

High-throughput screening of small interfering ribonucleic acid identifies important modulators in islet dysfunction and apoptosis

In eukaryotes, the intracellular environment is maintained in a constant state by the homeostatic mechanism. This is attributable to the fact that, when the extracellular environment changes, stimuli generated by the changes are transmitted into cells through specific or non-specific pathways, and then induce modification and degradation of intracellular proteins, through which cells adapt to changes in the environment. There are, broadly, two systems involved in the protein degradation mechanism for this adaptation. They are the selective degradation through the ubiquitin–proteasome system and the non-selective degradation through the autophagy–lysosome system. Both systems are essential for maintaining the physiological functions of cells. When cells suffer extensive impacts of viral infection, autoimmune damage or metabolic stress, however, the repair mechanisms fail, leading to impaired physiological functions of organelles and eventually cell death. It is important to discover the molecular mechanisms underlying these processes for elucidation of the pathogenesis of various diseases and the development of new treatments for them. Thus, these mechanisms are attracting attention in many fields of study.

In the latest studies of metabolism, important issues are not only the identification of a single metabolite involved in certain pathological conditions, but also elucidation of regulatory networks associated with the relevant metabolite. The reason for this trend is that once a study

finds the regulatory mechanism and the cellular remodeling process of the pathway, it could develop new therapeutic tools by intervening the targets. In terms of methodology, studies for this objective used to be carried out by selecting genes as candidates by biochemical assessment and then analyzing them to know the regulatory pathways¹. However, recent studies have aimed to elucidate regulatory factors involved in certain metabolic pathways. In other words, studies first focus on the identification of a certain metabolic pathway to identify unknown regulatory factors, with the next step being analysis of the regulation of gene expressions involved in the identified pathway. Thus, recent studies, namely functional genomics, have been carried out in the order opposite to that of conventional study approaches. Most notably, functional genomics is a field that has been attracting research attention^{2,3}, in which genes associated with the regulation of metabolic pathways are screened specifically by applying the ribonucleic acid interference method (RNAi). Researchers have started to identify corresponding genes with RNAi technology, assuming the presence of regulatory key elements in some biochemical pathway. It has allowed wider application compared with the previous approaches.

In diabetes mellitus, a typical example of metabolic disorders, dysfunction and apoptosis of pancreatic islet cells are the basis of the pathogenesis regardless of the type of diabetes. In type 1 diabetes, injury leading to apoptosis of pancreatic islet cells is caused by an autoimmune mechanism. When immunocompetent cells are activated, pro-inflammatory cytokines, such as interleukin-1 β , tumor necrosis factor- α and interferon- γ , are secreted. In islet β -cells, stimulation by

these cytokines causes changes in intracellular signaling pathways including cell death through nuclear factor- κ B (NF- κ B). This cytokine-induced damage has been the subject of important articles published in recent years on studies using the aforementioned new technology. The article by Beck *et al.*⁴ is noteworthy, serving as a pioneering report in this type of diabetes research. Binding of the pro-inflammatory cytokines to their specific receptors on the islet β -cell causes phosphorylation of the inhibitor of κ B (I κ B). The phosphorylated I κ B then becomes a target of ubiquitination, which is followed by degradation in proteasomes, and thereby enables nuclear translocation of NF- κ B to occur. Adding to this process, ubiquitination also serves to regulate the functions of a number of proteins along this pathway (e.g., tumor necrosis factor receptor associated factor and receptor-interacting protein 1). Conversely, de-ubiquitinating enzymes exert the opposite effect by restricting NF- κ B activity and inhibiting apoptosis of β -cells. As their report pointed out, the signaling elements through the entire process of this cytokine-induced β -cell death have yet to be elucidated.

One of the features of the study is that genes were screened by using pro-inflammatory cytokines to achieve extracellular stimulation of pancreatic islets with the assumption of autoimmunity in type 1 diabetes. Genes involved in the regulation of signaling pathways leading to cytokine-induced cell apoptosis were comprehensively screened on human pancreatic islet cells *in vitro*. When pancreatic islet cells are isolated from the body and left untreated, they show patterns of gene expression reflecting the systemic environment immediately before isolation. Thus, methods using simply isolated

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pancreatic islets are likely to yield differences in gene expression among individual samples. Furthermore, cells in tissue samples of pancreatic islets vary according to differentiation state, which is likely to account for the observed heterogeneity in gene expressions and signaling pathways. In order to overcome these problems, the authors isolated and further dispersed pancreatic islet cells before using them in their experiments. Then, in order to make cellular conditions consistent among the samples *in vitro*, the cells were briefly placed in the culture system to homogenize gene expression levels. These processes reduced noise in the analysis of responses to cytokines, contributing to success in the identification of relevant small interfering RNA (siRNA), or genes, associated with changes in caspase 3 activity. Furthermore, the application of high-throughput screening of siRNA is another feature of

this study. In order to carry out the high-throughput screening, the authors transfected as many as 730 non-redundant siRNAs into pancreatic islet cells. The effects on caspase 3 activity were then assessed for corresponding well to each siRNA to identify potentially anti- and pro-apoptotic genes. The effects of the siRNAs on caspase 3 activity were compared with those observed in the control samples with an indicator called the mz score. As a consequence of this procedure, candidate genes were narrowed down to 21 genes with large mz score changes. They included an anti-apoptotic gene with an mz score of +16.6, the highest score. When the substance corresponding to this gene was identified, the gene was found to encode otubain 2 (OTUB2), an important modulator. OTUB2, identified in human islets, was also confirmed to be involved in the corresponding pathway in cultured

murine β -cells (MIN6 cells) by similar siRNA screening for cytokines. Furthermore, a knockdown experiment of OTUB2 by siRNA confirmed the function of OTUB2 so as to maintain the viability of MIN6 and human islet β -cells, to protectively act against decreased glucose-induced insulin secretion from β -cells treated with cytokines, and to prevent activation of NF- κ B. Thus, OTUB2 was considered to be a cytokine-induced modulator molecule (Figure 1).

OTUB2 is a de-ubiquitinating enzyme that participates in chromatin remodeling against genetic mutation and cell death after cellular injury as a result of deoxyribonucleic acid double-strand breaks. In general, the OTUB family negatively regulates double-strand break-dependent ubiquitination. A recent study⁵ showed that OTUB2 functions as a modulator of the cellular response by controlling the speed of double-strand break-induced

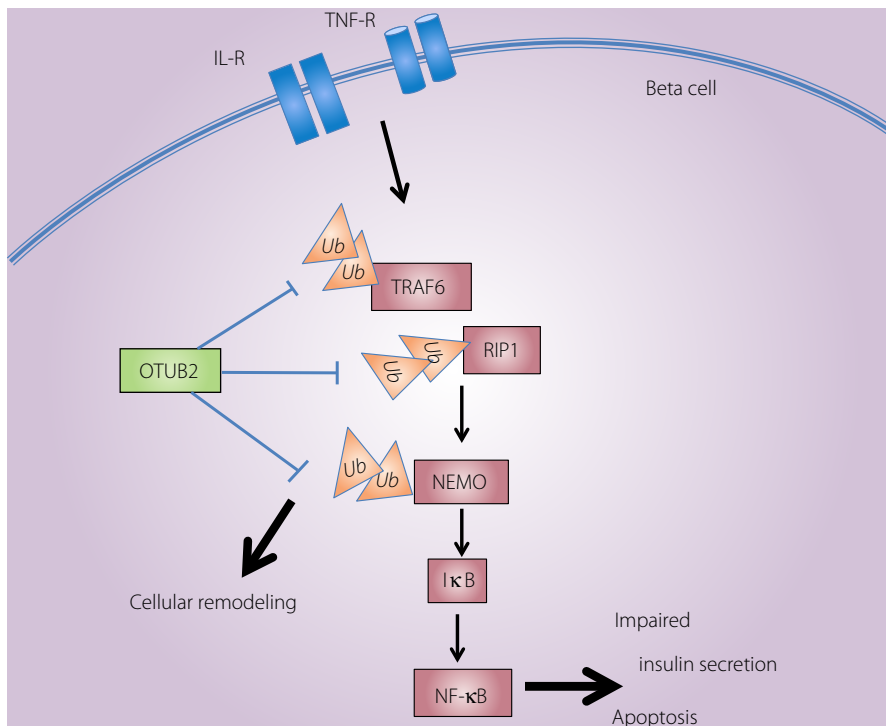


Figure 1 | Binding of cytokines to their receptors on islet cells activates a number of signaling pathways including transcription nuclear factor- κ B (NF- κ B) through ubiquitination of elements, which should provoke cytokine-induced cell death. The pathway, however, might be modified by de-ubiquitinating elements, such as OTUB2. This modifier executes remodeling of the damaged islet cells. I κ B, inhibitor of κ B; IL-R, receptor for interleukins; OTUB2, otubain 2; NEMO, nuclear factor- κ B essential modulator; RIP1, receptor-interacting protein 1; TNF-R, receptor for tumor necrosis factor superfamily; TRAF, TNF receptor-associated factor; Ub, ubiquitin.

ubiquitination, allowing the appropriate deoxyribonucleic acid repair pathway to be selected. This also supports the crucial role of OTUB2 in promoting viability after cytokine-induced β -cell damage through de-ubiquitination of the elements involved.

Meanwhile, β -cell injury in type 2 diabetes is not assumed in the article. However, it could be considered that, in type 2 diabetes, metabolic stressors, such as fatty acids, activate specific signaling pathways triggering cell damage, which consequently causes dysfunction of β -cells and also a decreased β -cell number as a result of apoptosis. Thus, if high-throughput siRNA screening similar to that used in the present study is carried out by using palmitate and other substances to achieve extracellular stimulation of β -cells, molecules specific to type 2 diabetes that are completely different from those identified in the study

and specifically associated with β -cell injury caused by metabolic stress could well be identified.

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