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Original Article

CD56-positive Cells with or without Synaptophysin Expression are Recognized in the Pancreatic Duct Epithelium: A Study with Adult and Fetal Tissues and Specimens from Chronic Pancreatitis

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We observed the distribution of CD56+ epithelial cells in the pancreatic duct system using 25 fetal, one infantile, 3 normal adult, 4 diabetic, and 8 chronically inflamed pancreatic tissue samples. In the early stage of gestation (12 to 17 weeks), CD56+ cells were commonly seen in the immature tubular structures. They were often continuous to pancreatic islets, and their distribution was similar to that of synaptophysin (Syn)+ cells, suggesting that they are precursors of islet neogenesis. Their number decreased in proportion to gestational age. Instead, from 24 weeks of gestation, luminal cell clusters that were common in interlobular ducts revealed CD56+. These cell clusters were unrelated to islet neogenesis and Syn expression. Similar CD56+ luminal cell clusters were also observed in cases of chronic pancreatitis, whereas they were scarce in normal adult and diabetic tissues. CD56+ cells were also occasionally seen in intralobular ducts, intercalated ducts, and centroacinar cells in cases of chronic pancreatitis. We conclude that there are two types of CD56+ epithelial cells in the pancreatic duct system: CD56+ endocrine cells are numerous during the early stage of gestation, when islet neogenesis appears, while CD56+ luminal cells may represent developmental and regenerative changes of pancreatic ducts.

Key words: CD56, pancreas, development, pancreatitis, islets

CD56, also known as the neural cell adhesion molecule, is a member of the immunoglobulin supergene family, and mediates homophilic binding between cells or between cell and matrix. It is expressed in various normal and neoplastic tissues, notably in neural and endocrine tissues and subsets of lymphoid cells [1]. In human pancreas, CD56 expression has been well known in normal islet cells and endocrine tumors [1] and can also be identified in fetal duct epithelium [2], the pancreatic buds of the early rat embryo [3, 4], and tumors such as solid-pseudopapillary tumors, acinar cell carcinomas, and pancreatoblastomas [5].

Given that pancreatic islets derive from pancreatic ducts, CD56+ epithelial cells in the duct system may contribute to islet neogenesis. In a preliminary study with fetal tissues, however, we found that CD56+ cells in pancreatic ducts do not necessarily correspond to endocrine cells that are identified with synaptopysin (Syn) stain

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[5]. In order to ascertain the biological significance of CD56+ epithelial cells found in the duct system, we made an immunohistochemical study of fetal and normal adult pancreatic tissues as well as specimens from cases with diabetes mellitus and chronic pancreatitis.

Materials and Methods

Selection of tissues. Histologically normal pancreatic tissues were collected from patients with a pulmonary or mediastinal disease in the autopsy file of the Department of Pathological Research, Okayama University Graduate School of Medicine and Dentistry. Pancreas tissues of fetuses and infants as well as of adults with diabetes mellitus were retrieved from the same autopsy file. The surgical pathology file of Kurashiki Central Hospital, Kurashiki, Japan, was searched for cases of chronic pancreatitis, and a total of 3 normal adult (59 to 64 years of age), 25 fetal (12 to 40 weeks of gestation), and one infantile (7 months of age) pancreatic tissue samples, 4 specimens from cases with diabetes mellitus, and 8 specimens from cases with chronic pancreatitis were collected. All of the specimens were fixed in formalin, embedded in paraffin, and processed routinely.

Immunohistochemistry. For each case, 5-μm-thick sections of a representative block were deparaffinized and rehydrated with gradient alcohol. Immunohistochemical staining was carried out by hand using the avidin-biotin-peroxidase complex method. Briefly, after rehydration, slides were immersed in citrate buffer (pH 6.0) and irradiated in a microwave oven for 15 min. After cooling for an hour at room temperature, sections were incubated for 3 h with anti-CD56 (Novocastra, Newcastle upon Tyne, U.K.) and anti-synaptophysin (Novocastra; or DAKO Japan, Kyoto, Japan) monoclonal antibodies. Slides were also incubated with phosphate-buffered saline as negative controls. Diaminobenzidine was adopted as a chromogen, and nuclei were counterstained with hematoxylin. Peripheral nerves and pancreatic islets served as internal positive controls. Compared to the former, the staining of the latter was usually less intense for CD56. One fetal tissue sample was eliminated from the study, because there was no positive control reaction for CD56.

The slides were reviewed by 2 authors (M.F., K.N.). The pancreatic duct system was subdivided into interlobular, intralobular, and intercalated ducts and centroacinar cells [6, 7] (Fig. 1), and each compartment was estimated separately. The main pancreatic duct was excluded from the study, because some slides lacked it, and, if present, its epithelium was often denuded. The difference between interlobular and intralobular ducts is their location; interlobular ducts (IrLs) are located between the lobules, while intralobular ducts (IaLs) exist within the lobules (Fig. 1a). Intercalated ducts (ICs) are present between acini in the lobules and are composed of small cuboidal cells with a narrow lumen (Fig. 1b). Centroacinar cells (CAs) have clear or eosinophilic cytoplasm and are situated at the center of acini.

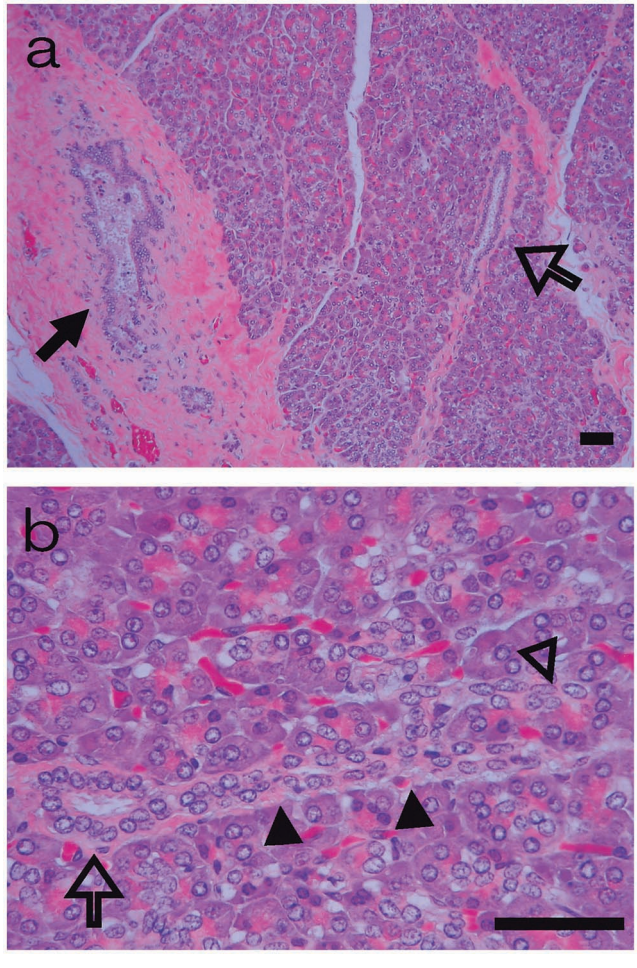

Fig. 1 Classification of the pancreatic duct system. The bar indicates 50 μm. a, Interlobular (arrow) and intralobular (open arrow) ducts. b, Intralobular duct (open arrow), intercalated duct (arrowhead), and centroacinar cells (open arrowhead).

Results

CD56 expression was predominantly membranous, although weak stains could be seen within the cytoplasm. In the fetal tissues, CD56 expression was noted in the duct system, except in ICs and CAs, throughout the entire gestational period. Histologically, in the 2 most immature fetuses at 12 and 14 weeks, pancreatic lobules were incomplete and consisted of immature tubular structures. Acinar cells with basophilic granular cytoplasm were not identified yet, although islets cells were already present. In the third immature fetus, at 17 weeks, acinar cells appeared among the tubules. In all of these fetal tissues, CD56+ cells were encountered in connection with the tubules (Fig. 2a). These cells were small and oval, often clustered preferentially at the basal portion of the tubules, and they frequently protruded into the stroma to comprise a pancreatic islet. Islets were sometimes found to be circumscribed by peripheral nerves, which were distinct from the islets by their more intense expression of CD56. Syn+ cells were distributed in a similar, but more intense and numerous fashion (Fig. 2b). From 20 weeks, when the lobular architecture became apparent, scattered cells or tiny clusters with CD56 expression were found at the basal portion of the duct epithelium. Their cellular features and staining intensity for CD56 were similar to that of CD56+ cells before 20 weeks, but the number of CD56+ cells as well as the incidence of continuous islet neogenesis was markedly decreased. These cells were initially noted in both IaLs and IrLs, but, after 30 weeks, usually existed in IrLs. A similar distribution of positive cells was observed with Syn stain. In contrast to the decrease of CD56+ cells at the basal portion, from 24 weeks of gestation, intense CD56 expression was visible in some luminal cells found at the luminal side of the epithelium to assemble a duct lumen (Fig. 2c). They were cuboidal or columnar and were predominant in IrLs. When numerous, these cells were seen gathering. They were also present and numerous in the single infant tissue sample we studied. Syn+ cells were almost never seen among luminal cells (Fig. 2d).

In the normal adult pancreas, a few single or grouped CD56+ cells were noted in occasional IrLs. They consisted of both luminal and basal cells. The distribution of Syn+ cells was similar to that of the basal cells. Less frequently, small foci of CD56+ cells were also encountered in IaLs, ICs and/or CAs of the lobules. In patients with diabetes mellitus, the distribution and extent of CD56+ cells was not different from the ductal system of the normal pancreas tissues.

In 5 of 8 patients with chronic pancreatitis, CD56+ luminal cells were increased in IrLs, often in a clustered pattern (Fig. 2e). Morphologically, islet neogenesis was not obvious in these foci, and, in addition, their distribution was completely different from that of Syn+ cells, which were seen exclusively in a few cells at the basal epithelial portion. CD56+ cells were, in addition, seen occasionally, but more frequently than in the normal tissues, in IaLs, ICs, and/or CAs of the lobules (Fig. 2f). We occasionally encountered pancreatic ducts that were continuous to an islet, but CD56+ epithelial cells in such an area were, akin to the islet, less intensively stained than were peripheral nerves, and they were restricted at the basal portion (Fig. 2g, h), in contrast to the intensely stained luminal cells.

Discussion

CD56 expression in pancreas has been reported in islet cells [1], scattered single ductal cells of fetuses[2], and ventral and dorsal pancreatic buds of the early rat embryo [3, 4]. In the former 2 situations, CD56 expression is related to endocrine differentiation of cells, in which CD56 expression is common, while the last condition probably reflects its wide expression in mesodermal and endodermal derivatives in organogenesis [3]. Given that pancreatic islets derive from pancreatic ducts, CD56+ cells in the duct epithelium may be the precursors of the endocrine cells. However, in a preliminary study we found that CD56+ cells in the duct system do not always correspond to endocrine cells that are recognized with Syn stain. These results are mentioned briefly in the Discussion section of that study [5].

Endocrine cells of human pancreas have been observed from 6 or 7 weeks of gestation [2, 8]. Initially they are single, but these cells aggregate to form islets at 10 to 12 weeks, when acinar cells with granular cytoplasm are still indistinct [9, 10]. With light microscopic and ultrastructural observations, endocrine cells have been confirmed to originate from the immature tubules [11, 12]. Likewise, in this study, we found CD56+ cells at the basal portion of the immature tubules. Some of them formed clusters and protruded into the stroma to assemble islets. These cells were small and oval, and, akin to islet cells, the intensity of CD56 expression was weaker compared to that in peripheral nerves. Syn+ cells were

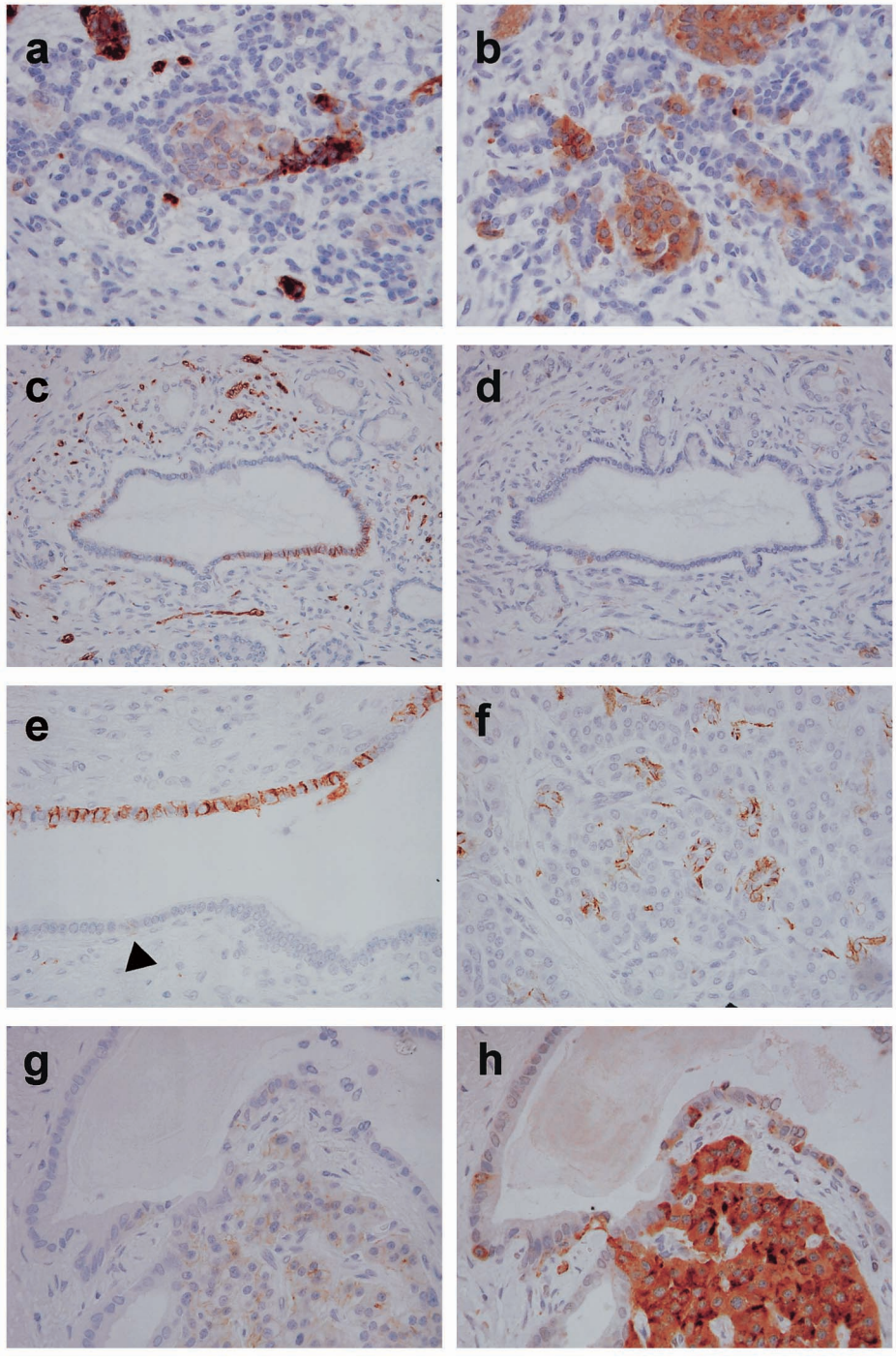

Fig. 2 Immunohistochemical staining for CD56 (a, c, e-g) and synaptophysin (b, d, h). a, CD56 + cells in the immature tubules of a fetus at 14 weeks of gestation. Some of the cells are giving rise to pancreatic islets. The right pole of the islet (center) is circumscribed by a peripheral nerve. b, Endocrine cells positively stained for synaptophysin of the same case shown in (a). c, Numerous CD56 + luminal cells in an interlobular duct found in a fetus at 33 weeks. d, Synaptophysin stain in the same area shown in (c). e, Numerous CD56 + luminal cells in the interlobular duct of a chronic pancreatitis tissue sample. One basal cell with a weak reaction (arrowhead), which probably represents an endocrine cell, is also noted. f, CD56 + intercalated ducts in a case of chronic pancreatitis. g, Islet formation continuous to an interlobular duct seen in a case of chronic pancreatitis. Although endocrine cells are weakly immunolabeled, the luminal cells are CD56−. h, Endocrine cells positively stained for synaptophysin in the same areas shown in (g).

distributed similarly, but more intensely and extensively. Thus we conclude that these CD56+ cells are endocrine cells that participate in islet neogenesis. Some authors have assumed that expression of certain adhesion molecules such as CD56 and cadherins contributes to cell aggregation and islet neogenesis [10, 13]. However, the islet size and number have been found to be normal in CD56-deficient mice, although the segregation of constituent cells is disrupted within the islets [4]. As a result, CD56 may not be mandatory for the endocrine cell adhesion during islet neogenesis. Interestingly, we revealed that, when they arose from the tubules, peripheral nerves circumscribed some immature islets. There has been ample evidence that autonomic nerves innervate islet cells and play an important role in the regulation of their hormonal function [14]. Our observation suggests that this innervation may start early in islet neogenesis. The autonomic nerves may also facilitate the outpouching of the endocrine cell clusters to form islets through homophilic bindings of CD56 molecules on both of these tissues.

We further demonstrated another type of CD56+ cell in the fetal and infantile duct epithelium, mainly of IrLs. In contrast to CD56+ endocrine cells that scarcely existed at the basal portion of IrLs after 30 weeks of gestation, these were luminal cells that constituted a lumen of the duct system. They were intensely positive for CD56 and were often seen clustered. Portions with numerous CD56+ luminal cells were unrelated to islet neogenesis, and Syn stain was almost always negative among luminal cells. Similar CD56+ luminal cells were also increased in IrLs of specimens from chronic pancreatitis compared to normal adult and diabetic pancreatic tissue samples. In fact, in cases of chronic pancreatitis, we sometimes encountered islets that were continuous to a pancreatic duct. In such a focus, only cells at the basal portion of the duct epithelium were positive for CD56, and luminal epithelial cells were negative. We are not aware of the biological significance of these CD56+ luminal cells. We collected fetal tissues from the autopsy file, but increased CD56+ luminal cells were not related to specific diseases (data not shown). Some of the extrainsular endocrine cells in pancreatic ducts have been reported to border the lumina and have been considered to secrete pancreatic hormones such as insulin, somatostatin, and pancreatic polypeptide directly into the pancreatic juice [7, 15]. However, these cells are a minor component, and it is very unlikely that Syn stain could not demonstrate the presence of such numerous endocrine cells at all, because, regardless of the cell type, pancreatic endocrine cells are considered to express Syn diffusely [16]. Thus we speculate that CD56+ luminal cells are unrelated to either islet neogenesis or endocrine cells.

There are sporadic reports of CD56+ epithelial structures most likely unrelated to neuronal or endocrine differentiation. Immature tubuloglomerular structures of fetal kidney [17] and bile ductular proliferation in liver [18, 19] are such examples. In the latter condition, CD56+ bile ductular cells are indicated to represent a regenerative state. Given that CD56+ luminal cells were numerous in patients with chronic pancreatitis, these cells may also represent a regenerative process in the pancreatic duct system. In addition, in cases of chronic pancreatitis, the peripheral portion of the duct system including IaLs, ICs, and CAs was also occasionally positive for CD56, suggesting that regeneration is a possible cause of CD56 expression. Similarly, in fetuses and infants, these cells may indicate a developmental change.

In conclusion, we found 2 types of CD56+ cells in the pancreatic duct system. In the earlier stage of gestation, they were mostly endocrine cells and are related to islet neogenesis. In contrast, the CD56+ luminal cells, which are common in the later stage of pregnancy and infancy and in chronic pancreatitis, are probably unrelated to endocrine cells. CD56 expression in the latter cells may indicate a developmental or regenerative change of the pancreatic ducts.

References

1. Shipley WR, Hammer RD, Lennington WJ and Macon WR: Paraffin immunohistochemical detection of CD56, a useful marker for neural cell adhesion molecule (NCAM), in normal and neoplastic fixed tissues. Appl Immunohistochem (1997) 5: 87–93.
2. Gaidar YA, Lepekhin EA, Sheichetova GA and Witt M: Distribution of N-cadherin and NCAM in neurons and endocrine cells of the human embryonic and fetal gastroenteropancreatic system. Acta Histochem (1998) 100: 83–97.
3. Lackie PM, Zuber C and Roth J: Polysialic acid of the neural cell adhesion molecule (N-CAM) is widely expressed during organogenesis in mesodermal and endodermal derivatives. Differentiation (1994) 57: 119–131.
4. Esni F, Täljedal IB, Perl AK, Cremer H, Christofori G and Semb H: Neural cell adhesion molecule (N-CAM) is required for cell type segregation and normal ultrastructure in pancreatic islets. J Cell Biol (1999) 144: 325–337.
5. Notohara K, Hamazaki S, Tsukayama C, Nakamoto S, Kawabata K, Mizobuchi K, Sakamoto K and Okada S: Solid-pseudopapillary tumor of the pancreas: Immunohistochemical localization of neuroendocrine

markers and CD10. Am J Surg Pathol (2000) 24: 1361–1371.
6. Klimstra DS: Pancreas; in Histology for Pathologists, Sternberg SS ed, 2 nd Ed, Lippincott-Raven, Philadelphia (1997) pp 613–647.
7. Kodama T: A light and electron microscopic study on the pancreatic ductal sytem. Acta Pathol Jpn (1983) 33: 297–321.
8. Bocian-Sobkowska J, Zabel M, Wozniak W and Surdyk-Zasada J: Prenatal development of the human pancreatic islets. Immunocytochemical identification of insulin-, glucagon-, somatostatin- and pancreatic polypeptide-containing cells. Folia Histochem Cytobiol (1997) 35: 151–154.
9. Klöppel G and Lenzen S: Anatomy and physiology of the endocrine pancreas; in Pancreatic Pathology, Klöppel G and Heitz PU eds, Churchill Livingstone, New York (1984) pp 133–153.
10. Peters J, Jürgensen A and Klöppel G: Ontogeny, differentiation and growth of the endocrine pancreas. Virchows Arch (2000) 436: 527–538.
11. Robb P: The development of the islets of Langerhans in the human fetus. Q J Exp Physiol (1961) 46: 335–343.
12. Laitio M, Lev R and Orlic D: The developing human fetal pancreas: An ultrastructural and histochemical study with special reference to exocrine cells. J Anat (1974) 117: 619–634.
13. Dahl U, Sjödin A and Semb H: Cadherins regulate aggregation of pancreatic β-cells *in vivo*. Development (1996) 122: 2895–2902.
14. Ahrén B: Autonomic regulation of islet hormone secretion— implications for health and disease. Diabetologia (2000) 43: 393–410.
15. Bendayan M: Presence of endocrine cells in pancreatic ducts. Pancreas (1987) 2: 393–397.
16. Navone F, Jahn R, Di Gioia G, Stukenbrok H, Greengard P and De Camilli P: Protein p38: An integral membrane protein specific for small vesicles of neurons and neuroendocrine cells. J Cell Biol (1986) 103: 2511–2527.
17. Satoh F, Tsutsumi Y, Yokoyama S and Osamura RY: Comparative immunohistochemical analysis of developing kidneys, nephroblastomas and related tumors: Considerations on their histogenesis. Pathol Int (2000) 50: 458–471.
18. Chedid A, Mendenhall CL and Moritz TE: The antigenic heterogeneity of the bile duct epithelium in alcoholic liver disease. VA Cooperative Study Group 275. Arch Pathol Lab Med (1999) 123: 411–414.
19. Van Den Heuvel MC, Slooff MJH, Visser L, Muller M, De Jong KP, Poppema S and Gouw ASH: Expression of anti-OV6 antibody and anti-N-CAM antibody along the biliary line of normal and diseased human livers. Hepatology (2001) 33: 1387–1393.