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ORIGINAL RESEARCH

Growth Factor Independence-1 (*Gfi1*) Is Required for Pancreatic Acinar Unit Formation and Centroacinar Cell Differentiation

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SUMMARY

In a knockout mouse model, growth factor independence-1 (*Gfi-1*) plays an important role in regulating the development of pancreatic centroacinar cells and the formation and structure of the pancreatic acinar/centroacinar unit.

BACKGROUND & AIMS: The genetic specification of the compartmentalized pancreatic acinar/centroacinar unit is poorly understood. Growth factor independence-1 (*Gfi1*) is a zinc finger transcriptional repressor that regulates hematopoietic stem cell maintenance, pre-T-cell differentiation, formation of granulocytes, inner ear hair cells, and the development of secretory cell types in the intestine. As *GFI1*/*Gfi1* is expressed in human and rodent pancreas, we characterized the potential function of *Gfi1* in mouse pancreatic development.

METHODS: *Gfi1* knockout mice were analyzed at histological and molecular levels, including qRT-PCR, in situ hybridization, immunohistochemistry, and electron microscopy.

RESULTS: Loss of *Gfi1* impacted formation and structure of the pancreatic acinar/centroacinar unit. Histologic and ultrastructural analysis of *Gfi1*-null pancreas revealed specific defects at the level of pancreatic acinar cells as well as the centroacinar cells (CACs) in $Gfi1^{-/-}$ mice when compared with wild-type littermates. Pancreatic endocrine differentiation, islet architecture, and function were unaffected. Organ domain patterning and the formation of ductal cells occurred normally during the murine secondary transition (E13.5–E14.5) in the $Gfi1^{-/-}$ pancreas. However, at later gestational time points (E18.5), expression of cellular markers for CACs was substantially reduced in $Gfi1^{-/-}$ mice, corroborated by electron microscopy imaging of the acinar/centroacinar unit. The reduction in CACs was correlated with an exocrine organ defect. Postnatally, *Gfi1* deficiency resulted in severe pancreatic acinar dysplasia, including loss of granulation, autolytic vacuolation, and a proliferative and apoptotic response.

CONCLUSIONS: *Gfi1* plays an important role in regulating the development of pancreatic CACs and the function of pancreatic acinar cells. *(Cell Mol Gastroenterol Hepatol 2015;1:233–247; http://dx.doi.org/10.1016/j.jcmgh.2014.12.004)*

Keywords: Centroacinar Cells; Claudin 10; Growth Factor Independence-1 (*Gfi1*).

The digestive functions of the pancreas are provided by acinar cells. Structurally, glandular acini constitute the main mass of pancreatic parenchyma, organized within pancreatic lobuli. Acini are composed of pyramid-shaped cells that surround a centroacinar lumen. Drainage of digestive juice is initially performed by a small duct that is commonly referred to as the intercalated duct. The intercalated ducts invaginate the acini, and the distal-most cells of the intercalated ducts have been referred to as centroacinar cells (CACs). Electron microscopy has allowed for three-dimensional visualization of the acinar unit structure, revealing that intercalated duct-type cells are not obligatorily connected to the main ductal tree but may intersperse within the larger acinar structure.

Acinar cells secrete directly into the luminal portion at places lined with intercalated ductal cells. The developmental origin of intercalated ductal cells has not been established, but such are generally viewed as being thought to develop from the identical origin as that of the main ductal tree, which then would argue for an early developmental fate allocation presumably occurring at the time of ductal fate assignment in early embryogenesis.[1] A general absence of markers to distinguish between CACs and the intercalated duct cells of the pancreas has not allowed a clear separation of the two cell types, and studies investigating a possible differential, or identical, origin of such cells through lineage tracing has not been possible. Genetic components identifying the mechanism of CACs development have not been found.[2]

Production of low protein/high bicarbonate fluid by the intercalated ductal cells and CACs helps to solubilize acinar cell secretions, and the neutralizing effect of bicarbonate helps to normalize pH in duodenum after gastric emptying. Neutralization of pH locally may be important for neutralizing the content of exocrine secretory granules. Although mature exocrine granules are at neutral pH, immature granules are known to be acidified[3] through the activity of the vacuolar V-ATPase.[4] Such granule acidification is a requirement for the pathological intracellular activation of

Abbreviations used in this paper: **BPL, Bauhinia purpurea lectin; BrdU, bromodeoxyuridine; CACs, centroacinar cells; DIG, digoxigenin; EM, electron micrographs; *Gfi1*, growth factor independence-1; PBS, phosphate-buffered saline; qRT-PCR, quantitative real-time polymerase chain reaction; rER, rough endoplasmic reticulum; SD, standard deviation; TipPC, tip progenitor cells; TrPC, trunk progenitor cells; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; WT, wild type.**

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zymogens that occurs after supramaximal cholecystokinin or caerulein treatment,[5,6] which eventually leads to acinar cell death. The structural manifestation is one of intracellular, acidified vacuoles in which cathepsin B catalyzes the intracellular activation of zymogens. The insufficient neutralization of secretory juice is related to acinar disease. For example, intraductal acidosis is a manifestation of acute biliary pancreatitis.[5,7]

There is mounting evidence on the ontogeny of the major pancreatic cell fates through genetic lineage tracing, but knowledge of ductal cell-type specification in the pancreas is sparse.[2,8] The major ductal population of the pancreas has been shown to occur during a process referred to as organ domain patterning, which prefigures the secondary transition and helps segregate multipotent pancreatic progenitor cells into two distinct subsets, called trunk progenitor cells (TrPCs) and tip progenitor cells (TipPCs). Notch signaling is required for TrPC formation, and TipPC form upon Notch signaling abrogation.[9,10] Expression of specific transcription factors is spatially controlled during TrPC/TipPC formation, where *Hnf1β* (*Tcf2*), *Hnf6* (*Oc1*), and *Sox9* are expressed in the TrPC population, in contrast to *Ptf1a* which is expressed only by TipPC (as reviewed elsewhere[8,11,12]).

As *Hnf1β*, *Hnf6*, and *Sox9* remain expressed in ductal descendants of TrPC but are not expressed in endocrine descendants, these markers are useful for tracking ductal cell development. These factors are also expressed in CACs, which are also dependent on Notch signaling.[13–15] Therefore, it seems plausible that CACs are defined as a subpopulation of ductal descendants originating from the TrPC population. However, it also remains possible that TipPC acinar descendants could generate the CACs population, if such could reactivate Notch signaling, which subsequently could involve activation of the aforementioned intrinsic factors. The latter possibility is intriguing, considering multiple reports of acinar cell plasticity during adult organ regeneration, including reactivation of Notch signaling.

We have observed that *Gfi1* plays a role in the control of the functional acinar/centroacinar cell unit. *Gfi1* is critical during hematopoiesis and inner ear cell development, and it also plays a role in maintaining the functions of the lungs and intestines.[16–19] *Gfi1* is expressed during development in distalized pancreatic progenitors, corresponding to TipPC, and remains expressed in acinar descendants. The absence of *Gfi1* does not abrogate acinar differentiation, and a complement of all pancreatic lineages, including endocrine, duct, and acinar cells, develops normally. However, after differentiation of the exocrine pancreas of *Gfi1* nulls, acinar cells develop structural abnormalities in which they lose apical polarity. Electron microscopy analysis of *Gfi1*-null acinar cells has identified a general loss of rough endoplasmic reticulum (rER) associated with excessive cellular content of immature secretory granules. Postnatally, *Gfi1*-null exocrine cells become highly vacuolated. The exocrine phenotype is characterized by a particular reduction of CACs, as evidenced by an apparent elimination of the expression of several markers such as *Hnf6*, *Hnf1β*, and *Sox9* in the position of CACs. We conclude that *Gfi1* is required for the formation of CACs, and we believe *Gfi1* is the first factor in forming the genetic requirements for creating and maintaining the acinar/centroacinar structural unit.

Materials and Methods

Animals

The *Gfi1*$^{+/-}$ mouse line (*Gfi1*^{tm1sho}, MGI: 2449921) was provided by Dr. Stuart Orkin. This targeting model contains a deletion of exon 2–3.[20] Vaginal plugs were checked the next morning before 10:00 AM, and the time-mated embryos were dissected as described elsewhere.[21] The animals were housed in the animal facility at the Cleveland Clinic. All animal procedures and experiments were approved by the Cleveland Clinic Animal Care and Use Committee.

Histology and Immunofluorescence Analysis

Tissues were fixed in 4% paraformaldehyde at 4°C overnight, were washed in 1× phosphate-buffered saline (PBS), and were equilibrated in 30% sucrose before embedding in optimal cutting temperature compound, before the cryosectioning. The immunostaining protocol was followed as described elsewhere.[21] Antigen retrieval was performed with a neutral-pH antigen-retrieval agent.

The tissue samples were stained with the primary antibodies as detailed in Supplementary Table 1. For the anti-HNF6 antibodies staining, the Tyramide Signal Amplification Fluorescence system was used (PerkinElmer, Waltham, MA). Before applying the blocking reagents, the tissue samples underwent peroxidase (3% in PBS) treatment for 7 minutes. After incubating in the primary antibody (rabbit anti-HNF6) for overnight and being washed with PBS for 3 times (5 minutes/wash), the slides were incubated in biotinylated secondary antibody for 30 minutes at room temperature. After washing 3 times in 1×PBS (5 minutes/wash), the slides were further incubated in streptavidin-peroxidase conjugate for 15 minutes, which was then followed by three washes (5 minutes/wash). Then the slides were incubated in fluorophore tyramide (amplification reagent) for 3 to 10 minutes, which was followed by washing. Binding of the primary antibodies was detected by immunofluorescence by incubating with Texas Red-, Cy2-, or aminomethylcoumarin-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA). After washing with 1×PBS, tissue samples were mounted (Slowfade Antifade kit; Molecular Probes/Life Technologies, Eugene, OR).

Immunohistochemical analysis for cell apoptosis was performed by using an in situ apoptosis detection kit with TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) according to the manufacturer's instructions (Roche, Indianapolis, IN). To evaluate the cell proliferation, we intraperitoneally injected mice with bromodeoxyuridine (BrdU) (50 μg/g of body weight). Two hours after the injection, the mice were dissected, and the pancreas were fixed and processed following the same protocol as previously mentioned for immunofluorescence analysis. Images were obtained using either an Olympus BX51 upright epifluorescence microscope (Olympus