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Original article

β-Galactoside-mediated tissue organization during islet reconstitution

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ABSTRACT

We have previously reported that multi-cellular heteroaggregates comprising murine pancreatic α (αTC1.6) and β (MIN6-m9) cell lines spontaneously acquired islet-like architecture and displayed higher insulin secretion rates. However, the mechanisms of self-organization remain unclear. The objective of this study is to examine the possibility that a sugar chain participates in the mutual recognition of the cells during reconstitution of the islet-like structure *in vitro*. Using a lectin-binding assay, we identified *Erythrina cristagalli* agglutinin (ECA), which particularly recognizes the β-galactoside structure on the surfaces of MIN6-m9 cells. The self-organization of αTC1.6 and MIN6-m9 was obstructed using ECA-bound MIN6-m9 cells. Lactose neutralized the ECA's inhibitory effect on the autonomous rearrangement of αTC1.6 and MIN6-m9 cells, indicating that the inhibition of cell arrangement by ECA was mediated via β-galactoside. We concluded that a β-galactoside sugar chain was central to the reconstitution of the pancreatic islet-like architecture *in vitro*.

1. Introduction

In the pancreas, the islets of Langerhans play a pivotal role in glycemic homeostasis. Type 1 diabetes mellitus is a disease associated with hyperglycemia, which develops through the loss of insulin-producing β cells from the islets. Effective treatments of the disorder include pancreas transplantation and islet transplantation [1–5]. However, a rate-limiting factor is the donor shortage. Insulin-secreting cells derived from stem/progenitor cells have also been suggested as alternative resources of islets [6]. However, for the precise tuning of normal β cell function [7], it is essential to understand the mechanisms of mutual interactions between β and other islet cells such as α cells.

In previous studies, we reported a rapid aggregation system using a 3% methylcellulose medium to form islet-like tissues comprising both a murine α cell line (αTC1.6) and a murine β cell line (MIN6-m9) [8]. These aggregated tissues rebuild a specific architecture, which resembles the mouse pancreatic islet by self-organization of cells. Interestingly, insulin secretion ability was upregulated about three-fold when α cells and β cells were mixed at the ratio of 1:8, suggesting that cell-to-cell association in specific tissue structures affects cellular functions. It is obvious that cell surface molecules engaged in the association.

Sugar chains can be particularly recognized by lectins, and these are one of the most important factors for morphogenesis in a number of species [9–15]. However, the role of the sugar-chain in islet development or regeneration remains unclear. In this study, we attempted to identify a specific sugar chain that is involved in tissue self-organization and to reveal the effect of the islet-like structure on insulin secretion activity.

2. Materials and methods

2.1. Cell culture

The mouse pancreatic α cell line αTC1.6 was obtained from the American Type Culture Collection. The mouse pancreatic β cell line MIN6-m9 was a gift rom Prof Seino [16]. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; 041-29775, Wako, Osaka, Japan) supplemented with 10% fetal bovine serum (Cellgro, 35-010-CV, CORNING, Corning, NY, USA), 100 U/mL penicillin, and

Abbreviations: ECA, *Erythrina cristagalli* agglutinin; DMEM, Dulbecco's Modified Eagle's Medium; FITC, fluorescein isothiocyanate; ConA, concanavalin A; LCA, *Lens culinaris* agglutinin, α-D-mannosyl group; WGA, wheat germ agglutinin; MAA, *Maackia amurensis* agglutinin; SSA, *Sambucus sieboldiana* agglutinin; UEA, *Ulex europaeus* agglutinin; RCA, *Ricinus communis* agglutinin; MC, methylcellulose.

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100 μg/mL streptomycin (168-23191, Wako). Cells were maintained at a subconfluent density, allowing recovery every 2 or 3 days. αTC1.6 and MIN6-m9 cells were stained with fluorescent cell membrane markers, PKH26 or PKH67 (Sigma–Aldrich, St. Louis, MO, USA), as required.

2.2. Evaluation of lectin-binding ability and lectin toxicity

Fluorescein Isothiocyanate (FITC)-conjugated plant lectin library (INCJ106, J-OIL MILLS, Osaka, Japan) was diluted with DMEM and used to detect sugar chains, concanavalin A (ConA) for α-D-mannosyl and α-D-glucosyl groups; *Lens culinaris* agglutinin (LCA) for α-D-mannosyl group; wheat germ agglutinin (WGA) for *N*-acetyl-D-glucosamine and sialic acid residues; *Maackia amurensis* agglutinin (MAA) and *Sambucus sieboldiana* agglutinin (SSA) for sialic acid residues; *Ulex europaeus* agglutinin (UEA) for α-L-fucosyl residues; and *Ricinus communis* agglutinin (RCA) and *Erythrina cristagalli* agglutinin (ECA) for β-D-galactoside. We incubated MIN6-m9 cells in DMEM with 10 μg/mL of a lectin at room temperature for 10 min. The cells were observed using fluorescent microscopy (LAS AF with DMI6000B, Leica microsystems, Wetzlar, Germany) and the intensities of the FITC were evaluated as no (−), weak (+), or strong (++) binding. Lectin toxicity was evaluated in two ways. One was whether the lectin treated cells formed aggregates or not. The lectin treated cells were centrifuged, resuspended with culture medium and put on a culture dish. If there were many aggregated cells compared to the non-treated cells, we decided there was toxicity. The other was whether the lectin treated cells adhered to a culture dish and grow or not. The lectin treated cells were plated and the loss of adhesion was evaluated as toxicity at 24 h later. The cell growth was also observed.

2.3. Self-organization of islet-like tissues

We employed a rapid cell aggregation system using a 3% methylcellulose (MC; M0512, Sigma–Aldrich) medium [17]. The MC medium was poured into a Petri dish or a cover glass chamber (5222-004, Asahi Glass Co., Tokyo, Japan) with the use of a positive-displacement pipette (Gilson, Middleton, WI, USA). MC-free culture medium (1 μL) containing 1000 cells of αTC1.6 and 8000 cells of MIN6-m9 cells was injected into the MC medium to assemble heteroaggregates. The αTC1.6 and MIN6-m9 cells were pre-stained with PKH67 and PKH26, respectively. Injected suspension cells were gathered in the MC medium within 30 min and the aggregates were cultured without contacting the bottom of the dish or chamber. After 2 days culture in the MC medium, the hetero-aggregates formed islet-like tissue with specific cell architecture. We observed the tissue with a confocal microscope (SP5, Leica microsystems). To isolate islet-like tissues from the 3% MC medium, 5 U/mL cellulase solution (Onozuka RS; Yakult Pharmaceutical Industry, Tokyo, Japan) was added to the MC medium, and the mixture was incubated for 30 min at 37 °C to digest the MC. As required, lectin-treated MIN6-m9 cells or lectin and lactose (Kanto Chemical Co., Tokyo, Japan)-treated MIN6-m9 cells were used instead of intact MIN6-m9 cells.

3. Results

3.1. Evaluation of the sugar chain on cell surfaces

To reveal molecules, which were included in the hetero-aggregate self-organization comprising αTC1.6 and MIN6-m9 cells, we surveyed the types of sugar chains that were displayed on the surfaces of these cells. Both cell types were incubated with various plant lectins (ConA, LCA, WGA, MAA, SSA, UEA, RCA, and ECA) conjugated with FITC. Several lectins bound to the cell surfaces (Table 1). WGA bound both αTC1.6 and MIN6-m9 cells. There was no lectin it was able to bind only αTC1.6 cells, whereas both RCA and ECA lectins strongly bound to only MIN6-m9 cells. This fact suggested that β-galactoside was particularly expressed on the surfaces of MIN6-m9 cells.

3.2. Verification of the lectin concentration for toxicity avoidance

To understand the role of β-galactoside in the self-organization of islet-like tissues, we tried to use RCA- or ECA-binding ability to neutralize the function of the molecule harboring β-galactoside. It is known that lectin binding is often a cause of cell toxicity as well as unexpected cell aggregation. Therefore, we carefully checked the concentration of lectin during the binding step of MIN6-m9 cells (Table 2). When the RCA lectin was used at a concentration of 1.0 μg/mL, cells were aggregated and cell adhesion to the dish was prevented. Unexpected cell aggregation was disappeared at the concentration was 0.1 μg/mL, but the prevention of cell adhesion was maintained even when RCA lectin was diluted to 0.001 μg/mL. In contrast, ECA lectin did not block cell adhesion at 10 μg/mL, and cell growth was normal. We decided to use 10 μg/mL ECA lectin to mask the β-galactoside on the surfaces of MIN6-m9 cells (ECA-treated MIN6-m9) in subsequent experiments.

3.3. Inhibition of islet-like structures by lectin binding

As we reported previously, αTC1.6 and MIN6-m9 cells autonomously migrate and form an islet-like structure when they are aggregated and cultured for 2 days in the MC medium [8]. To confirm whether β-galactoside is involved in the autonomous pattern formation, ECA-treated MIN6-m9 cells were utilized instead of intact MIN6-m9 cells. Fig. 1 shows that the autonomous remodeling was not observed when MIN6-m9 cells were coupled with ECA lectin, whereas remodeling did occur when intact MIN6-m9 cells were used. The influence of the lectin was abrogated when lectin binding was performed with lactose-absorbed ECA because lactose has a β-galactoside structure. This confirmed that the effect of lectin was particularly mediated by the β-galactoside structure of the sugar chain.

4. Discussion

The self-organization of tissue aggregates comprising α and β cells (and also other types of islet component cells) is a well-known phenomenon [18,19]. We found that the same type of event occurred when we composed a heteroaggregate of αTC1.6 and MIN6-m9 [8]. It is easy to predict that surface molecules of both αTC1.6 and MIN6-m9 should be involved in the mechanism. In this study, we hypothesized that some sugar chains that are bound to cell surface molecules play an important role in tissue self-organization. We identified that β-galactoside is one such sugar chain, and we also found that the neutralization of β-galactoside by ECA was sufficient to inhibit self-organization. These results are

Table 1
Binding ability of the lectin to αTC1.6 and MIN6-m9 cells.

	ConA	LCA	WGA	MAA	SSA	UEA	RCA	ECA
αTC1.6	−	−	++	−	−	−	−	−
MIN6	−	+	++	+	−	+	++	++

αTC1.6 and MIN6-m9 cells were incubated in a medium with FITC-labeled lectin and the binding property was evaluated as no binding (−), weak binding (+), or strong binding (++) with a fluorescent microscope.

Table 2
Cell toxicity of RCA and ECA.

RCA conc. (μg/ml)	0.001	0.01	0.1	1.0
Aggregation	Not tested	Not tested	−	+
Cell adhesion inhibition	+	+	+	+
ECA conc. (μg/ml)	10	100		1000
Aggregation	−	−		−
Cell adhesion inhibition	−	+		+

Toxic effects such as cell aggregation and inhibition of cell adhesion were evaluated as not toxic (−) or toxic (+).

was mediated by the β-galactoside structure on MIN6-m9 cell surfaces. Using the ECA-binding activity, we might identify the molecule that is displaying β-galactoside on the cell surfaces and plays an important role in the interaction between αTC1.6 and MIN6-m9 cells. This method is limited because it is an artificial system using cell lines. However, similar mechanisms may exist in intact islets, which help to maintain its architecture as well as morphogenesis potential in pancreatic development. The molecules related with the mechanisms may be potential therapeutic targets in pancreatic disorders, because dysfunction of the molecules leads the loss or abnormality of the islet architecture.

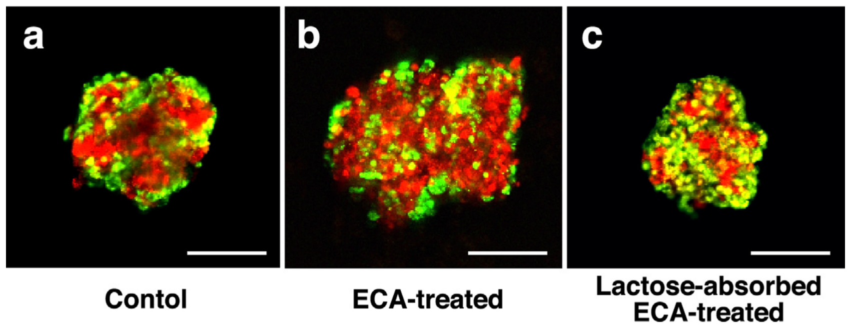

Fig. 1. Inhibition of self-organization of islet architecture by blocking of β-galactoside structure. Heterospheroids comprising αTC1.6 and MIN6-m9 cells were injected into the MC medium and cultured for 2 days to induce self-organization. (a) Intact MIN6-m9 cells were used and an islet-like architecture was observed. (b) ECA-treated MIN6-m9 inhibited self-organization in heteroaggregates. (c) Lactose-absorbed ECA failed to conjugate with MIN6-m9 and self-organization did not occur in control conditions. All tissues in the MC medium were observed using confocal laser microscopy, and αTC1.6 and MIN6-m9 cells were pre-stained with green and red fluorescent dye, respectively. Bar: 100 μm. MC: methylcellulose, ECA: *Erythrina cristagalli* agglutinin.

valuable in understanding the tissue engineering aspects of islet architecture.

Sugar chains are very prevalent on the surfaces of cells because they are conjugated to various cell surface proteins and lipids, and they can regulate the association of cell surface molecules [20]. There are cell types displaying different sets of sugar chain even in the same organ. For example, in the developing kidney, metanephric mesenchyme cells and ureteric bud cells display specific sugar chains, and therefore, we can discriminate these two parts using the lectins, peanut agglutinin and *Dolichos biflorus* agglutinin, respectively [21,22]. In our system, β-galactoside was detected only on the surfaces of MIN6-m9 cells; however, it was not clear whether β-galactoside bound to a single type of molecule or various types of molecules. We have not yet confirmed the existence of β-galactoside on the β cells in mouse or human islets in the pancreas. Although the expression of β-galactoside in vivo might be different from that of *in vitro*, the sugar chain approach can be useful to identify and isolate islet component cells, including the β cell.

ECA and RCA lectins were used to detect β-galactoside structure. In this study, we tried to use these lectins as a method of neutralization of the sugar chain-binding molecule. Unfortunately, RCA has strong toxicity in this study; MIN6-m9 covered with this lectin failed to adhere to the culture dish even at a very low concentration. In contrast, ECA was easy to use because of its low toxicity. This is an appropriate example to use because the plant lectin can neutralize cellular alignment without notable toxicity. Considering that plant lectins are relatively cheap compared with antibodies, their usage might result in reduced costs for research and development as well as medical expenses.

In previous study, we found heteroaggregates of αTC1.6 and MIN6-m9 cells showed self-organization and formed islet-like structures [8]. ECA-binding to MIN6-m9 cells inhibited the self-organization. In addition, the adsorption of ECA by an excessive amount of lactose neutralized the inhibition. These results clearly show that an autonomous migration of αTC1.6 and MIN6-m9 cells

5. Conclusion

MIN6-m9 cells expressed cell surface molecules conjugating with a β-galactoside structure. The acquisition of islet-like architecture in the heteroaggregates comprising αTC1.6 and MIN6-m9 cells is controlled through a β-galactoside sugar chain. Such type of sugar chain is a potential therapeutic target in pancreatic disorders.

Conflict of interest

The authors have no conflict of interest to declare.

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