Diversity of pathophysiology in type 2 diabetes shown by islet pathology

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ABSTRACT
The etiology of type 2 diabetes is multifactorial, in which environmental and genetic factors are involved to varying degrees. This suggests that its pathophysiology might vary depending on the individuals. Knowledge of the differences is critical, because these differences are directly linked to the care and treatment of the patients. Recent studies have attempted to carry out subclassifications of type 2 diabetes based on clinical and genetic differences. However, there is no pathological evidence to support these subclassifications. The pathophysiology of type 2 diabetes is generally divided into insulin resistance in peripheral tissues and pancreatic islet dysfunction. Among them, islet dysfunction causes a deficit in insulin secretion from β-cells. In particular, a deficit in insulin secretion is ascribed to a combination of disruption of the insulin secretory machinery and a decrease in β-cell volume in type 2 diabetes. Recent research has suggested that transdifferentiation and dedifferentiation are involved in the decrease in β-cell volume, and that it might change dynamically depending on the glucose metabolic state. However, it is possible that the numbers of islet cells are decreased in type 2 diabetes. In particular, the loss of endocrine cells due to islet amyloid deposits is an important pathological change in type 2 diabetes in humans. These results show that pathological changes of the islets can be different in each individual with type 2 diabetes and reflect each pathophysiology, which is useful in establishing further subclassifications and developing tailor-made therapies for type 2 diabetes.

DIVERSE PATHOGENESIS OF TYPE 2 DIABETES
The current epidemic of type 2 diabetes is a serious concern in modern society worldwide†. Effective prevention or cure of this disorder has yet to be established. One of the reasons for this difficulty might be the diversity of the clinical and pathophysiological background. Such heterogeneity might largely be influenced by different ethnicities or life customs. Peripheral insulin resistance and deficient insulin secretion are salient features of type 2 diabetes‡. Insulin resistance is associated with a high body mass index (BMI; average 27.5) in white type 2 diabetes patients, but not in Japanese type 2 diabetes patients (average BMI 23.4)§–‖. Several studies disclosed that impaired insulin secretion rather than insulin resistance was more closely associated with the development of diabetes in Japanese individuals§–‖. Differences in islet pathology between white patients and Japanese patients with or without type 2 diabetes have also been recently presented§–‖. For example, the prevalence of islet amyloid deposition is >80% in white type 2 diabetes patients, whereas it is just 30% in Japanese type 2 diabetes patients§–‖. An obesity-associated increase in β-cell volume density is well documented in white individuals§, whereas it is not the case in Japanese individuals§–‖. Thus, the diversity of pathological changes in the islets might reflect the background pathophysiology of each individual.

SUBCLASSIFICATION OF TYPE 2 DIABETES
Recently, many attempts have been made to subclassify type 2 diabetes. Swedish investigators classified type 2 diabetes into five subgroups of autoimmune-based type, insulin deficient type, aging-related type, obesity-related type, and insulin-resistant type by measures of glutamic acid decarboxylase antibody, age, BMI, glycated hemoglobin, homeostasis model assessment of β-cell function and homeostasis model assessment of insulin resistance§. Among these, they found that a group of type 2 diabetes patients with severe insulin resistance developed a high prevalence of chronic kidney disease and cardiovascular events with poor prognosis§. The German group also classified type 2 diabetes into five subgroups of mild
age-related diabetes, mild obesity-related diabetes, severe autoimmune diabetes, severe insulin-resistant diabetes and severe insulin-deficient diabetes based on age, BMI, glycemia, homeostasis model estimates and islet autoantibodies. Interestingly, the prevalence of diabetic complications, particularly diabetic neuropathy, was the highest in the severe insulin-resistant diabetes group among all groups. The underlying change in pathology in the islets for the aforementioned subgroups of type 2 diabetes remains, however, to be addressed. It is important to clarify the difference in pathologies in those classifications, because it will determine the therapy for each patient. In the present review, we discuss the diversity of islet pathology relating to clinical characteristics, which can promote further subclassification and tailor-made therapies for type 2 diabetes.

**ALTERATION OF ISLET PATHOLOGY IN TYPE 2 DIABETES**

A main pathogenesis of type 2 diabetes is abnormal hormone secretion in response to glucose from the islet, such as the deficiency of insulin secretion in β-cells and no suppression of hyperglycemic glucagon hypersecretion in α-cells. Pathologically, it is known that the β-cell volume decreases, the α/β-cell ratio increases and the α-cell volume increases (Figure 1). Decreased β-cell volume is directly linked to less insulin secretion, whereas the increase in α-cell volume might interfere with the reduction of glucose levels after meal ingestion. In contrast, it is known that alteration of endocrine cell composition in the islets also changes endocrine cell cross-talk in the islets and affects hormone secretion. β-cells secrete humoral factors, such as insulin, gamma aminobutyric acid, zinc and glutamate, which can inhibit glucagon secretion from cells. Therefore, the alteration of endocrine cell composition in the islets is directly linked to the function of the islets.

Several mechanisms for β-cell reduction have been proposed, including β-cell death and transdifferentiation (Figure 2). In type 2 diabetes, islet neogenesis can occur to compensate for β-cell reduction, and β-cell proliferation is not influenced. The decrease in β-cell volume can depend on the blood glucose status. The frequency of β-cell apoptosis is extremely low or difficult to find. In addition, the islet volume itself is not significantly reduced in type 2 diabetes. These findings might suggest that transdifferentiation and dedifferentiation could be major players in β-cell loss rather than β-cell death in the short term. Nevertheless, because type 2 diabetes is a multifactorial disease, islet pathological changes might also vary with different involvement of pathophysiological factors among patients with type 2 diabetes.

**ISLET AMYLOID DEPOSITS IN TYPE 2 DIABETES**

Amyloid deposits are characteristic pathological changes in type 2 diabetes. Amyloid in type 2 diabetes consists of islet amyloid polypeptide. Islet amyloid polypeptide, also known as amylin, is a 37-amino acid peptide hormone. Amylin is the second most abundant peptide secreted by β-cells. Amylin is released in response to nutrients, including glucose, lipids or amino acids (arginine). Pro-amylin is processed by cleavage at basic residue pairs at both its amino and carboxyl termini with prohormone convertase 1/3 and prohormone convertase 2, and its C-terminus is amidated by carboxypeptidase E and peptidylglycine α-amidating monoxygenase. Mature amylin is secreted along with insulin, which is stored in the same granules. The molecular mechanisms that result in refolding of the peptide to convert it from a normally soluble monomer into insoluble fibrils have not been identified. Amyloid deposition increases the frequency of apoptotic β-cells in white individuals, but can increase deoxyribonucleic acid fragmentation of β-cells in Japanese type 2 diabetes patients. Similarly, amylin aggregates drive inflammation elicited by pro-inflammatory macrophages, resulting in β-cell dysfunction. Interestingly, amyloid deposition suppresses the increase in α-cells and decreases chromogranin-A endocrine cells (Figure 3). These results suggest that aggregation of this peptide results in the formation of islet amyloid deposits, which can be

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**Figure 1** | Morphological changes in the islets of type 2 diabetes patients. Quadruplicate immunostained sections (red, glucagon; black, insulin; green, somatostatin; blue, PP; brown, K67) are shown. (a) Individuals without diabetes. (b) Individuals with diabetes. The occupancy of insulin-positive cells is decreased and that of glucagon-positive cells is increased in type 2 diabetes patients compared with individuals without diabetes.
toxic to not only β-cells, but also other kinds of endocrine cells in type 2 diabetes patients. The individuals with amyloid deposition in the islets present with specific etiologies and would have different pathophysiology from the individuals without amyloid deposition (Figure 3). Thus, type 2 diabetes patients can be classified according to amyloid deposition in the islets.

**ETHNICITY**

As aforementioned, islet pathology can be different between white patients and Japanese patients. In response to obesity, islets in white patients can show hyperplasia to compensate for the demand for insulin from peripheral tissues. In contrast, the morphology of islets in Japanese individuals is less influenced by obesity. Furthermore, the prevalence of amyloid deposits in the islets of white individuals is more >80%, whereas that in Japanese individuals is approximately just 30–40%.

Butler et al. showed that islet amyloid deposition was not increased in type 2 diabetes patients with a BMI of 27 kg/m² compared with lean patients with a BMI of 25 kg/m², nor was it increased in obese individuals with impaired glucose tolerance compared with either obese or lean individuals without diabetes in a recent autopsy study of 124 white individuals. In contrast, as shown in a Chinese article, BMI is correlated with amyloid occupancy in Japanese individuals. These results suggest that amyloid deposition is dependent on ethnic differences. To date, genetic differences to explain the morphological changes in islets have not been identified among different ethnicities.
OBESITY AND INSULIN RESISTANCE

Islet amyloid deposition might be a result of obesity-associated insulin resistance. Heterozygous transgenic mice expressing human amylin in pancreatic β-cells show islet amyloid deposition when crossed with an obese, insulin-resistant diabetic strain. As is the case with insulin, patients with obesity have high basal and stimulated levels of plasma amylin. As mentioned before, insulin secretion rather than insulin resistance was more closely associated with the development of diabetes in Japanese individuals. In contrast, there might be individuals who maintain sufficient insulin secretion among Japanese individuals with obesity, particularly in the initial stage of the disease, where hyperinsulin and amylin secretion can occur to compensate for peripheral insulin resistance.

HYPERAMYLINEMIA AND CARDIOVASCULAR DISEASE

Elevation of systolic and diastolic blood pressure, and the presence of hypertension are risk factors for amyloid deposition in Chinese individuals. These findings can be interpreted either as a cause or effect of hyperamylinemia. Hyperamylinemia promotes amylin deposition in the heart, causing alterations in cardiac myocyte structure and function. Amylin deposition negatively affects cardiac myocytes by inducing sarcomemmal injury, generating reactive aldehydes, forming amylin-based adducts with reactive aldehydes and increasing the synthesis of interleukin-1β independent of hyperglycemia. Amylin deposition in the heart failure of non-human primates activates hypoxia-inducible factor-1α and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 signaling. Verma et al. reports that hypersecretion of human amylin is associated with amylin deposition in the microvasculature of the kidney and red blood cells, leading to impaired red blood cell–capillary interactions and activation of hypoxia signaling pathways, and resulting in the disruption of microvasculature. Thus, hyperamylinemia directly influences the function and cell viability of cardiovascular systems. Hyperamylinemia can also be associated with single-nucleotide polymorphisms in the amylin gene. The −132 G/A mutation is located within an activator domain of the amylin gene promoter. The prevalence of the mutation was more frequently observed in diabetes patients than in the control population with high serum amylin levels (10.1 vs 0.9%). Interestingly, hypertension was higher in a population of diabetes patients carrying the mutation than in diabetes patients who are non-carriers (74 vs 57%; P < 0.05). These results suggest that hyperamylinemia induces cardiovascular disease, including hypertension and heart failure.

ISLET AMYLOID DEPOSITION AND MACRO- AND MICROANGIOPATHIES OF DIABETES

Conversely, as insulin resistance is a risk factor for both macroangiopathy and amyloid deposition in the islets, there is a possibility that the presence of circulatory disease itself is a risk factor for amyloid deposition in the islets. Recent studies have shown that macroangiopathy is a risk factor for microangiopathy in type 2 diabetes. Thickened basement membrane (BM) and loss of pericytes are hallmarks of microangiopathy in diabetes, and long-standing metabolic aberrations, such as increased polyol pathways and glycation, might underlie its development. Generally, thickened BM obstructs drainage of amyloid-related protein, resulting in amyloid formation. Nevertheless, to date, the correlation between diabetic microangiopathy and amyloid deposition has not been fully clarified in type 2 diabetes. As the pancreatic islet is a highly vascularized endocrine microorgan, it is plausible that microangiopathy of the islet vasculature contributes to amyloid deposition in type 2 diabetes patients.

The islets of ob/ob mice, which is a model of an obese type 2 diabetes similar to white type 2 diabetes patients, show dilation of islet capillaries and a reduction in pericyte lining, which might be an adaptive response to a high demand for insulin secretion due to insulin resistance. In Goto-Kakizaki rats, a lean type 2 diabetes model, hypercholesterolemia interacts with chronic hyperglycemia to induce islet microangiopathy, which can be associated with a reduction in the β-cell mass. In white patients with type 2 diabetes, islet microvessels showed thickened walls similar to those of microangiopathy observed in other tissues. These alterations of islet microvasculature might contribute to amyloid deposition in the islets of human type 2 diabetes.

EXAMINATION OF CARDIOVASCULAR DISEASE AND AMYLOID DEPOSITION USING AUTOPSY PANCREAS SAMPLES

To explore the hypothesis described earlier, a study was carried out on the autopsy pancreas samples of Japanese type 2 diabetes patients with complications of acute myocardial infarction (AMI) as a hallmark of cardiovascular disease. Clinically, systolic blood pressure, total cholesterol and prevalence of renal failure were more elevated in type 2 diabetes patients with AMI than in those without AMI. Furthermore, in pathological evaluations, amyloid deposits, BM thickening, loss of pericytes and increase in vascular density in the islets were significantly observed in type 2 diabetes patients with AMI (Figure 4). These results suggest that type 2 diabetes patients with AMI might be closely related to the insulin resistance group classified by Ahlqvist et al. (Table 1). Namely, amyloid deposits might reflect islet pathological features of the insulin-resistant group. In the evaluation of the microvessels in the islets of type 2 diabetes patients with AMI, the thickening of BM and the loss of pericytes were remarkable. These changes are similar to the microangiopathy of diabetes. It is conceivable that microangiopathy-like changes in pancreatic islets might be exerted due to macroangiopathy complications. Such vascular changes can reduce vascular permeability for amylin and promote amyloid deposition.

RENAL FAILURE AND AMYLOID DEPOSITION

Systemic amyloidosis can occur when there is a high serum concentration of protein precursors, as seen in long-term hemodialysis patients with increased levels of β-2-microglobulin.
Under normal circumstances, amylin is released into the circulation and is excreted through the kidney\(^{40,57}\). Similarly, the presence of renal insufficiency is known as a risk factor for amyloid deposition in the islets of type 2 diabetes patients\(^{23,58}\). The measured serum amylin levels were high in individuals with chronic kidney disease\(^{59}\). This is ascribed to augmented insulin resistance in patients with end-stage renal failure on dialysis treatment, which is associated with hypersecretion of amylin concurrently with reduced renal excretion of amylin. However, the increase in serum amylin was not directly associated with hypoinsulin secretion or an increase in insulin resistance, whereas amyloid deposition in the islets was pronounced in patients on hemodialysis. Although increased levels of circulating amylin are therefore unlikely to be a direct pathogenetic factor in the development of type 2 diabetes, increased amylin can contribute to the formation of amyloid in the islets.

**AGING AND AMYLOID DEPOSITION**

The prevalence of diabetes in the elderly is currently on the rise. Type 2 diabetes in the elderly can be divided into two types\(^{60}\). In one group, type 2 diabetes manifests in middle age and continues to old age, and in the other group, there is no metabolic disorder in middle age and diabetes first develops in old age. It is known that diabetes in the elderly has a different pathological condition from diabetes that develops in middle age\(^{59}\). For example, older-onset type 2 diabetes is characterized by low involvement of genetic factors and high susceptibility to hypoglycemic attacks. In contrast, aging is a factor that enhances amyloid formation in the body\(^{61}\). For example, the accumulation of amyloid fibrils, such as amyloid-β and amylin, plays key roles in the pathogenesis of fatal age-related diseases, such as Alzheimer’s and Parkinson's diseases\(^{62}\). The prevalence of amyloid deposition in the islets is increased with aging in Japanese individuals without diabetes\(^{63}\). A total of 6% of individuals without diabetes aged >80 years showed amyloid deposition in the islets\(^{64}\). Therefore, the exploration of the involvement of aging in the process of fibril accumulation in islets is extremely important in view of the increased life expectancy in our societies. It is also important to define the pathological and clinical characteristics of amyloid deposition in the elderly, which might help to explore the pathogenesis of islet amyloid deposition.

**CHARACTERISTICS OF ISLET PATHOLOGY OF ELDERLY DIABETES PATIENTS**

We evaluated the pathological changes of the islets in the autopsy pancreas samples of elderly type 2 diabetes patients\(^{21}\). The average age of the group was 88.0 ± 0.6 years, and the age at type 2 diabetes onset was 80.8 ± 1.4 years. BMI was comparable between individuals with and without diabetes. Pancreatic weight was reduced by half in the senile-onset type 2 diabetes group compared with the elderly group without diabetes. Islet amyloid deposits were significantly increased in middle-aged type 2 diabetes patients compared with age-matched individuals without type 2 diabetes. Amyloid deposition was significantly increased in the senile individuals with diabetes group compared with the senile individuals without diabetes group (Figure 5a). A comparison between the middle-aged diabetes patients group and the senile diabetes patients group also showed that the senile diabetes group exhibited marked amyloid deposits. Both β-cell and α-cell masses were also significantly reduced in senile diabetes patients compared with middle-aged diabetes patients.

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**Table 1 | Similarity of acute myocardial infarction complicated type 2 diabetes to insulin resistance type**

| Insulin resistance | Type 2 diabetes + AMI |
|--------------------|-----------------------|
| High prevalence of cardiovascular events | + | + |
| High prevalence of renal failure | + | + |
| Islet amyloid deposition | Possibly | + |
| Insulin resistance | + | Possibly |

AMI, acute myocardial infarction.
Interestingly, in senile diabetes patients, pancreatic intraepithelial neoplasia (PanIN) was frequently found in the pancreatic duct (Figure 5b-d). Acinar cells were atrophic and fibrotic around the duct complicated with PanIN (Figure 5c,d). PanIN is thought to result in outflow disorder of pancreatic juice, leading to localized pancreatitis, exocrine atrophy, fibrosis and weight loss of the pancreas. From the aforementioned results, it was considered that senile-onset diabetes mellitus shows a unique pancreatic pathology along with amyloid deposition of the islets. The present results show that the pathogenesis of older-onset type 2 diabetes shares some features with pancreatic diabetes. Ueberberg et al. reported that the prevalence of islets containing amyloid deposits was significantly higher in both diabetes due to exocrine pancreatic disorders compared with healthy individuals. This suggests that PanIN-induced pancreatic atrophy might accelerate amyloid deposits in older-onset type 2 diabetes.

In the present review, the diversity of islet pathological changes in human type 2 diabetes, especially in cases of amyloid deposits, and the similarity to subclassification of type 2 diabetes described by Ahlqvist et al. were shown (Table 1). The loss of β-cell mass is a hallmark of the pathological change in islet endocrine cells in type 2 diabetes. However, a detailed pathological examination of the clinical course and cells other than β-cells might clarify further pathophysiology and subclassification of type 2 diabetes. Recently, comprehensive molecular pathological analysis using paraffin blocks has become widespread in the field of cancer. We hope that the clinical and genetic classification of type 2 diabetes based on pathological exploration with such a new analysis method will be further advanced, and that the understanding of these pathological conditions and application of tailor-made treatments will be established.

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DISCLOSURE
The authors declare no conflict of interest.

The protocol for this research project has been approved by a suitably constituted Ethics Committee of the institution and it conforms to the provisions of the Declaration of Helsinki. Committee of Hirosaki University School of Medicine, date of approval: 20 October 2017, approval number #2017–121.

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