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Commentary on:
A live-cell, high-content imaging survey of 206 biologic factors across 5 stress conditions reveals context-dependent survival effects in primary beta-cells

Tuning to the right signal

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Abbreviations: 5'HT, serotonin; CNTF, ciliary neurotrophic factor; DPP4, dipeptidyl peptidase 4; GFP, green fluorescent protein; GH, growth hormone; GIP, gastric inhibitory polypeptide; GLP1, glucagon-like peptide 1; IFNγ, interferon gamma; IL1R1, interleukin 1 receptor 1; IL-1β, interleukin-1beta; MIP, mouse insulin promoter; Olfm1, olfactomedin 1; PI, propidium iodide; PRL, prolactin; SEMA4a, Semaphorin4a; Th1, T helper 1; TNFα, tumor necrosis factor alpha; VIP, vasoactive intestinal peptide.

Abstract

Pancreatic beta cells are clustered in islets of Langerhans together with alpha cells in an arrangement that facilitates the tight coordination of insulin and glucagon secretion at the source of their release. Other secretory cells, including somatostatin-secreting delta cells and pancreatic polypeptide cells, co-localize with alpha and beta cells in the islet and serve to modulate islet endocrine output. A multitude of non-secretory cell types, including endothelial cells, pericytes, stromal cells, glial cells and macrophages complete the cellular make up the islet, which is further enhanced by (para)sympathetic nerve terminals that impinge on the islets via neurotransmitters released in the islet microenvironment. While this islet architecture is relatively simple in

comparison to the vast complexity of the central nervous system, the constellation of cell types united in the islet nevertheless provides a rich substrate for local paracrine and autocrine interactions that affect diverse aspects of islet physiology, ranging from the modulation of hormone secretion to the regulation of islet cell mass via proliferation and death. In this issue of Diabetologia, Yang et al., take the notion of rich cross-talk within the islet as their point of departure for a systematic evaluation of the beta cell-protective properties of an extensive panel of over 200 factors, with some surprising and highly interesting results.

Maintenance of beta cell mass is determined by the net effect of beta cell proliferation and beta cell death. Beta cell proliferation is abundant in juvenile rodent islets, declines rapidly with age, but can be stimulated by a variety of factors including incretins, prolactin (PRL) and insulin growth factors [1]. However, stimulation of human beta cell proliferation has proven much more elusive [2], which provides all the more incentive to prevent loss of beta cells from death in response to diabetes-associated insults that are major contributors to disease [3]. In this issue of *Diabetologia*, Carol Yang, Quin Wills and Jim Johnson systematically evaluate the beta cell-protective properties of an extensive panel of over 200 factors on primary mouse beta cells [4]. These factors were selected on the basis of the expression of their cognate receptors in mouse or human islets, derived from prior work by the authors [5] among many others [6-10]. Recognizing that beta cell stress in diabetes can occur in many different shapes and forms [3], each factor was systematically evaluated in five distinct islet cell culture conditions, reflective of different type 1 or type 2 diabetes-associated stresses. To prevent confounding actions of survival factors in fetal bovine serum, each beta cell stress condition was designed as an extension of serum starvation under 5.5 mM glucose. Tested paradigms included 20 mM glucose (glucotoxicity), palmitate (lipotoxicity), thapsigargin (ER stress) and treatment with a cocktail of IL-1β, TNFα and IFNγ (reflective of pro-inflammatory insults associated with both type 1 and type 2 diabetes). To enable the recognition of beta cells, the entire study was conducted on freshly isolated primary mouse islets isolated from the mouse insulin promoter (MIP)-driven GFP transgenic reporter mouse [11], which was one of several lines recently reported to contain a human growth hormone (GH) minigene that drives local production of GH and 5'HT [12]. While this represents a possible confound, addition of GH and 5'HT in the context of the screen in several instances conveyed robust beta cell protection,

suggesting that the levels of GH/5'HT attributable to the MIP-GFP transgene did not maximally protect beta cells from death. Cell cultures were stained with Hoechst and propidium iodide (PI), with the loss of Hoechst-positivity and the accumulation of PI among GFP-positive beta cells quantified every 3 hours as indices of beta cell death. The result is an impressive screen that features high content live cell imaging data of primary beta cells that is unique in scope and ambition. Such a comprehensive overview of factors precludes the depth we normally expect from papers that focus on individual factors, but this was not the purpose of this study. Instead, the value and novelty of this work is in its breadth, complemented by the fact that so many factors are benchmarked directly against each other for their ability to protect primary beta cells against death induced by several treatments.

All these factors are tested in a systematic high-content imaging screen that is conducted on primary islet cells, a significant improvement upon the majority of screening efforts that are carried out on cell lines. The authors opted to carry out their study on mouse islets (and not human islets) for entirely justifiable reasons, as it is difficult to control for the variation between human islet donors when conducting screens of this magnitude that are conducted over the course of several years. However the use of rodent models as a proxy for human disease comes at the risk of identifying signals that protect mouse but not human beta cells against death. For example, systematic comparison of the transcriptomes of purified mouse and human beta cells revealed that human beta cells, in contrast to mouse beta cells, express very little to no receptors for the related cytokines prolactin (PRL), GH or ciliary neurotrophic factor (CNTF) [13, 14]. This would suggest that potential protective effects of these factors may not translate to human beta cells. Similarly, mouse beta cells express very high levels of the IL-1β receptor (*Il1r1*) [13], while the expression of IL1R1 in human islets is markedly lower and enriched in non-beta cells [14], which raises the question if human beta cells would respond similarly robust to an insult mediated by a cytokine cocktail that includes IL-1β. Of course, mouse beta cells in most studies [4, 13] are isolated from adults whose age is measured in months; a confound that is difficult to avoid when using mouse beta cells to model diseases of the adult human islet.

Perhaps the most surprising outcome of this study is that relatively few factors emerged with pan-protective effects across each of the distinct beta cell insults that was

tested [4]. Among these were adiponectin and vasoactive intestinal peptide (VIP), which are known to stimulate beta cells, and somatostatin. The latter thus promotes beta cell survival despite inhibiting insulin release. Of course somatostatin is a potent inhibitor of glucagon release [15], which opens up the possibility that some of the actions of somatostatin and possibly other factors in this screen are mediated through intermediates derived from non-beta cells in co-culture. Notably absent from the list of pan-protective factors were the incretins gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP1) and oxyntomodulin, which also derives from the same pre-pro-glucagon precursor and promotes glucose-stimulated insulin release [16]. This in spite of the fact that incretins are often regarded as beneficial for their ability to promote rodent beta cell proliferation and survival [17]. Perhaps the known peptide instability of the natural pre-pro-glucagon-derived peptides contributed to their inconsistent performance as pan-beta cell protectors across all conditions tested; there is significant dipeptidyl peptidase 4 (DPP4) gene expression in mouse beta cells [13, 18]. More stable incretin analogs such as exendin4 were not included in the screen, as it focused on endogenous factors. One of the lesser known factors that emerged to convey good overall protection against all beta cell insults tested in the screen on primary mouse beta cells is olfactomedin 1 (OLFM1). Olfm1 is a member of a larger family of glycoproteins that was initially purified from olfactory neuro-epithelium. Olfm1 is implicated in early neurodevelopment and axon growth [19], but had no known function in islets. In following up on the identification of Olfm1 as a beta cell protective factor in a screen on mouse beta cells, the authors show that its beta cell protective properties translate to human beta cell protection under similar conditions *in vitro* [4].

In addition to the identification of factors that prevent beta cell death across a broad spectrum of insults, Yang *et al*., find that each of the different beta cell stress paradigms is associated with relatively distinct sets of protective factors [4]. This suggests that beta cells are protected from different types of insults through mechanistically distinct pathways. Case in point is Semaphorin 4a (SEMA4a), which was found to strongly protect from beta cell lipotoxic insults, but actually promoted beta cell death in other conditions [4]. Members of the semaphorin family were originally discovered as repulsive axon guidance cues, but SEMA4a also plays important roles in the immune system, where it is expressed on the membrane of dendritic cells and interacts with T cell surface receptors to provide co-stimulation in Th1 differentiation [20].

However, as is the case for OLFM1, SEMA4a had not previously been associated with beta cell function, which illustrates the power of non-biased efforts such as the one by Yang *et al*., to identify entirely novel factors that contribute to beta cell survival. More generally speaking, prevention of palmitate-induced beta cell death was more strongly associated with tyrosine kinase receptors, while protection against pro-inflammatory cytokines was more strongly associated with Gαq-mediated G protein-coupled receptors. The finding that relatively distinct signaling pathways are associated with protection against different types of beta cell insults is unexpected. It also suggests significant synergistic potential by co-stimulation with factors that are selectively protective against glucotoxicity and lipotoxicity to maximize beta cell protection in type 2 diabetes. These results by Yang *et al*., will hopefully serve as a catalyst to the field of islet biology, by providing a wealth of novel information regarding the beta cell protective effect of several hundred signals endogenous to the islet. As diabetes is a disease that manifests itself in many shapes and forms, an increased understanding of the specific pathways that ensure maximal protection in the face of death precipitated by distinct beta cell insults would be a significant step towards the development of personalized medicine for the treatment of diabetes.

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The author was the sole contributor to this paper.

The author declares that there is no duality of interest associated with this manuscript

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