituting both myenteric (Auerbach's) plexus and mucosal (Meissner's) plexus in almost all specimens examined (Fig. 1). CA-RP XI was not expressed in any cell type included in both plexuses. There was no difference in the expression pattern of CA-RPs VIII and X among the esophagus, stomach, duodenum, small intestine, and colon.

Table 1: Immunohistochemical analysis of CA-RP VIII, X, X! expressions in the human enteric autonomic nervous system and the central nervous system

System	Specific cell type	CA-RP VIII	CA-RP X	CA-RP XI
The enteric nervous system	Auerbach's plexus	+	+	-
	Meisner's plexus	+	+	-
The central Nervous system	Neural cells	+	+	+
	Glial cells	-	+	-

Abbreviations: CA, carbonic anhydrase; CA-RP, carbonic anhydrase-related protein; CNS, central nervous system; RPTP, receptor-type protein tyrosine phosphatase

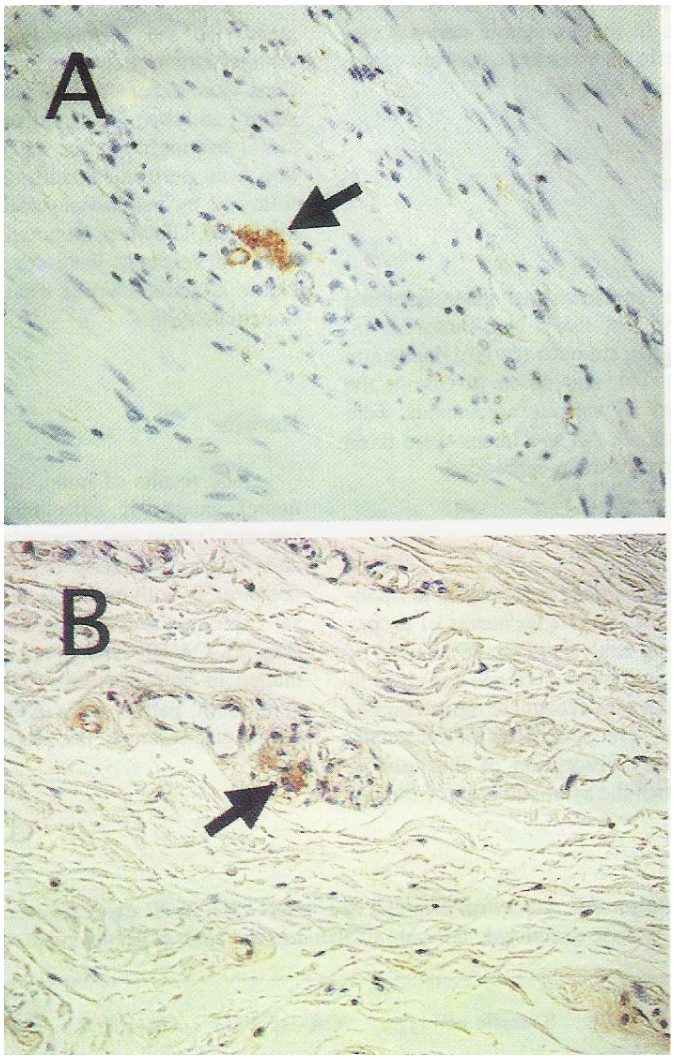

Fig. 1

Fig 1: *Immunohistochemical staining of the enteric nervous system with the monoclonal antibodies to CA-RPs VIII and X. Representative signal for CA-RP VIII expression was seen in the neural cell body included in the Auerbach's plexus (A, original magnification: x63), and significant signal for CA-RP X expression was observed in the neural cell body included in the Meissner's plexus (B, original magnification: x30).*

Discussion

This is the first report to show CA-RPs expression in the peripheral nervous system. In previous studies, significant and developmental expressions of all three CA-RPs have been demonstrated in the human and murine brains [8,10]. Although the exact biological function of CA-RPs is uncertain, they are postulated to play important roles in various biological processes in the CNS. Similar to the CNS, CA-RPs were significantly expressed in the enteric nervous system. Among three CA-RP isoforms, interestingly, CA-RPs VIII and X were expressed in the neural ganglionic cells included in both the myenteric (Auerba ch's) plexus and mucosal (Meissner's) plexus, but CA-RP XI was not expressed in both plexuses. As demonstrated in previous studies [8,10] and summarized in Table 1, CA-RP XI was significantly expressed only in the CNS. CA-RP XI was shown to demonstrate a similar distribution to that of CA-RP VIII in the CNS. It is of great interest that these two CA-RPs showed a distinct expression pattern in the enteric nervous system, suggesting different biological roles between these two CA-RPs in the peripheral nervous system and also possibly in the CNS.

Informative studies regarding the biological function of CA-RP have been limited. A few but important findings regarding the extracellular region of (CA-RP)RPTPβ (designated phosphacan in the rat) have been reported. Rat phosphacan was shown to bind to neurons and neuronal cell adhesion molecules (Ng-CAM and N-CAM) and to the extracellular matrix protein, tenascin, which is a strong inhibitor of cell adhesion and neurite out-growth [12-15]. Furthermore, the extracellular region of RPTPβ was re-

ported to bind to a GPI membrane-anchored neuronal cell recognition molecule, contactin [16]. These findings suggest that CA-RPs VIII and X also have some role in the nervous ganglionic cells in Auerbach's and Meissner's plexuses in binding to neighboring cells or to the interstitial matrix protein.

Recently, C-KIT and CD34 were discovered as molecular markers for the diagnosis of gastrointestinal tumors [17,18]. Since these markers were also demonstrated in the interstitial cells of Cajar, which are known as gastrointestinal pacemaker cells, it has been suggested that gastrointestinal tumors might be derived from this cell type. Gastrointestinal tumors have been reported to show various histogeneses; some were myogenic, some were neural (autonomic neuronal differentiation), some seemed to show bidirectional differentiation, and some had a "null" phenotype [18]. Usually, immunohistochemical analyses with a combination of various molecular markers, such as smooth muscle actin, S-100 protein, and desmin, are em ployed to diagnose their differential tumor types. In some clinical cases, however, diagnosis is difficult, since structural and immunohistochemical findings are complicated. There is a possibility that immunohistochemical analysis of CA-RP expression may contribute to the diagnosis of gastrointestinal tumors.

In conclusion, the present study showed specific expression of CA-RPs VIII and X in the neural ganglionic cells in the Auerbach's and Meissner's plexuses in the enteric wall . Although their exact biological function is uncertain, in contrast to the consistent expression of all three CA-RPs in the CNS, selective expression among CA-RPs in the enteric autonomic nervous system suggests that each CA-RP has different biological function.

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Abbreviations: CA, carbonic anhydrase; CA-RP, carbonic anhydrase-related protein; CNS, central nervous system; RPTP, receptor-type protein tyrosine phosphatase

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Correspondence to:

Dr. Isao Nishimori
First Department of Internal Medicine
Kochi Medical School
Nankoku, Kochi 783-8505
Japan.

Phone: and fax: +81-88-880-2338.
e-mail address: nisao@kochi-ms.ac.jp

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