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Original contribution

HIP/PAP, a member of the reg family, is expressed in glucagon-producing enteropancreatic endocrine cells and tumors

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Summary Hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein (HIP/PAP) protein, a member of the reg family, is constitutively expressed by some specialized epithelial cell subsets in the digestive tract and the pancreas. We performed a detailed analysis of the expression of HIP/PAP protein in normal digestive endocrine cells according to their localization, lineage, and differentiation stage, and in digestive endocrine tumors according to their site of origin and hormonal profile. In both adult and fetal normal tissues, HIP/PAP expression was detected only in endocrine cells of the small intestine, ascending colon, and pancreas. Two different expression patterns were identified: (*a*) a strong cytoplasmic labeling observed in the endocrine cells of the digestive mucosa and the outer rim of Langerhans islets specialized in the synthesis of glucagon and glucagon-like peptides; (*b*) a weak cytoplasmic immunoreactivity observed in the other pancreatic endocrine cell populations. HIP/PAP expression was detected in 36 of the 184 cases of digestive endocrine tumors examined; 32 of these cases (89%) were pancreatic. The 2 patterns observed in the normal state were retained: (*a*) a strong labeling was observed in 5% to 100% of tumor cells in 26 tumors, all expressing glucagon or glucagon-like peptides; (*b*) a weak labeling was present in 10 tumors, presenting various hormonal profiles. In conclusion, a strong expression of HIP/PAP is characteristic of glucagon-producing normal and neoplastic enteropancreatic endocrine cells. Our results lend further support to the concept that members of the reg family play regulatory roles in various endocrine cell populations and that their expression in endocrine cells is lineage-specific.

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1. Introduction

The human hepatocarcinoma-intestine-pancreas (HIP) protein has been initially identified because of its overexpression in hepatocellular carcinoma and cholangiocarcinoma [1,2]. This protein eventually proved to be identical with the human pancreatitis-associated protein (PAP), previously identified as an acute phase reactant expressed by exocrine pancreatic cells during acute pancreatitis [3]. The HIP/PAP protein is a lactose-binding C-type lectin belonging to a family of closely related proteins, the reg multigene family [4]. In humans, this family contains several members belonging to distinct subfamilies [4,5]. The regI subfamily contains reg1α (also called pancreatic stone protein or lithostatine) and the closely related reg1β and regL. HIP/PAP (whose gene is tandemly ordered at chromosome 2p12 with those coding for the members of the regI family [6,7]) belongs to the regIII subfamily. The recently characterized regIV [8,9] is the sole member of the regIV subfamily.

Like that of the other members of the reg family, the distribution of HIP/PAP protein presents a marked tissue specificity. In the normal state, human HIP/PAP has been detected only in the intestine and the pancreas, where its expression is restricted to a few specialized cell populations: the Paneth cells located in the small intestine, the appendix, and the ascending colon, and some populations of endocrine cells [10]. The expression pattern of HIP/PAP presents marked alterations in various pathological situations. As a stress protein with cytoprotective effects, HIP/PAP is induced in various digestive cell populations after cell injury: the best-studied example is the induction of HIP/PAP in acinar cells of the pancreas during acute pancreatitis [3]. As a paracrine growth regulator with mitogenic and antiapoptotic effects [11], HIP/PAP is induced in various examples of cell regeneration [12] and in carcinogenesis. In particular, ectopic expressions of HIP/PAP have been documented in various types of digestive cancers, including gastric, colorectal, and pancreatic adenocarcinomas [13,14], hepatocellular carcinoma [12,15], and cholangiocarcinoma [12].

So far, most studies have focused on the physiopathological roles of HIP/PAP. Very little is known about the patterns of expression of HIP/PAP and its regulation in the various cell subpopulations of digestive cells in which the protein is normally expressed. We were therefore prompted to undertake a detailed analysis of the expression of HIP/PAP protein in normal enteropancreatic endocrine cells, in both adult and fetal tissues, and in their neoplastic counterparts, through the study of a large series of endocrine tumors of the digestive tract and the pancreas. Our aims are to (*a*) determine the patterns of expression of HIP/PAP in the various subpopulations of enteropancreatic endocrine cells, according to their localization, lineage, and differentiation stage; and, (*b*) analyze HIP/PAP expression in enteropancreatic endocrine tumors according to their site of origin and hormonal profile.

2. Materials and methods

2.1. Tissue material

Formalin-fixed, paraffin-embedded archival tissue samples filed in the Department of Pathology, Hôpital Edouard Herriot, Lyon, were used throughout. To analyze the expression of HIP/PAP in normal gastrointestinal tissues, we tested formalin-fixed, paraffin-embedded archival samples of the following organs: stomach (n = 10), jejunum (n = 3), ileum (n = 10), colon (n = 10), rectum (n = 5), pancreas (n = 10). For comparison purposes, formalin-fixed, archival samples from pancreatic resections performed for acute pancreatitis were also examined (n = 5). To compare with other endocrine tissues, we also examined formalin-fixed, paraffin-embedded archival samples samples from adrenal tissue (n = 5) and thyroid tissue (n = 5) obtained during surgical resections for therapeutic purposes.

To evaluate the expression of HIP/PAP during organogenesis, samples from 25 human embryos and fetuses of various gestational ages, from 5 to 40 weeks, were used. Samples were obtained after informed consent from legal voluntary, therapeutic, or spontaneous abortions. The ages of the fetuses were determined according to the time from ovulation to the day of abortion. Ages were corrected according to crown-rump, hand, and foot lengths.

To study HIP/PAP expression in digestive endocrine tumors, archival specimens from 184 cases were tested. Complete clinical information was available for all patients. Tumors were classified according to the World Health Organization (WHO) criteria [16]. In all cases, the endocrine nature of the tumor was verified by the immunodetection of chromogranin A and synaptophysin, and its hormonal profile was assessed by immunohistochemistry (Table 1). Hormone-producing tumors were classified according to their main hormonal product. As in previous works [17], we considered "non–hormone-producing tumors" all tumors in which, in our technical conditions, none of the hormones tested was detectable or in which one

Table 1 List of antibodies used in the study

	Clone	Source
Chromogranin A	Polyclonal	Boehringer
Synaptophysin	SVP38	Sigma
Insulin	Polyclonal	ICN
Glucagon	Polyclonal	INSERM U45, Lyon
Somatostatin	Polyclonal	INSERM U45, Lyon
Pancreatic polypeptide	Polyclonal	INSERM U45, Lyon
VIP	Polyclonal	INSERM U45, Lyon
Serotonin	5HT-H209	Dako
Cholecystokinin	Polyclonal	Novocastra
Gastrin	Polyclonal	INSERM U45, Lyon

NOTE. Boehringer, Mannheim, Germany; Dako, Glostrup, Denmark; ICN, Costa Mesa, Calif; Immunotech, Marseille-Luminy, France; Novocastra, Newcastle, United Kingdom; Sigma, St. Louis, Mo.
Abbreviation: VIP, vasoactive intestinal peptide.