

How does glucagon-like peptide 1 stimulate human β -cell proliferation? A lesson from islet graft experiments

The incidence of type 2 diabetes increases with age. The age-dependent decline in functional β -cell mass contributes to the increased risk of onset of diabetes, reflecting the central role of pancreatic β -cells in glucose homeostasis. Indeed, the replication rate of human and rodent β -cells is known to decline sharply with age, and such a characteristic of β -cells might explain the increased onset of type 2 diabetes in the older population. The molecular mechanism involved in the age-dependent decline of β -cell proliferation has been extensively studied, mainly using rodents and *in vitro* culture systems, but its molecular basis is still largely unknown. A mechanism by which glucagon-like peptide-1 receptor activation induces human β -cell proliferation only within a restricted time window was recently suggested in a study in which human islets were grafted into immunodeficient mice. The authors found that the mitogenic effects of exendin-4 require calcineurin/nuclear factor of activated T-cells signaling, and that only in juvenile islets, exendin-4 induced the expression of nuclear factor of activated T-cells signaling components, as well as downstream target genes that facilitate β -cell proliferation. These findings provide a mechanistic

explanation as to why glucagon-like peptide 1 exerts mitogenic effects only in juvenile human β -cells.

The replication rate of human pancreatic β -cells is believed to be at 3–4% during the first several years of the postnatal period, and then after 10 years-of-age it dramatically declines to a low level, at 0.05–0.1%. In adulthood, the replication rate of β -cells remains low, at 0.01–0.1%. Previously, the lifespan of rat β -cells was assessed by the observation of lipofuscin granule accumulation using electron microscopy. The authors estimated the half-life of β -cells in 12-month-old rats to be 30–60 days. In contrast to rats, the lifespan of human pancreatic β -cells was estimated to be >20 years, consistent with the lower replication rate of human β -cells. However, human adult β -cells show active proliferation when an individual is pregnant or has liver disease. The presence of obesity increases insulin resistance, and thus an excess secretion of insulin is required to maintain normal glucose tolerance. Therefore, obese individuals require hypertrophy of their β -cells to maintain normal glucose tolerance. Indeed, the β -cell volume density of obese non-diabetic Caucasians was shown to be increased by 1.5-fold compared with lean non-diabetic control individuals. Mechanistic analysis of β -cell proliferation largely depends on data from rodents, as it has been very difficult to experimentally reconstitute human β -cell proliferation, and systems that enable the assessment of proliferation potency of human β -cells have been greatly anticipated.

Whereas an age-associated decline in human β -cell proliferation has been

previously reported, its molecular basis has never been tested *in vivo*, and no mechanistic explanation has been provided to support the idea. An age-associated increase in p16^{INK4A} has been implicated in the impaired proliferation of aged β -cells. P16^{INK4A} is a tumor suppressor protein and a cyclin kinase inhibitor that prevents phosphorylation of the retinoblastoma protein by cyclin-dependent kinase 4, thus preventing E2F transcription factors from activating the cell division cycle. The expression of p16^{INK4A} in β -cells is suppressed by the polycomb genes, *Ezh2* and *Bmi1*, both of which decline with age. The postnatal decline of *Ezh2* and *Bmi1* appears to be mediated by the decline of signaling through the platelet-derived growth factor receptor.

Glucagon-like peptide-1 (GLP-1) has a well-known role in stimulating insulin secretion, which is known as the incretin effect, in addition to enhancing insulin biosynthesis and preventing β -cell apoptosis¹. GLP-1 and its analogs, such as Ex-4, have been reported to induce mouse β -cell proliferation². Previous studies investigating whether GLP-1 stimulates human β -cell proliferation have provided conflicting results.

A recent study by Dai *et al.*³ at Vanderbilt University reported the establishment of a system that enables the assessment of human β -cell replication *in vivo*. In this system, human pancreatic islets were grafted into a renal capsule from immunodeficient non-obese diabetic scid gamma mice, these mice were treated with Ex-4 for 2 weeks using osmotic pumps, and Ex-4-induced β -cell replication was assessed by Ki67 immunostaining. They found that Ex-4 shows mitogenic effects on juvenile human β -cell grafts (from 0.5 to 7 years-of-age), but not on adult β -cell grafts

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(>20 years-of-age). Here, another important finding was that Ex-4 enhanced glucose-stimulated insulin secretion similarly in juvenile human β -cell grafts and in adult human β -cells. Accordingly, these results indicated that the Ex-4-induced promotion of β -cell proliferation was age-dependent. Their results also suggested that both β -cell proliferation, as well as insulin secretion induced by GLP-1 receptor (GLP-1R) activation, are regulated by two distinct signaling pathways³.

Then, what is the molecular mechanism that mediates the age-dependent human β -cell proliferation induced by GLP-1R agonists? To address this question, ribonucleic acid sequencing analysis was carried out and identified 1,440 genes showing enhanced expression in juvenile human islet grafts stimulated by Ex-4. Gene ontology analysis showed that genes involved in the calcineurin/nuclear factor of activated T cells (NFAT) signaling pathway were on the top of the gene list, which were preferentially expressed in juvenile islet grafts stimulated by Ex-4. Among the NFAT family members, the messenger ribonucleic acid of NFAT1, NFAT2 and NFAT4 is abundantly expressed in juvenile human islets, but is markedly declined in adult islets. Furthermore, in juvenile human islets, Ex-4 significantly induced the expression of the target genes of NFAT, including cyclin A, cyclin-dependent kinase and Forkhead box protein M1, which are involved in stimulating β -cell proliferation. A crucial role for the calcineurin/NFAT pathway in Ex-4-induced proliferation of human β -cells was shown by the observation that the addition of FK-506, a strong inhibitor of the NFAT signaling pathway, almost completely eliminated Ex-4-induced β -cell proliferation, concomitantly with the disruption of Ex-4-induced activation of downstream effectors. Taken together, the authors concluded that the calcineurin/NFAT pathway mediates the age-dependent human β -cell proliferation induced by GLP-1R activation (Figure 1)³.

GLP-1R agonists, when used chronically, have been shown to inhibit

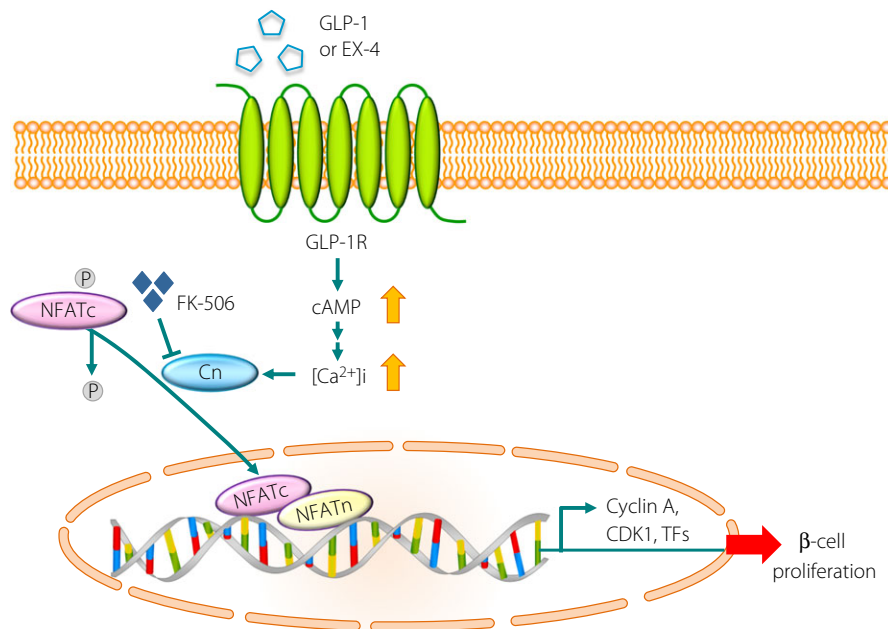


Figure 1 | Model of glucagon-like peptide-1 (GLP-1) receptor-induced mitogenesis of juvenile human β -cells through the calcineurin/nuclear factor of activated T cells (NFAT) pathway. Binding of GLP-1 (or exendin-4 [Ex-4]) to the GLP-1 receptor (GLP-1R) increases intracellular calcium concentrations through cyclic adenosine monophosphate (cAMP) production. An increase in intracellular calcium concentration potentiates calcineurin (Cn), a calcium-sensitive phosphatase. Activated Cn dephosphorylates the cytoplasmic component of NFAT (NFATc), which triggers its rapid entry into the nucleus. In the nucleus, NFATc proteins assemble on deoxyribonucleic acid with partner proteins termed NFATn, thereby activating target genes, including cell cycle activators (*cyclin A* and *CDK1*) and proliferation-activating transcription factors (TFs), such as Forkhead box protein M1, which are all produced at low levels in adult islets. FK-506, a well-known inhibitor of calcineurin, inhibits β -cell proliferation and thus might predispose individuals to diabetes. CDK, cyclin dependent kinase; P, phosphorylation.

bodyweight gain and enhance liver insulin sensitivity, and thereby decrease insulin demand of the whole body⁴. A previous study² reported that 4 weeks of Ex-4 administration at the same dose as that used in the report by Dai *et al.*³ (300 pmol/kg bodyweight/day) to 8-week-old C57BL/6 mice did not stimulate any β -cell proliferation, although 3 days of Ex-4 administration significantly stimulated β -cell proliferation assessed by Ki67 immunostaining. The observed difference in 3 days vs 4 weeks of administration might be explained by the fact that 4 weeks of Ex-4 administration significantly suppressed bodyweight gain in C57BL/6 mice compared with control phosphate-buffered saline-treated groups². Therefore, the results obtained from graft

experiments reported by Dai *et al.*³ showing that the 4-week-administration of Ex-4 did not induce proliferation of adult human β -cells needs to be interpreted with caution; the possibility cannot be ruled out that mitogenic effects of Ex-4 on adult human β -cells might have been underestimated by the decreased demand of insulin secretion as a result of the increased insulin sensitivity of host non-obese diabetic scid gamma mice achieved by 4 weeks of treatment with Ex-4³. It is of special importance that juvenile human β -cells showed significant proliferation under the same condition.

It has been reported that β -cell proliferation induced by GLP-1 is dependent on the function of *pdx1*. The stimulation of insulin-1 (INS-1) insulinoma cells and

human pancreatic cells with GLP-1 results in induction of the *pdx1* gene, and mouse β -cells deficient for *pdx1* cannot proliferate in response to GLP-1. Insulin receptor substrate 2 (IRS2), a crucial adaptor molecule for β -cell survival and proliferation, is also implicated in GLP-1-induced β -cell replication. Signaling through insulin-like growth factor, which is activated by GLP-1, contributes to GLP-1-induced β -cell proliferation. It is well known that activation of the GLP-1R provokes Ca^{2+} signaling. A previous study reported the crucial role for calcineurin signaling in controlling human β -cell survival⁵. Although the authors detected no human β -cell replication in the islet culture system, treatment with the calcineurin inhibitor, tacrolimus, significantly induced human β -cell apoptosis. Furthermore, a crucial role for calcineurin/NFAT signaling in human β -cell survival has been suggested by the observation that 10–30% of patients treated with the immunosuppressant, FK-506, developed diabetes mellitus⁶. It is therefore intriguing that the study by Dai *et al.*³ directly connects the Ca^{2+} signaling elicited by GLP-1R activation to human β -cell proliferation by using a novel human islet graft technique. The development of an

engraftment system that enables the assessment of human β -cell replication *in vivo* will significantly facilitate prospective analyses of the proliferative potency of human

β -cells, and might eventually provide avenues towards discovering novel therapeutic strategies for functional β -cell expansion in humans.

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DISCLOSURE

The author declares no conflict of interest.

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