CHAPTER 1

Chemical Approaches for Beta-cell Biology

AMEDEO VETERE,[a] VISHAL S. PAREKH,[a]
ASHLEY E. MODELL,[a,b,c] VERONIKA M. SHOBA,[a]
AMIT CHOUDHARY[a,b,c] AND BRIDGET K. WAGNER*[a]

[a] Broad Institute, Chemical Biology & Therapeutics Science Program, 415 Main Street, Cambridge, MA 02142, USA; [b] Department of Medicine, Harvard Medical School, Boston, MA 02115, USA; [c] Divisions of Renal Medicine and Engineering, Brigham and Women's Hospital, Boston, MA 02115, USA
*Email: bwagner@broadinstitute.org

1.1 Introduction

Diabetes is a staggering epidemic that is constantly increasing. In its recent ''Global Report on Diabetes,'' the World Health Organization (WHO) estimated that about 422 million adults were living with diabetes in 2016, compared to 108 million in 1980.[1,2] Despite this grim forecast, the pharmacologic toolkit available is somewhat limited. The stringent boundaries established by the FDA in terms of cardiovascular effects for any new antidiabetic drugs have made the development of novel chemical entities a ''high-risk, low-reward'' endeavor. Novel small-molecule approaches in islet biology, however, may prove fruitful in the future. In this chapter, we review the principles of chemical biology that enable small-molecule discovery, the current state of the art for therapeutic approaches, novel beta cell-focused approaches to use small molecules that improve phenotypes, and more recent efforts to deliver therapeutics selectively to the beta cell.

Nanoscience & Nanotechnology Series No. 59
Nanotechnology for Diabetes Management
Edited by Amar Abderrahmani, Sabine Szunerits, Rabah Boukerroub and Abdelfattah El Ouaamari
© The Royal Society of Chemistry 2023
Published by the Royal Society of Chemistry, www.rsc.org

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

1.2 Principles of Chemical Biology

Chemistry and biology have long been considered independent disciplines, missing the important point that without chemistry we cannot have biological processes and *vice versa*. The emergence of the concept of ''chemical biology'' filled this gap. Chemical biology as a scientific discipline is a relatively new field; it only emerged about 20 years ago when scientists became interested in applying the principles of chemistry to study biological systems.[3] At its core, chemical biology is the process of discovering and using small molecules to modulate and understand how biological systems work. With this broad definition in mind, it is understandable to associate the process of small-molecule discovery with the concept of drug discovery. However, this is only part of the story. Small molecules as ''drugs'' have a very specific therapeutic scope and must meet stringent requirements in term of pharmacokinetics, safety, and bioavailability.[4] On the contrary, small molecules as ''probes'' can have more lenient pharmacokinetic requirements, but often even more stringent requirements for protein and cell selectivity. Chemical probes have the potential to provide important discovery tools to delve into biological phenomena.[5,6]

Chemical biology, as a discipline, and chemical probes, as its experimental tools, have provided an enormous boost to the expansion of the druggable genome. The completion of the Human Genome Project in 2003 coincided with the rise of chemical biology. The publication of a draft human genome sequence[7,8] provided the basis for understanding the roles of genes in normal physiology and in pathology. At the same time, chemical biology (or, as it is often referred to, chemical genetics) emerged as the way to modulate ideally each protein product emerging from all human genes.[9]

Chemical modulation of targets can be quite flexible, since it can provide narrow control of biological events on both temporal and concentration scales. At a temporal level, in the majority of cases, the effect of the chemical probe is rapid and reversible, as the effect of a chemical probe will last for the time it is present (covalent modulators are the exception). Further, the effects of small molecules are immediate, which helps determine the role of a cellular target. Data from knockout mice, on the other hand, can be confounded by developmental effects or compensation from a related isoform. At a concentration level, a key feature of chemical probes is their ability to show dose–response effects, where phenotypes are gradated by varying the concentration of the probe. Both of these properties are difficult to obtain by genetic manipulation. Temporal control of genetic manipulation would require conditional alleles, which are difficult to obtain and are prone to unwanted pleiotropic phenotypes.[10]

1.3 High-throughput Screening (HTS)

Genetics has been inspirational to the development of chemical biology as a modern discipline. From an experimental point of view, genetics studies are

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

performed in a ''forward genetics'' or ''reverse genetics'' setting. In ''forward genetics,'' random mutations are introduced in the experimental model and the subsequent phenotypes are observed. Subsequent sequencing analysis allows one to associate a mutation with a specific phenotype. In ''reverse genetics,'' the mutations are introduced in a specific gene followed by analysis of the arising phenotype. Similarly, chemical genetics can be divided into ''forward'' and'' reverse'' chemical genetics. The procedural concept is similar. In ''forward chemical genetics,'' the perturbation is obtained treating the experimental model with small molecules and the readout is the change of phenotype. With ''reverse chemical genetics,'' a well-characterized and purified target protein is probed with small molecules followed by assessment of the specific activity of the target protein. A more general transposition of these concepts can be applied in chemical biology, and here we will discuss ''target-based screening'' and ''phenotypic screening,'' for the concepts of ''reverse chemical genetics'' and ''forward chemical genetics,'' respectively.

1.4 Target-based Screening

As the name suggests, in target-based screening a well-characterized protein target involved in a biological phenomenon or causative of a disease is used to interrogate chemical libraries to find high-affinity binders or modulators. Subsequently, active compounds are assessed in tissues and whole organisms. Target-based screening has been successfully used in both academic and industrial settings. The use of target-based screening has been particularly appealing to the drug discovery world since it seemed that targeting directly specific proteins would streamline the development of novel therapeutics. This concept stemmed from the observation that there were many examples of mutated proteins that were responsible for some cancers. Some notable examples of successful drugs that were discovered by target-based screening and that are in clinical use include inhibitors of Rho-dependent kinase (fasudil),[11] BCR-Abl (imatinib),[12] and EGFR (gefitinib).[13] Many more are approved or are at different stages of clinical trials[14] but the numbers are far from covering the entire known kinome. This is true not only for the kinome and but also for most of the druggable genome.

1.5 Phenotypic Screening

An alternative to target-based screening is phenotypic screening. In its general definition, phenotypic screening uses an agnostic approach by perturbing a phenotype with small molecules. Before the advancement of target-based drug discovery, new medicines were discovered using mainly phenotypic approaches. In an interesting analysis of drugs developed over the first decade of the 2000s, it emerged that 56% of the novel small-molecule approved drugs that constituted ''new chemical entities'' were discovered using phenotypic screening.[15] The past 20 years have seen an

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

increase in this approach, with the discovery of first in class drugs for pathologies such as spinal muscular atrophy,[16] cystic fibrosis,[17] and hepatitis C.[18] Academic laboratories were also successful in identifying probes with novel mechanisms of action by using phenotypic screening as has been well summarized in a recent report.[19]

1.5.1 Hit Triage

Regardless of its final use (as a probe for basic research or as novel drug for clinical applications), a small molecule must satisfy basic criteria in terms of potency, solubility, toxicity, and specificity. Exhaustive descriptions of what constitutes a ''good'' probe have been recently reported.[5,20,21] Recently, the concept of ''nuisance'' has been added to the pool of factors to be considered in evaluating the quality of a probe.[22] Nuisance compounds are those that interfere with the assays used for the screening or, in more general terms, any compounds that show undesirable bioactivity. Nuisance compounds have always plagued phenotypic screening campaigns[23,24] but only recently has a systematic approach been pursued to categorize and characterize them.[22]

1.5.2 The Sources of Chemical Probes

1.5.2.1 Natural Products Libraries

Although the concept of chemical probes emerged as tools to realize the full potential of chemical biology, they were not strangers to either chemists or biologists. There are many examples of natural products whose discovery and use have enabled the understanding of important biological events. Some notable examples are colchicine, which helped study cytoskeleton organization,[25] FK506 and cyclosporine, which were instrumental to study immunosuppression,[26,27] rapamycin, which elucidated the role of mTOR,[28] and many others. Not surprisingly, the majority of the early studies focused on natural products. For centuries humankind has turned to natural compounds, mainly from plant extracts, as therapeutics. Far from being ''old-fashioned,'' this trend continues and, not surprisingly, a great many FDA-approved drugs are natural products or their derivatives.[29] The search, purification, and eventually chemical synthesis of natural products, however, is an ''art'' that competes with the scope and need of chemical genetics, which aims to target every single human gene.[9,30–32]

1.5.2.2 Diversity-oriented Synthesis (DOS) Libraries

To increase the throughput of targeted genes or proteins it is necessary to access a wider and more diversified chemical space that can be achieved only by screening richer libraries of structurally diverse compounds. This goal has been approached by the introduction of diversity-oriented synthesis

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

(DOS) libraries of small molecules.[33–35] The idea behind DOS is to create, with few synthetic steps, rich libraries of small, conformationally diverse compounds. Part of what makes DOS compounds unique is the introduction of stereochemistry as a further level of diversity.[34,35] Since their inception, DOS libraries have been successful in identifying novel mechanism-of-action compounds and chemical probes. Some notable examples are the discovery of novel multistage antimalarial inhibitors,[36] a novel compound that protects cytokine-induced beta-cell apoptosis,[37] a highly specific inhibitor of the insulin-degrading enzyme,[38] and selective Cas9 inhibitors.[39]

1.5.2.3 Repurposing Collections

One of the paradigms of small-molecule discovery is to screen libraries of diverse compounds to identify those that show a high specificity toward the cellular target or phenotype. A more pragmatic approach has recently emerged with the introduction of the concept of ''repurposing'' screening. The idea behind repurposing screening is to exploit the relative promiscuity of well-annotated small molecules by using them to probe targets or phenotypes different from those for which they were originally developed. Repurposing screening represents an attractive and economical way to find novel therapeutic applications for compounds that had already gone through the painstaking process of different stages of preclinical development or even clinical trials. Although useful, large campaigns involving repurposing screening are still in their infancy. As recently reported,[40,41] the major hurdles to a widespread use of repurposing screening are (1) the lack of repositories of all available drugs, (2) low quality of drug annotations, and (3) the lack of clear readouts of drug activity. In the past few years there have been attempts to solve some of these problems. For example, today a few repurposing repositories are already available.[40–42]

1.5.2.4 DNA-encoded Libraries

A further improvement in the ability to identify novel compounds arose with the advent of DNA-encoded libraries (DEL).[43] The core concept behind DEL screening is to combine the diversity of organic chemistry with the uniqueness of DNA barcodes. The presence of unique DNA barcodes allows screening to be performed in a pooled fashion rather than in an arrayed manner. Pooled approaches have the advantage of allowing screening of millions, if not billions, of compounds in a single test tube.[44] The identification of hits is then achieved by evaluating the enrichment of unique DNA tags after PCR amplification and next-generation sequencing.[45] DEL screening represents a significant breakthrough in the field of chemical biology with the identification of novel protein ligands.[45] Despite its potential, DEL screening has some limitations, mainly the historical lack of structural diversity of chemical scaffolds and a chemical liability associated

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

with the need for DNA-compatible chemistry.[46,47] In the past few years significant progress has been made to resolve both issues.[48,49]

1.5.2.5 *Molecular Glues*

A further breakthrough in the field of chemical biology arose with the realization of a phenomenon that was observed in the early 1990s, known as ''chemical-induced proximity'' or the ''molecular glue'' concept. As reported above, the discovery of cyclosporin A was instrumental to understanding immunosuppression, but it was years later that it was found that it acts in a unique mode by creating a protein–protein interaction, hence ''molecular glue''.[50] From there, chemical-induced proximity became the new frontier of chemical biology, and with all of its various applications, it is having profound effects on both biology and therapeutics.[51,52] The most recent example of chemical-induced proximity is the proteolysis targeting chimera (PROTAC) approach. PROTACs were first developed in the early 2000s.[53] In its original conception, it was based on the creation of a chimeric structure between a small molecule, specific for a target protein, and a peptide that had specificity toward E3 ligases. Thus, due to proximity, the lysines of the target protein are ubiquitinated by the E3 ligase complex and subsequently targeted to the proteasome to be degraded. Despite success in demonstrating proof-of-concept for PROTAC technology, peptide-based PROTACs had some disadvantages, such as high molecular weight, lability of peptide bonds, poor cell permeability, and low potency.[54] Over the last decade, we have witnessed a dramatic improvement in the PROTAC technology with the introduction of small molecule-based PROTACs.[55–57] The small molecule-based PROTAC technology relies on the construction of a heterobifunctional molecule from small-molecule ligands for the Von Hippel Lindau (VHL)[58,59] and cereblon (CRBN) E3 ligases[57] instead of peptide binders. We envision that the development of screening approaches and libraries for the discovery of such proximity inducers will tremendously benefit the field of drug discovery.

1.5.3 Target Identification and Mechanism-of-action Studies

The unbiased approach of phenotypic screening represents a powerful tool to identify compounds that affect a phenotype of interest, but a major challenge of this approach is the need to identify the target and mechanism of action for a new compound. Target identification is an important task needed to evaluate the specificity and selectivity of the probe and to, eventually, develop target-based screening strategies. Furthermore, target identification helps to better strategize medicinal chemistry efforts. Target identification is not an easy task but today there are at least three complementary approaches that are used for target identification: direct biochemical methods, genetic interaction methods, and computational inference methods.[60–64]

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

1.5.3.1 Label-based Target Identification

Direct biochemical methods rely on the affinity of the small molecule of interest for its protein target. This approach can be used to perform ''label-based'' or ''label-free'' target identification. Label-based approaches depend on a solid contribution from synthetic organic chemistry, since it typically requires the introduction of appropriate functional groups in the lead compound. Historically, this approach has involved immobilizing the small molecule of interest on a physical support that is used to ''fish out'' target proteins from a cellular extract,[65] whose identity can be detected by mass spectrometry.[61,66] This approach brings with it a risk of missing biologically significant but weak interactors, due to the stringent washes generally required by these protocols. To overcome these limitations, label-based approaches have shifted toward methods in which the small molecule of interest can establish covalent interactions with target protein(s) within a cellular context. This goal can be reached either by introducing electrophilic groups that can react with their nucleophilic counterparts in target proteins (activity-based probes), or by introduction of a photo-reactive group that can cross-link with the target protein upon UV-irradiation (photo-affinity probes).[67]

Activity-based probes possess common features, including (1) a reactive group for covalent binding, (2) a core structure that confers specificity, and (3) a reporter group (*i.e.*, biotin, or a generic fluorophore) for detection.[68–70] The reactive warhead can be designed to target key groups of active sites of a specific category of enzymes. The reporter group can be a constitutive part of the activity-based probe or can be added subsequently once the probe is covalently bound to its target.

To avoid high hit rates due to false positives, the majority of compounds used in high-throughput screening are based on chemistries that avoid covalent binding to target proteins. Nevertheless, covalent binding can become a useful instrument for target identification. One way to exploit this possibility is to introduce UV-reactive groups.[71,72] There are many factors that have to be considered before choosing the right photo-affinity probe,[72] but ultimately the nature of the core structure will dictate the choice based on the chemical manipulation it can withstand without losing activity and specificity. Label-based approaches have been used successfully for target identification deriving from unbiased phenotypic screening, as described recently.[73]

1.5.3.2 Label-free Target Identification

Label-based target identification approaches have undoubted advantages, but they typically require organic chemistry efforts that are not always available. To overcome this limitation, and to make target identification more accessible to biology-focused laboratories, many groups have embarked on efforts to develop label-free target identification methods. These approaches revolve around the ability of a small molecule, upon protein binding, to protect that protein from (1) protease digestion, (2) chemical

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

denaturation, or (3) thermal denaturation; the respective methods are (1) drug affinity responsive target stability (DARTS), (2) stability of proteins from rates of oxidation (SPROX), and (3) cellular thermal shift assay (CETSA). Each of these methods has the option of using high-resolution mass spectrometry to determine and quantify the relative differences in proteins in compound-treated samples, with respect to control samples.

DARTS was developed based on the observation that when a small molecule interacts with its cognate protein target, the protein becomes less susceptible to the action of proteolytic enzymes.[74] Cell extracts are subjected to partial proteolysis after treatment with compound or DMSO. After SDS-PAGE, the bands differentially expressed in the treated sample *versus* the DMSO controls are excised and analyzed by mass spectrometry. SPROX uses a similar principle, but in this case is based on the susceptibility of exposed methionines on denatured proteins to oxidation by hydrogen peroxide.[75] Here, the assumption is that the presence of a small molecule will prevent denaturation and methionine oxidation. Cell extracts are treated with an increasing concentration of a denaturing agent, such as guanidinium chloride, and the exposed methionine residues are then oxidized with hydrogen peroxide. The samples are then digested, and peptides are analyzed by mass spectrometry. The readout is a shift of the so-called ''transition midpoint,'' which is the concentration of denaturant required to reach 50% oxidized methionine and 50% native methionine.[75]

CETSA represents the latest approach introduced for target identification, with the first having been reported in 2013.[76] The principle behind CETSA is based on the well-appreciated understanding that the thermal stability of proteins can be modulated by a bound ligand. This observation forms the basis of the widely used biochemical thermal shift assay (TSA).[77] The major limitation of TSA is that it involves the use of purified protein. On the other hand, with CETSA, the concept of TSA was transposed to a cellular context. In its original iteration, CETSA was developed to study target engagement for known interactions in a cellular context.[76,78,79] Here, cells treated with the desired compound are subject to a brief thermal gradient. The cells are then lysed, and the soluble fraction is used to detect the desired target protein by western blotting. Plotting the band intensities as a function of temperature results in a melting profile, and the degree of shift in the melting temperature (T_m, corresponding to 50% denatured) in treated samples *versus* controls serves to evaluate the degree of target engagement. CETSA could not be applied to unbiased target identification until recently, when its principle was extended to proteome-wide applications with the introduction of thermal proteome profiling (TPP).[80] In a similar fashion as CETSA, cells treated with a specific compound are heated to increasing temperatures and then lysed, with the soluble fractions analyzed this time by mass spectrometry. The differential abundance of peptides in the treated *versus* the controls provides a melting profile for thousands of proteins and ultimately can help identify target protein(s). Since its first introduction, TPP has been used to identify novel targets for small molecules identified by phenotypic screening.[81–83]

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

1.5.3.3 Genetic Interaction Methods

An original goal set forth by leaders in chemical biology was to identify a chemical probe for each human protein.[9] Recent progress in functional genomics is bringing chemical biology closer to this goal. This is particularly true with the advent of CRISPR/Cas9 technology to manipulate the mammalian genome.[84–86] In brief, the methodology uses Cas9 as an effector protein that is ''guided'' to its target gene by the association of a guide RNA (sgRNA) complementary to the locus of interest. Cas9 introduces, then, a double-stranded DNA break, which triggers activation of the repair mechanisms of the cell. In the majority of cases, this results in frameshift mutations and subsequent loss of the corresponding protein.

Chemogenetic screens in the presence of genome-wide CRISPR can yield genes, whose corresponding protein knockout results in a loss of compound activity. With this approach, target cells are infected, in a pooled fashion, with lentivirus expressing Cas9 along with sgRNAs targeting each known gene. The infection is performed at a MOI that allows each cell to express only one sgRNA. In the setting of target identification, the pooled infection of CRISPR/Cas9/sgRNA is performed in the presence of a small molecule. This approach is particularly useful to identify targets of cytotoxic compounds, but the method is being expanded to other phenotypes as well. The outcome of the genetic perturbation may be the development of resistance to the compound (a suppressor interaction), or it may result in an increased susceptibility to the compound (an enhancer interaction). This approach has been used to identify novel targets of small molecules and directly correlate the gene to the effect of the small molecule.[64,87]

For cytotoxic small molecules, resistance screens also can be very valuable to identify targets. Here, the aim is to identify protective mutations in a specific gene that are induced after long-term treatment with a cytotoxic drug. In brief, cells are treated with a sublethal concentration of toxic compound until resistant clones emerge. After expansion, the transcriptomes of the resistant clones are then sequenced. The result of this approach is a series of clusters of genes of interest. CRISPR/Cas9 can then be used to deconvolute these clusters to pinpoint which mutation is essential for drug activity. This approach has been successfully used to identify novel target of small molecules.[87–89] As stated above, both chemogenetic and resistance screening approaches are particularly apt to identify the protein target of cytotoxic compounds. However, the phenotypic scale of application is rapidly expanding, and we expect that applications in the beta cell will arise in the near future.

1.5.3.4 Computational Inference Methods

Computational approaches are critical to a successful target identification campaign, regardless of the methods used. At the same time, computational approaches can serve as stand-alone methods for target identification. These approaches are based on the principle that structurally similar compounds,

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

or phenotypically similar compounds, are likely to have similar mechanisms of action, and hence similar protein targets. In the past few years, many computational approaches have been successfully developed for target deconvolution.[90–92] Here we will discuss two methods, Cell Painting and the Connectivity Map.

Cell Painting is a target-naïve image-based profiling approach based on the analysis of up to 1500 morphological features (*i.e.*, size, shape, texture, intensity, *etc.*). These fingerprints are derived from six different fluorescent dyes specific for distinct cell organelles and compartments (nucleus, endoplasmic reticulum, mitochondria, cytoskeleton, Golgi apparatus), imaged with orthogonal fluorescent channels.[93] After treating with the compound of interest, cells are fixed, stained, and imaged. The feature pattern is compared with a reference database of previously acquired fingerprints from compounds with known mechanism of action, and pattern similarity allows inference of the targets for novel compounds.[94]

The Connectivity Map (CMap) takes a conceptual approach similar to Cell Painting. In this case, the readout is a gene-expression signature rather than a morphological fingerprint. CMap was first developed by using 164 distinct small molecules, selected among a broad range of activities (FDA-approved drugs, nondrug bioactive compounds). As a proof of concept, compounds sharing molecular targets were included to determine whether such compounds would share a molecular signature. Furthermore, the pool included compounds with the same clinical indication, to determine whether therapeutic class could determine connections.[95] These compounds were tested in MCF7 (breast cancer epithelial cell line), PC3 (prostate cancer epithelial cell line), and SKMEL5 (melanoma cell line) cell lines. More recently, transcriptional analysis was reduced to 1000 so-called ''landmark'' genes and termed an L1000 profile. It emerged that the L1000 profile was enough to cover 82% of the information of the entire transcriptome.[96] From a target deconvolution point of view, CMap is used by comparing the profiles of unknown compound with those derived from known perturbagens and has been successfully used to uncover novel mechanisms of action.[97]

Together, the judicious use of assay development, high-throughput screening, target identification, and mechanism-of-action studies provides a powerful blueprint for identifying new small molecules with beneficial activity in the islet or probes to further our understanding of disease. Further, these compounds can furnish the diabetes community with information about new cellular targets whose modulation may be of interest in islet biology. The application of these chemical biology approaches to islet biology and diabetes has become increasingly important to the advancement of the field.

1.6 The Chemical Biology of Diabetes

The development and use of chemical biology tools have deepened our knowledge of many diseases, including diabetes. Diabetes is one of the

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

oldest reported human diseases, with the first reports emerging from Egypt around 1500 B.C.E.[98] Over time, there has been significant progress in understanding the pathology of diabetes, but perhaps not at the pace we would hope from a disease known for over 3000 years. Until the end of the 1980s, the basic idea about diabetes pathophysiology pivoted around the concept known as the triumvirate.[99] This concept stems from the basic knowledge of glucose homeostasis. Under normal conditions, after ingestion of a meal, the pancreas is primed to secrete insulin in response to hyperglycemia. The subsequent physiological hyperinsulinemia promotes uptake of glucose by the liver and muscle tissues and inhibits hepatic glucose synthesis. Therefore, the three pillars of glucose homeostasis are beta cell, liver, and muscle, hence the triumvirate model. An impairment at any of these levels can cause overt diabetes.[99] In real life, it was common to categorize diabetic patients based on these criteria. For example, individuals showing beta-cell dysfunction as causative of diabetes were typically the lean diabetic patients, while individuals whose diabetes started because of a reduced peripheral insulin sensitivity were typically the obese patients. Over the past 30 years, this vision of the pathogenesis of diabetes appeared reductive, and the triumvirate became the ominous octet.[100] Beta cells, liver, and muscle were joined by adipocytes, the gastrointestinal tract, alpha cells, the kidney, and the brain. Each of these can play an important role in the pathogenesis of diabetes.

Adipocytes are the major site of fatty-acid storage (as triglycerides) in the body. They are very efficient in their storage capacity in response to insulin, as long as the physiological conditions are normal. Obesity creates an imbalance where adipocytes enlarge and become insensitive to the antilipolytic effect of insulin. The excess of free fatty acids will, in turn, impair the ability of peripheral tissues to respond to insulin, and over time will promote beta-cell apoptosis.[101] Aside from its mechanical role, the gastrointestinal tract can be considered as an endocrine structure responsible for the synthesis and secretion of at least two key hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). GLP-1 is secreted by the L-cells of the distal small and large intestine, while GIP is secreted by the K-cells located in the early small intestine.[102] Both GLP-1 and GIP are at the forefront of insulin secretion, and collectively they are responsible for >70% of the insulin secreted after a meal. In overt diabetes, GLP-1 and GIP secretion are normal, but beta cells are insensitive to their action.[103] Like the gastrointestinal tract, the kidney plays a role in the regulation of metabolism. In physiological conditions, after an overnight fast, the kidney is responsible for ~20% of glucose production. This process is tightly negatively regulated by insulin itself. As in other peripheral tissues, the kidney becomes resistant to the effect of insulin in the case of diabetes. This loss of control increases the already present hyperglycemia, contributing to the severity of diabetes.[104]

The role of the brain in the pathogenesis of diabetes is not immediately evident, but not necessarily less important. Along with its direct effect on

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

glucose uptake, insulin also has a potent effect on reducing food intake, at the level of hypothalamus. In the presence of obesity, which often accompanies diabetes, the hypothalamus becomes resistant to the anorectic effect of insulin, leading to an increase in food intake and contributing ultimately to the demise of beta cells.[105] Finally, alpha cells are rightly considered the counterpart of beta cells in the pancreatic islet. Alpha cells are responsible for the synthesis and secretion of glucagon, which is responsible for counteracting the effects of insulin and for inhibiting insulin secretion. In diabetic patients, glucagon remains elevated, even in presence of hyperglycemia.[106] Furthermore, elevated glucagon levels will prime the liver to start gluconeogenesis, contributing to hyperglycemia.

The pancreas is a mix of digestive enzyme-producing exocrine and hormone-producing islets. Both exocrine and endocrine compartments of the pancreas are essential for the homeostatic function. The major cells in the exocrine cells are acinar cells and ductal cells. Here, digestive enzymes produced in the acinar cells are drained *via* ducts into the duodenum. Pancreatic islets consist of four major endocrine cell types; beta cells, alpha cells, delta cells, and PP cells, which secrete insulin, glucagon, somatostatin, and pancreatic polypeptide hormone, respectively. Insulin and glucagon are major glucose-regulating hormones. Glucagon has a direct effect on the liver and stimulates gluconeogenesis under hypoglycemic conditions. The beta cells are the only cell types in the body to synthesize, process, store, and release insulin in response to glucose. Impaired or diminished insulin secretion leads to the development of impaired glucose tolerance and subsequently fasting hyperglycemia. Thus, insulin deficiency and fasting hyperglycemia are clinical features of diabetes and are related to low beta-cell mass, beta-cell dysfunction, and/or death. Thus, finding small molecules that impact on these phenotypes could have a major impact on diabetes understanding and treatment.

Diabetes is classified based on the clinical presentation, and there is considerable variation in disease progression due to its underlying etiology. The American Diabetes Association has classified diabetes into four categories: (1) type 1 diabetes (T1D), (2) type 2 diabetes (T2D), (3) gestational diabetes, and (4) specific types of diabetes due to other causes.[107] This last category includes neonatal diabetes, monogenic diabetes, disease relating to the exocrine pancreas (cystic fibrosis and pancreatitis), and chemical-induced diabetes, for example due to the use of glucocorticoids. T1D and T2D are the predominant diabetes types and are polygenic diseases; despite the baseline genetic risk, the numbers of individuals with T1D and T2D are rising due to changes in the environment and lifestyles.[108]

1.6.1 Type 1 Diabetes (T1D)

In type 1 diabetes, insulin deficiency is absolute, and it occurs as a result of autoimmune-mediated destruction of beta cells. Exogenous administration of insulin or insulin analogs is the only current therapy available for

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

individuals with T1D. Several dogmas exist for the etiology and development of T1D.[109] For example, islet-intrinsic mechanisms[110] have been proposed as important to the development of T1D. Beta-cell stress and inflammatory cytokines trigger the generation of reactive oxygen species and nitrosative species, which lead to beta-cell dysfunction, attraction of immune cells, and beta-cell death. Islet-extrinsic mechanisms are associated with defective exocrine pancreatic function[111] and dysregulation in autoimmune factors.

The autoimmune basis of beta-cell death has gained much favor. As per this theory, naïve autoreactive T cells resident in the islet or islet lymph nodes are activated by beta cell-derived peptides. Autoreactive T cells transform B cells into plasma cells, which subsequently generate antibodies against one or more epitopes targeting beta cells. The predominant epitopes are insulin itself, glutamic acid decarboxylase (GAD65), islet antigen 2 (IA-2), and zinc transporter 8 (ZnT8, encoded by *SLC30A8*). Naïve autoreactive T cells also activate other T cells and B cells, which subsequently migrate to the islets – in a process known as insulitis – and release proinflammatory cytokines, which further increase beta-cell cytokine stress and subsequent killing by cytotoxic T cells.

Our early knowledge about the etiology of type 1 diabetes was enhanced by the use of the non-obese diabetic (NOD) mouse model, as well as immune profiling, and isolation of beta cells, islets, and pancreas from T1D individuals. It is interesting to note that recent studies indicate that beta-cell intrinsic mechanisms leading to overexpression of HLA1 and autoantigen presentation are likely involved in the recruitment and activation of CD4+ and CD8+ T cells.[110] While beta cell HLA1 overexpression is an important trigger,[112] a defining cause of T1D onset remains elusive.[113,114] Beta cells can express class II MHC molecules which are sufficient to interact with the circulating CD4+ T cells in islets at the onset of T1D.[115]

T1D has been defined as a polygenic disease, with underlying genetic risk factors (*e.g.*, *INS*), genetic linkages (to HLA1), and the presence of circulating autoantibodies (aAb) against beta-cell antigens.[116,117] However, several other hypotheses have been suggested to explain the increased incidence of T1D globally, including the decline of infectious diseases – the hygiene hypothesis[118] – and vitamin D deficiency.[119] Accumulating evidence indicates that loss of functionally mature beta cells precedes and may contribute to insulitis and the demise of beta cells themselves. For example, aberrant proinsulin processing has been observed in new-onset (<2 years) T1D,[120] and the presence of proinsulin-positive beta cells has been observed in long-standing T1D pancreata.[121] The acquisition of a senescence-associated secretory phenotype by beta cells[122] and activation of the HIF1a/PFKFB3 pathway[123] also argue for an early beta-cell dysfunction preceding the onset of T1D. Thus, protection of mature beta-cell function at the onset of, or during early stages of T1D, is an attractive therapeutic strategy for T1D. While previous studies have confirmed that a T1D-like cytokine storm can induce profound beta-cell stress, followed by autoimmune-mediated cell death, the auto-activation of oxidative stress and interferon signaling in beta

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

cells *per se* is thought to provoke an antiviral response and trigger beta-cell death.[124]

Because T1D is considered an autoimmune-mediated irreversible loss of beta cells, efforts to treat T1D have been limited to modalities such as exogenous insulin, islet transplantation, or restoration of normal immune function. However, genome-wide association studies have shown that >50% of genes near loci associated with T1D are expressed in beta cells, indicating that beta cells can play an active role in the development of T1D.[125] Indeed, in prediabetic NOD mouse models, beta cells display activation of NF-κB along with morphologically stressed ER, and defective insulin secretion as revealed by elevation of the proinsulin:insulin ratio.[126] Targeting the ABL-Ire1a signaling pathway protected beta cells from ER stress, preserved beta-cell mass, and reversed autoimmune diabetes in NOD mice.[127] Additional studies in NOD mice showed that beta cell-specific deletion of Ire1a induced beta-cell dedifferentiation and reduced islet immune cell infiltration, due to diminished levels of MHC class I antigen and beta-cell autoantigens.[128] Consistent with this finding, recent studies observed that beta cells adopt a senescence-associated secretory phenotype in NOD mice, which, when eliminated, can reduce immune infiltration and protect the remaining healthy beta cells.[122] Interestingly, NOD mice can be protected from autoimmune diabetes by small-molecule inhibition of the JAK1/JAK2 pathway[129] or with the ER chaperone TUDCA.[130] Future work will be needed to perform high-throughput screening to discover small molecules that ensure the functional maturity and health of beta cells.

1.6.2 Type 2 Diabetes (T2D)

Like T1D, type 2 diabetes has a strong polygenic component that increases baseline risk. T2D is characterized by insulin resistance and impaired glucose-stimulated insulin secretion (GSIS), compromised pulsatility of insulin secretion, and amyloid deposits in the islet. Interestingly, many genetic loci linked with T2D are near known transcription factors or proteins involved in beta-cell development, maturation, or function. Despite this finding, the global trend in diabetes is increasing and strongly associated with obesity. In this case, beta cells compensate for increased peripheral insulin resistance by increasing insulin secretion, but prolonged and sustained insulin resistance increases the stress on beta cells, due to the increased need for insulin synthesis, trafficking, and secretion. As a result, beta-cell function deteriorates and fails to produce enough insulin to overcome insulin resistance, leading to the development of diabetes.[131]

Glucose and free fatty acids can directly induce insulin secretion. However, sustained and uncontrolled hyperglycemia, combined with elevated circulating levels of free saturated fatty acids (C16 or greater),[132] exhaust beta cells, resulting in impaired insulin secretion (beta-cell dysfunction) and beta-cell death. These effects of glucose and lipids in beta cells have been termed glucotoxicity,[133] lipotoxicity, or glucolipotoxicity. An alternative

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

hypothesis has been posited to explain the growing number of diabetes cases in countries like India and China. Insulin is an important fetal growth factor, and the fetal insulin hypothesis suggests that impaired insulin secretion or increased insulin resistance results in low birth weights and contributes to impaired glucose tolerance, diabetes, and other cardiovascular complications.[134] Low birth size and subsequent obesity, which impacts on fatty acid and proinflammatory cytokines levels, can dramatically increase peripheral insulin resistance and the development of diabetes.[135] Together, there is good evidence that targeting the beta cell could be an important component to developing the future of diabetes therapeutics.

1.6.3 Modeling the Beta Cell for Small-molecule Discovery

1.6.3.1 Primary Human Islets

The availability of a good cellular model is critical for small-molecule discovery. Such models, however, have been difficult to obtain in the lab. Primary islets, isolated from the pancreata of cadaveric human donors, are the gold standard for *in vitro* beta-cell research, because any candidate hit from small-molecule screening may be more directly relevant to T1D or T2D. However, primary islets are fragile and difficult to culture for long periods of time, and in many parts of the world are not obtainable. Further, the quality of islet preparations has a high level of variation due to donor variability as well as potential differences in islet isolation procedures across clinical centers. Our group has developed an islet culture system suitable for high-throughput screening.[136,137] In our experience, using either dissociated human islet cells or small clusters has advanced our discovery activities.

1.6.3.2 Stem Cell-derived Beta Cells

Stem cell-derived islet-like aggregates are an attractive tool for screening a wide variety of compounds to study and/or improve beta-cell maturation, prevention of beta-cell degradation, and insulin secretion. In addition, pluripotent stem cell-derived beta cells offer expandability, allow genome editing, and enable transplantation in animal models of diabetes. Currently, several standard or reporter embryonic stem cell lines, with inherent genomic variability, are available in the islet research field.[138] On the contrary, induced pluripotent stem cells offer more flexibility in using patient-derived cells to derive beta cells that may more faithfully mimic the specific diabetes biology for drug discovery.[139]

1.6.3.3 Human Beta Cell Line

In stark contrast to the many immortalized cell lines available for cancer biology, it is only recently that an immortalized human beta-cell line (EndoC-βH1)[140] has become an alternative cell source for human beta-cell

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

research. EndoC-βH1 expresses markers similar to native human beta cells, exhibits glucose-induced insulin secretion, and ameliorates chemical-induced diabetes *in vivo*. Human pancreatic buds were transduced with lentivirus encoding SV40 gene under the control of the insulin promoter and transplanted into SCID mice for fetal beta-cell maturation and subsequent generation of beta-cell teratomas. These cells were then transduced with lentivirus encoding human telomerase reverse transcriptase (hTERT) and re-transplanted into the SCID mouse for further expansion to generate *in vitro* cell lines. The insulin content and secretion were further improved in EndoC-βH2, a second-generation cell line where immortalizing transgenes were removed with a Cre expression system.[141] Electrophysiological processes, insulin exocytosis, and ultrastructural morphology of insulin granules of both EndoC-βH1 and EndoC-βH2 were similar to primary human beta cells.[142] EndoC-βH3, a third-generation cell line, enables excision of immortalizing transgenes using tamoxifen.[143] These human beta-cell lines offer expandability, which is necessary for screening, glucose-stimulated insulin secretion, and have a relatively similar genome to primary beta cells. The main limitation of these lines has been the challenge of culturing them in most labs; the cell lines grow quite slowly and require expensive specialty reagents. Overall, these cell lines are a very valuable and complementary resource for small-molecule and genetic discovery.

1.6.3.4 Insulinoma Cell Lines

Rodent insulinoma cell lines, such as INS-1E (selected through subcloning of original INS-1 cells),[144,145] INS-1 832/13 (derived by stable transfection with the human proinsulin gene),[146] MIN6,[147,148] and βTC6[149] have insulin secretion capacity similar to rodent islets, can respond to diabetogenic stress, and are cheaper alternatives for small-molecule screening. Careful consideration should be made, however, of the phenotype being measured. Rodent insulinoma lines are not good models for beta-cell proliferation, as there are vast differences between rodent and human beta cells in this process. Apoptosis and insulin secretion, on the other hand, are two phenotypes that are well-suited to the use of rodent cell lines. Indeed, we used the INS-1E cell line to generate a model for luminescence-based insulin secretion and high-throughput screening.[150] To achieve this, we inserted *Gaussia* luciferase into the C-peptide portion of proinsulin, allowing equimolar co-secretion of both insulin and luciferase, as we will discuss below. Together, the use of appropriate assay readouts (Table 1.1) on physiologically relevant cell models is an important component of chemical discovery in the beta cell.

1.7 Therapeutic Approaches in the Beta Cell

From this brief overview, it is clear that diabetes is a very complex disease with systemic complications. The landscape is further complicated by the fact that diabetes is a progressive disease that may require years to become overt. At the

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

Table 1.1 The assay models and readouts used for small-molecule discovery, including through high-throughput screening.

Model		Phenotype		Assay
T1D	One or more inflammatory cytokines (TNF-α, IFNα, IFNγ, IL-1β) or poly I:C	Regeneration	Beta cell mass/ replication	EdU, CldU, Ki67, PCNA, pH3
			Transdifferentiation (non-beta stem cells)/maturity	*PDX1, UCN3, FoxO1, MafA, NKX6.1*
		Protection		HLA1, HLA2, b2M, cleaved caspase-3, ATP, Annexin V, propidium iodide
		Health and function		ATP content, proinsulin: insulin ratio, GSIS
T2D	Glucotoxicity, lipotoxicity, glucolipotoxicity	Insulin secretion		Insulin ELISA, luciferase assay
		Beta-cell rest		Insulin ELISA, membrane potential
		Amyloid clearance		Thioflavin S
		Beta-cell protection		Cleaved caspase-3, ATP, Annexin V, propidium iodide
		Dedifferentiation/ maturity		*PDX1, UCN3, FoxO1, MafA, NKX6.1*

time of diagnosis (especially with T1D), the progression of the disease is already at an advanced state of beta-cell loss and dysfunction. Historically, the majority of therapeutic approaches have been aimed at lowering blood glucose by focusing on peripheral tissues (Figure 1.1). However, a few therapeutic strategies have focused on insulin secretion itself. An interesting summary of various chemical biology approaches applicable to the study of beta-cell function has been recently reported,[151] including a very thorough perspective of the chemical probes available to study islet function. Here, we will start with a description of current clinical strategies and follow that with a description of research approaches focused on the beta cell.

1.7.1 First-generation Therapeutics

1.7.1.1 Insulin Secretagogues

The pharmacological induction of insulin secretion has historically been obtained more with the use of a sledgehammer rather than a scalpel.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

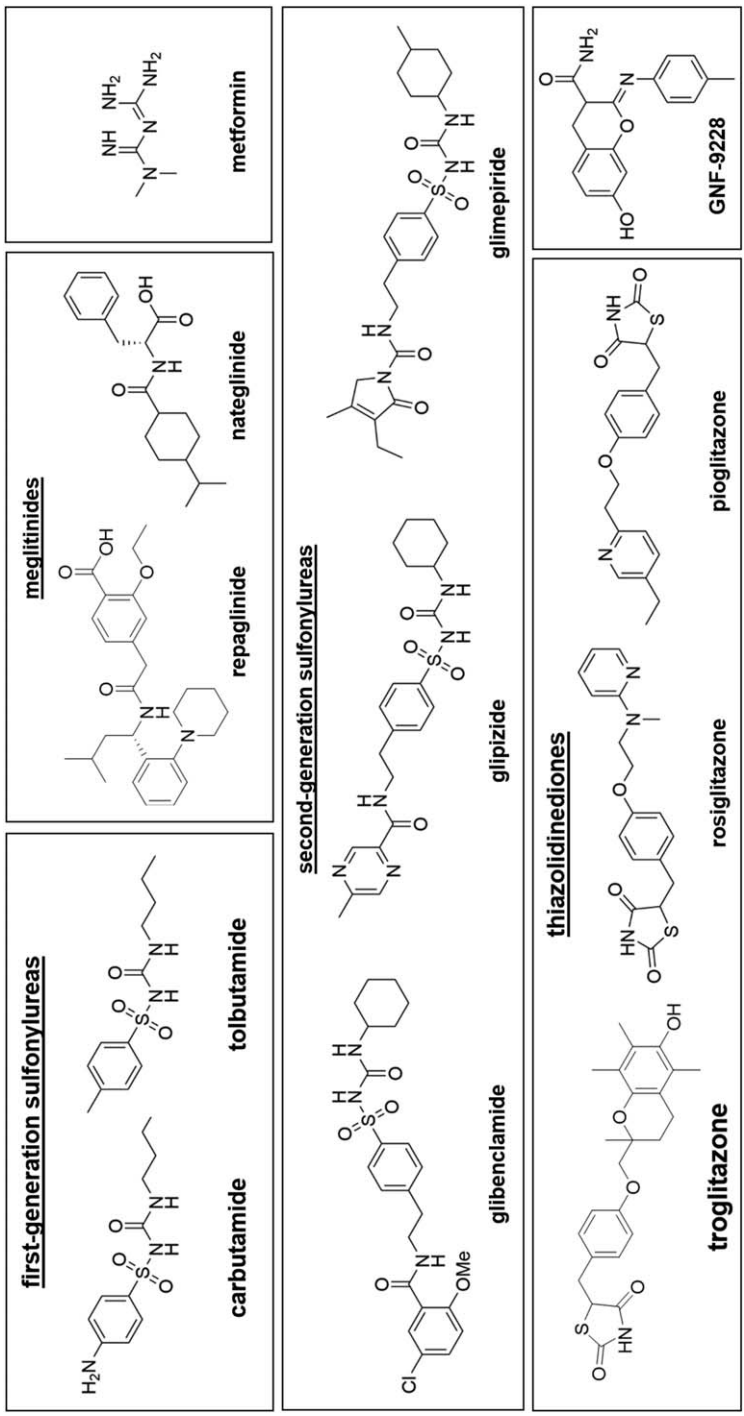

Figure 1.1 Chemical structures of current clinically used drugs for type 2 diabetes.

Therapeutic approaches promoting insulin secretion were (and still are today) based on sulfonylureas. In this process, insulin secretion from beta cells is induced by the increase in blood glucose. Oxidation of glucose drives an increase of ATP levels in the beta cell that in turn induces a closure of the ATP-sensitive potassium channel (K_{ATP}) and subsequent depolarization of cell membrane. This, in turn, induces calcium entry through L-type calcium channels and insulin secretion.[152] The sulfonylureas act by closing the K_{ATP} channel to induce insulin secretion.[153] The first generation of sulfonylureas is represented by tolbutamide and its analog carbutamide, introduced in the mid-1950s.[154] They are still available for prescription, but are no longer considered first-line therapeutics, due to side effects such as hypoglycemia, weight gain, hypersensitivity, and cardiovascular risks. Considering the high therapeutic value of sulfonylureas, much progress has been made to develop second-generation drugs that include, among others, glibenclamide, glipizide, and glimepiride. These second-generation sulfonylurea drugs were introduced around 1980. They are still widely prescribed although not as a first therapeutic approach. Second-generation sulfonylureas have increased potency but also suffer from side-effect profiles similar to the first-generation therapeutics, along with increased hepatotoxicity events.[155] Although sulfonylureas have been extensively used over many decades, their major liability is hypoglycemia. This effect is not surprising, since their mechanism of action is independent of both blood glucose levels and of glucose sensing by beta cells. The development of direct but glucose-dependent insulin secretagogues could have an important impact on treatment in the future, and as we will see below, the GLP-1-based incretin mimetics have an impact on this pathway.

Another class of insulin secretagogue is the meglitinides. Similar to the sulfonylureas, they act by blocking the K_{ATP} channel, but since they are short-acting, it is believed that they promote the first phase of insulin secretion. Physiologically, in response to glucose, beta cells secrete insulin in a biphasic mode. There is a first rapid secretion phase that occurs within minutes, and a second phase that occurs over a few hours.[156] Liver is more sensitive to the first phase of insulin secretion and in diabetes there is a marked loss of the first phase of insulin secretion.[157] Therefore, the idea behind the development of the meglitinides is that restoring the first phase of insulin secretion may create a therapeutic opportunity. In the clinic, the meglitinides appear to be efficacious both as monotherapy or in combination, and due to their fast-acting properties they are less prone to induce hypoglycemia.

1.7.1.2 Insulin Sensitizers

A second dimension of insulin-centric therapeutics is drugs that increase the insulin sensitivity of peripheral tissues. The archetype of this category is metformin. From a drug discovery point of view, metformin is quite unusual. Metformin is very poorly absorbed by the gut and therefore requires elevated

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

doses to reach plasma efficacy (about 500–2500 mg per day), it is not metabolized, and it is excreted intact, with a half-life of about 5 hours.[158] Metformin was introduced as a medication in the United States in 1995. The anti-diabetic effects of metformin mainly work by increasing the sensitivity of tissues to insulin and by inhibiting hepatic gluconeogenesis.[159,160] Metformin is the most widely prescribed anti-diabetic drug, often as first-line therapy.[161] Metformin is well tolerated, can promote weight loss,[162] and does not show significant side effects.[158] Despite its intense use, the molecular mechanism of metformin remains elusive, although some reports indicate that metformin may indirectly promote activation of AMPK.[163]

In the same category of insulin sensitizers are the thiazolidinediones (troglitazone, rosiglitazone, and pioglitazone). Approved in the late 1990s, they work mainly by sensitizing adipose tissue to the effects of insulin. Thiazolidinediones activate PPARγ.[164] The PPAR nuclear receptor family is known to be involved in the regulation of fatty acid metabolism.[165] Not all thiazolidinediones were created equal, though. While troglitazone was efficacious as monotherapy or in combination with sulfonylureas or metformin, it showed clear signs of hepatoxicity[166] and was withdrawn from use in the United States in 2000. In terms of efficacy, both rosiglitazone and pioglitazone are comparable to troglitazone, and while less hepatotoxic, they showed a tendency to induce an increase in body weight.[167] Although quite efficacious and less hepatotoxic, the fate of rosiglitazone followed that of troglitazone. Rosiglitazone was withdrawn from the US market in 2007 after a meta-analysis suggested it may be associated with an increase in heart failure.[168]

1.7.2 Second-generation Therapeutics

This overview might appear to provide a gloomy vision of drug discovery for anti-diabetic drugs. Cardiovascular complications are strongly associated with diabetes, and they are probably the major cause of mortality. Having on the market drugs that improve glycemic control but that may cause cardiovascular complications became a significant concern to the FDA. Based on these concerns, the FDA issued guidelines in 2008 requiring that any novel anti-diabetic agent, regardless of the mechanism of action, should bring evidence showing that a new drug, if not actually beneficial for cardiovascular effects, should at least be neutral to cardiovascular risks (https://www.fda.gov/media/71297/download). These guidelines set the bar quite high, probably limiting the investment, in terms of funding and effort, that drug manufacturers were willing to dedicate for anti-diabetic research.

1.7.2.1 GLP-1 Receptor Agonists

Despite this outlook, a significant paradigm shift in the development of a novel anti-diabetic therapeutic arose from the discovery of the incretin hormones GIP and GLP-1.[169] Incretin hormones play an important role in promoting insulin secretion after ingestion of a meal.[102] GLP-1 stimulated

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

particular interest due to its pleiotropic effects in the body. GLP-1 induces insulin secretion after oral ingestion of glucose and reduces glucagon secretion and glycogenolysis.[170] The GLP-1 receptor (GLP-1R) is a member of the class B of G-protein coupled receptors (GPCR). From a structural point of view, class B GPCRs are characterized by an N-terminal extracellular domain and a transmembrane domain that contain the GPCR motif (seven membrane-spanning α-helices). Signaling from class B GPCRs is mediated mainly by adenylate cyclase to increase the levels of intracellular cAMP.[171]

Class B GPCRs are famously resistant to drug discovery, which has hampered the identification of agonists; this has been true for GLP-1R as well. Efforts to identify small-molecule GLP-1R agonists in a drug-discovery setting were developed but have been slow to yield successes. There are numerous examples of small-molecule GLP-1R agonists reported in the literature,[172,173] but they have not yet reached clinical application. Nonetheless, intense efforts have been directed at developing biologic drugs based on the incretin biology. As a result, the landscape of GLP-1 analogs is quite impressive, with the introduction of first-in-class biologics such as exenatide and liraglutide. Both are GLP-1 analogs that have been engineered to have an extended half-life (2.5 hours, *vs.* 5 minutes for native GLP-1).[174] Aside from their ability to control glycemia, GLP-1 analogs are also showing some unexpected beneficial side effects, such as reduction of body weight and long-term cardiovascular benefits.

One of the basic concepts in the pathogenesis of diabetes is the failure of beta cells to accommodate increased insulin demand. The progressive decline in beta-cell function is accompanied by a loss of mass likely due to increased apoptosis. From a therapeutics point of view, it is emerging that inducing insulin secretion may be insufficient to prevent beta-cell exhaustion, and may even be harmful to the beta cell.[175] From these observations emerged the idea of ''beta-cell rest.'' If driving insulin secretion over the long term leads to a deterioration in beta-cell function, then beta-cell rest would likely be beneficial. Therefore, any therapeutic agent that is able to promote both insulin secretion and beta-cell rest may have a beneficial effect in preventing beta-cell exhaustion. Currently, GLP-1R agonists seem to meet these criteria.

1.7.2.2 Sodium-glucose Co-transporter Inhibitors

Another novel class of anti-diabetic drugs with exceptional promise is the class of sodium-glucose co-transporter 2 (SGLT2) inhibitors. Under physiological conditions, excess plasma glucose is filtered through the renal glomerulus. From here, it is reabsorbed into the blood *via* the sodium-glucose co-transporter SGLT2.[176] The idea behind SGLT2 inhibitors is that blocking the re-absorption of glucose may induce glucosuria, an increase of excreted glucose in the urine. The story of the discovery of SGLT2 inhibitors is a ''poster child'' for chemical genetics, as defined in the introduction to this chapter. First, an ''experiment of nature'' showed that inhibiting SGLT2 induced glucosuria. It had been shown that autosomal recessive mutations

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

in *SLC5A2* (the gene encoding SGLT2) are responsible for familial renal glucosuria. Even in its more extreme instances (complete absence of tubular glucose resorption), clinical manifestations are rare, and this condition is currently accepted to be relatively benign.[177] Second, phlorizin, a natural compound from the bark of pear, apple, and cherry trees and known to science since the late 1800s, was found to induce glucosuria and reduce plasma glucose. Mechanism-of-action studies showed that phlorizin acts as a competitive inhibitor of SGLT2.[178] As it stands, phlorizin lacks the properties of an ideal drug (*i.e.*, it has poor absorbance and low selectivity), but it provided an excellent starting point for a medicinal chemistry campaign. Over time, more successful derivatives of phlorizin were developed, such as canagliflozin, dapagliflozin, and empagliflozin, which reached the market around 2015.[178] Importantly, SGLT2 inhibitors have shown dramatic beneficial cardiovascular outcomes, including a reduced incidence of cardiovascular death and heart failure hospitalizations in people with and without diabetes, and those with and without prevalent heart failure.[179] These findings may lead to even wider clinical use of SGLT2 inhibitors and raise the bar even higher for the benefit that must be shown by novel therapeutic approaches.

1.7.3 New Therapeutic Approaches

The attention of the pharmaceutical industry has increasingly turned to the academic world to help de-risk novel potential targets for developing anti-diabetic agents. There are many examples of possible new avenues for the treatment of diabetes (Figure 1.2). Here, we will focus on beta cell-centered approaches, for which there are at least four major avenues being pursued: (1) glucose-dependent insulin secretion, (2) beta-cell proliferation, (3) beta-cell protection, and (4) beta-cell transdifferentiation.

1.7.3.1 Glucose-dependent Insulin Secretagogues

As we discussed above, GLP-1 analogs show glucose-dependent insulin secretion. In this competitive field, there are some novel opportunities that have been so far under-investigated, such as the quest for non-GLP-1R-agonist glucose-dependent insulin secretagogues. Surprisingly enough, there are limited data reported in the literature about screening campaigns to identify glucose-dependent insulin secretagogues.[180] In one instance, screening was performed in a low-throughput format, using insulin ELISA as readout and a collection of about 1200 small molecules from the Sigma library of pharmacologically active compounds. This effort identified several dopamine receptor antagonists that significantly enhanced glucose-dependent insulin secretion in rat islets.[180] Subsequent siRNA studies indicated that knockdown of *DRD2* (encoding dopamine receptor D2) had the same effect. However, these observations are in contrast with a body of literature showing that both pharmacological or genetic inactivation of *DRD2* impairs insulin secretion and causes glucose intolerance.[181,182]

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

Figure 1.2 Chemical structures of small molecules emerging in new therapeutic approaches to type 1 and type 2 diabetes.

In recent years, our group has performed high-throughput phenotypic screening to identify glucose-dependent insulin secretagogues. First, as mentioned above, we developed a high-throughput luminescent reporter-based system.[150] This system is based on a construct in which the sequence of *Gaussia* luciferase is placed within the C-peptide portion of mouse proinsulin, a fragment that is normally cleaved within secretory vesicles by pH-sensitive prohormone convertase enzymes and co-secreted with mature insulin during exocytosis. With this construct, luciferase tracks to the vesicles but is kept in an inactive state until cleavage of the peptide occurs by PC2 in the acidified vesicle. The freed luciferase is then co-secreted with endogenous mature insulin exocytosis. Using a lentiviral vector, we created a stable integration of the construct within the INS-1E beta cell line. After stimulation with glucose or a secretagogue, luciferase activity is measured on a plate reader in the supernatant using the luciferase substrate coelenterazine. Using this system, we performed high-throughput screening of over 380 000 compounds from the NIH Molecular Libraries Probe Production Centers Network and identified a small molecule that induces insulin secretion in rat INS-1E cells and human islets in a glucose-dependent manner, with an EC50 of ~1–2 μM. We also found that the compound strongly increases insulin secretion in islets from islet donors who had T2D, strengthening the notion that this compound could effectively modify human disease. These results indicate that novel glucose-dependent insulin secretagogues can be identified by high-throughput screening, and future work to determine the mechanism of action may have an impact on small-molecule discovery in this area.

1.7.3.2 Beta-cell Proliferation

Loss of beta-cell number and function is a key landmark of the pathology of diabetes.[183] Therefore, ways to expand pancreatic beta-cell mass and function could be an effective therapeutic approach. This goal is based on the premise that type 1 diabetes patients with long-standing disease exhibit residual beta-cell mass.[184–186] Small molecule-induced beta-cell proliferation has long been sought after, and there are some interesting examples in the literature.[187–189] Among the most notable examples are the discovery of dual-specificity tyrosine-regulated kinase1a (DYRK1A) inhibitors harmine and 5-iodotubercidine (5-IT) as potent inducers of human beta-cell proliferation,[190–192] including a series of aminopyrazines that were developed to increase potency in promoting human beta-cell proliferation.[192] Interestingly, this class of compounds showed inhibitory effects not only toward DYRK1A but also to GSK3β. Although GSK3β is a well-known target of beta-cell (but also general cell) proliferation,[193,194] it emerged that the simultaneous inhibition of DYRK1A and GSK3β proved more effective in inducing beta-cell proliferation than inhibition of either alone.[192] Additional studies on DYRK1A have shown that simultaneous inhibition of DYRK1A and DYRK1B is necessary for activity;[195] as it turns out, most current

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

chemical matter inhibits both kinases. These results indicate that polypharmacology may be a superior avenue to induce beta-cell proliferation.

In line with these observations, a recent report showed that the simultaneous pharmacological inhibition of DYRK1A and TGFβ[196] and DYRK1A and GLP-1R agonists[197] induces synergistic beta-cell proliferation in human cells, as inhibition of the latter also promotes beta-cell proliferation.[198] Although important as a proof of concept, targeting DYRK1A may have some disadvantages from a pharmacological point of view. First, DYRK1A has a broad tissue expression; therefore, any compound targeting it may induce undesirable proliferation events in peripheral tissues. Second, just about all DYRK1A inhibitors so far described are limited by a lack of specificity. In fact, both 5-IT and harmine target not only DYRK1A but also a subset of other kinases, comprising DYRK1B, DYRK2, DYRK3, DYRK4, CLK1, CLK2, CLK3, and CLK4.[190] Inhibiting these kinases may have as-yet unknown effects in humans.

Overall, though, these discoveries are paving the way to identify compounds with novel mechanisms of action with high selectivity for beta cells. For example, recently, GNF-9228 was discovered to promote DYRK1A-independent human beta-cell proliferation.[199] Along this line is the discovery that heterozygous inactivation of the Na^+/Ca^{2+} exchanger (isoform 1; NCX1) increases beta-cell proliferation in mice.[200] Based on these observations, recently the same group reported that chemical inhibition of the same target using phenoxybenzamyl-derivatives small molecules promoted beta-cell proliferation in mice.[201] The use of a more stringent approach resulted in the identification of glucocorticoids as inducers of beta-cell proliferation in rats and humans.[202] In this particular instance, beta cells from adult rats and humans were FACS purified and treated with a collection of 1280 bioactive compounds (LOPAC) for 6 days. At least four compounds were able to induce beta-cell proliferation, and these were glucocorticoid receptor ligands. Among those hits, hydrocortisone and methylprednisolone almost doubled the number of beta cells in 2 weeks. Glucocorticoids, however, induce insulin resistance on their own,[203] but in this case, the authors aimed to expand beta cells *ex vivo* for transplantation, so this systemic effect may not be as much of an issue.

1.7.3.3 Beta-cell Protection

Another approach to deal with the loss of beta-cell mass during diabetes is represented by protection from cell death. This kind of approach must be tailored based on the type of cellular insult imposed on the beta cell. While in T1D, the immune system recognizes the beta cell as foreign, resulting in destruction of the beta-cell population, in T2D, the insulin resistance that precedes overt disease will drive beta cells to exhaustion and ultimately cell death. In the case of immune attack, a model frequently used involves treatment with inflammatory cytokines such as interleukin-1β (IL-1β), interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) produced by macrophages and

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

T cells.[204,205] IL-1β and TNF-α induce NFκB expression, with downstream gene expression regulated by nitric oxide (NO) signaling, which increases endoplasmic reticulum stress-response pathways and decreases beta cell-specific function.[204,206] NFκB activation, along with IFN-γ-induced STAT1 signaling,[207,208] work together to abolish insulin secretion and to induce beta-cell apoptosis.[209] Efforts to inhibit individual cytokines with protein-based receptor antagonists had previously progressed to clinical trials, but did not lead to approved therapies.[210] For these reasons, we used Cas9 to engineer IL-10 to be secreted, along with insulin, beta cells.[211] On the other hand, small-molecule suppressors of cytokine-induced beta-cell apoptosis could lead to entirely new avenues for therapeutic intervention.

Studies have described small molecules, such as extracts from *Artemisia capillaris*[212] and St. John's wort,[213] that have protective effects against cytokines. Many of these natural products were discovered because of their general antioxidant or anti-inflammatory properties, but some of them have shown inhibitory activity toward JAK-STAT[214] or NFκB signaling.[215] Aside from these serendipitous approaches, more systematic ones were described. For example, we reported the identification of BRD0476 from high-throughput screening efforts to discover suppressors of beta-cell apoptosis.[216] BRD0476 inhibits IFN-γ-induced JAK2 and signal transducer and activator of transcription 1 (STAT1) signaling to promote beta-cell survival. Interestingly, BRD0476 does not have kinase inhibitory activity but we found that it works through an unusual mechanism of action by targeting the deubiquitinase ubiquitin-specific peptidase 9X (USP9X). In particular, we found that a competition between phosphorylation and ubiquitination on JAK2 explains the ability of BRD0476 to protect beta cells from death.[37] More recently, *TYK2* (tyrosine kinase 2) has been proposed as a genetic association with T1D,[217] and preclinical evaluation of TYK2 inhibitors for treatment of T1D is underway.[218] In this case, TYK2 activation occurs through IFN-α signaling in the beta cell, and these compounds show great promise.

The fate of the beta cell in overt T2D is no less dramatic. Glucotoxicity, lipotoxicity, and glucolipotoxicity are considered the main driving factors of beta-cell death in T2D. The underlying concept is that once the primary pathogenesis of diabetes is established, hyperglycemia and hyperlipidemia ensue, and thereafter exert additional damaging or toxic effects on the beta cells. In terms of small-molecule discovery, glucolipotoxicity-induced beta-cell death has been relatively neglected. At least two reports of high-throughput screening campaigns have been described.[219,220] The first report focused more on the protection from lipotoxicity. The rat insulinoma cell line INS-1 was exposed to palmitate to induce cell death in the presence of compounds. The collection comprised about 2000 compounds encompassing kinase inhibitors and other bioactive compounds. Hit prioritization and *in silico* optimization ultimately identified MAP4K4 inhibitors and the endocannabinoid anandamide as effective compounds at inhibiting palmitate-induced apoptosis in INS-1 cells as well as in rat and human islets.[219]

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

The second report focused on a more comprehensive approach aimed at protecting beta cells from glucolipotoxicity. Again, the INS-1E cell line was used as a model, and the compound collection included ~300 000 compounds. The readout, Caspase-Glo, was tailored to identify compounds that specifically prevent apoptosis rather than generic cell death. Further hit prioritizations identified six scaffolds that specifically protected beta cells from glucolipotoxicity-induced apoptosis.[220] Following systematic medicinal chemistry efforts, Compound D was obtained as the most potent and selective. Furthermore, mechanism-of-action studies indicated that Compound D works through a reduction of calcium influx, although definitive target proteins were not identified.

In terms of more general protection from beta-cell death, the thioredoxin-interacting protein (TXNIP) has emerged as a promising therapeutic target. TXNIP was found to be the top glucose-induced gene in a human pancreatic islet gene-expression microarray.[221] Its increased expression is associated with diabetes and beta-cell apoptosis, while its absence in beta cells is accompanied by a significant decrease in apoptosis and with an overall protective effect from diabetes. Interrogation of a 300 000-compound library identified SRI-37330 as a non-toxic small molecule, which effectively rescued mice from obesity-induced diabetes.[222] Moreover, SRI-37330 inhibited glucagon secretion, reduced hepatic glucose production, and reversed hepatic steatosis. The continued growth of this area will ensure that the validity of this approach will become more thoroughly vetted.

1.7.3.4 Beta-cell Transdifferentiation

The concept behind the idea of transdifferentiation is the plasticity of cell state. Advances in developing iPS cells have shown that cellular reprogramming no longer belongs to the realm of speculation. Furthermore, in the context of islet biology, many examples of spontaneous reprogramming have been described.[223] These findings support the idea that small-molecule methods may be used to reprogram cells to a beta-like state.

An intuitive starting point for beta-cell transdifferentiation is the alpha cell, since both share a common developmental lineage.[224] There are different examples of successful screening campaigns to identify small molecules promoting alpha- to beta-cell transdifferentiation. One of the first was reported in 2010 when BRD7389 was identified from a phenotypic screen of about 30 000 compounds.[225] From a molecular point of view, BRD7389 was identified as having a strong inhibitory effect on the RSK kinase family. Subsequently, another report showed that chronic treatment with GABA could induce alpha- to beta- transdifferentiation *in vivo*.[226] Similar results were obtained by another group, who also identified artemisinins as potentiators of GABA signaling by stabilizing gephyrin.[227] Following these reports, an effort rebutting these findings was reported, when lineage-tracing experiments failed to recapitulate the results, putting into question whether these agents may represent novel diabetes therapies.[228]

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

There is a long controversy around the idea that new beta cells may derive not only from replication but also from progenitors or stem cells.[229] In recent years, the scientific community seemed to reach the conclusion that these events are not mutually exclusive. From a therapeutic point of view, this opens more opportunities and chemical biology has taken advantage. A report described high-throughput screening of ~60 000 compounds. The human ductal cell line PANC-1 was used as a model and the readout was expression of *PDX1*, a master regulator of both beta-cell development and insulin expression. This effort identified BRD7552 as an inducer of PDX1 mRNA and protein expression in primary ductal cells. Furthermore, prolonged compound treatment induced both insulin mRNA and protein expression.[230]

1.7.4 Emerging Therapeutic Approaches

We have attempted to provide an overview of the most systematic unbiased approaches underway in four major phenotypic areas of intervention: glucose-dependent insulin secretion, beta-cell proliferation, beta-cell protection, and transdifferentiation. The complexity of beta-cell biology, though, means many more avenues may be open for small-molecule discovery as well. Here, we will provide some of the more recent and more relevant examples of possible therapeutic avenues that stem from basic science.

As described above, one of the most successful anti-diabetic drugs still prescribed is metformin. Although the precise mechanism of action of metformin remains mysterious, a body of evidence seems to indicate that, ultimately, metformin may work by activating AMPK, a key enzyme controlling glucose uptake and metabolism by peripheral tissues.[231] These premises were used to identify the compound O304 as a pan-AMPK activator. O304 is a small heterocyclic compound developed by Betagenon AB through rationale design for AMPK activators from an early hit molecule identified in a cellular screen. O304 showed beneficial effects in increasing glucose uptake, reducing beta-cell stress, and promoting beta-cell rest.[232] Moreover, O304 showed good performance in reducing fasting plasma glucose in patients with T2D and, interestingly, O304 improved peripheral microvascular complications.

Among the major players regarding anti-diabetic therapy, a central role is played by insulin itself. Since its discovery in 1922, insulin remains a life-saving drug for many. Therefore, it is no surprise that many approaches have aimed at improving the performance of endogenous insulin. One of the most notable examples is the targeting of insulin degrading enzyme (IDE), a ubiquitous zinc-metalloprotease responsible for the degradation of insulin, glucagon, and amyloid beta-protein.[233] Again, the proof of concept that IDE may be an interesting target for anti-diabetic drugs arose from the observation that a non-protein inhibitor of IDE extracted from liver was able to increase the effect of insulin *in vivo*.[234] Despite these observations, the discovery of small-molecule inhibitors of IDE proved quite challenging.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

In the first report from 2014, inhibitors were obtained by screening a DNA-templated library of 13 824 synthetic macrocycles.[235] From this screen, six candidate IDE-binding molecules were identified, and among those, the compound 6b had the highest inhibitory effect on IDE. *In vivo* studies showed 6b was able to improve glucose tolerance upon oral glucose administration. 6b, however, also prevented glucagon degradation, which is an undesirable effect for an effective anti-diabetic drug. In a subsequent iteration, the same group described novel small-molecule IDE inhibitors identified in a screen of ~17 000 compounds.[38] The top compound there showed greater efficacy when insulin was the substrate, as opposed to glucagon.

When administered exogenously, as often happens with diabetic patients, insulin is very effective, but it lacks the physiological control of being secreted only in the presence of elevated glucose. Although insulin analogs have improved glycemic control, glycemic excursions still cause major health problems and complications. Many attempts have been made over the years to develop so-called ''smart'' insulins, also known as glucose-responsive insulins (GRIs). The concept of GRIs is not new and was introduced for the first time in 1979.[236] So far, excellent GRIs can be obtained by embedding insulin in polymeric matrices containing glucose-responsive elements (*i.e.*, lectin), glucose oxidase, or phenylboronic acids (PBAs). PBAs are small molecules with high affinity for diols such as sugars. Since glucose is the most abundant sugar in the blood, PBAs are particularly apt to develop GRIs. The most recent progress in the development of GRI has been well described.[237]

1.8 Selectively Targeting the Beta Cell

1.8.1 Need for Beta-cell Targeting Moieties

As we have discussed, an attractive way to combat the reduced beta-cell mass in T1D or T2D is to develop small molecules that induce beta-cell regeneration. There is an abundance of chemical matter targeting DYRK1A, which is a kinase that, when inhibited, increases beta-cell proliferation. Efforts in the past several years of beta-cell biology have led to the development of leucettine, harmine, GNF4877, 5-iodotubercidin (5-IT), CC-401, and others.[190,191,238–245] Most of these compounds induce only 1–3% of beta cells to proliferate. More recently, DYRK1A inhibitors were combined with TGFβ inhibitors to have a synergistic mechanism of action to proliferate beta cells, resulting in a 5–8% increase.[196] However, DYRK1A and TGFβ receptors are ubiquitous and not specific. Additionally, DYRK1A inhibitors were used in conjunction with GLP-1R agonists and shown to increase beta-cell proliferation to 5–6%, but again, DYRK1A inhibitors are not specific to beta cells. It has been predicted that 10% of proliferating beta cells may be enough to maintain glucose homeostasis.[197] While these combinations increase the efficacy of beta-cell proliferation, they do not address the problem of off-targets and specificity.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

Thus, current beta-cell mitogens have therapeutic limitations because they have a low therapeutic window. Not only do they have low efficiencies in beta cells, but they are also not specific and proliferate other cells as well. Furthermore, the pancreas is composed of a heterogeneous mixture of cells (*e.g.*, alpha, beta, delta). One way to increase the therapeutic window of these compounds is by increasing the localization of the cargo to beta cells, or by creating pro-drugs for the specific release of the cargo in beta cells. In this chapter, we define ''cargo'' as a small molecule-based entity for delivery. Here, we will review compounds that take advantage of the unique features of beta cells. We focus on examples that (1) conjugate cargo to beta-cell surface markers, and (2) create pro-drugs that take advantage of the high levels of zinc in beta cells. Finally, we conclude with workflows to find new beta-cell markers to further increase specificity.

1.8.2 Beta-cell Targeting

There are several relatively specific cell-surface markers that could be used for beta-cell targeting (Figure 1.3). Among them, GLP-1R, VMAT2, dopamine receptor D2, and SUR1 are the most well-studied. One approach for cargo delivery stems from the concept of antibody–drug conjugates: tethering cargo to antibodies that are highly specific to cancer markers on a desired cell type is able to localize the cargo to the cell. Over the past few decades, several groups have developed radiolabeled or fluorescent ligands to help identify and sort beta cells. The logical next step is to conjugate these probes to cargo to specifically target them to beta cells.

A number of probes targeting cell surface markers exist (Table 1.2), including exendin-4, which targets GLP-1R; dihydrotetrabenazine (DTBZ), which targets VMAT2; (+)-4-propyl-9-hydroxynaphthoxazine (PHNO) and fallypride, which

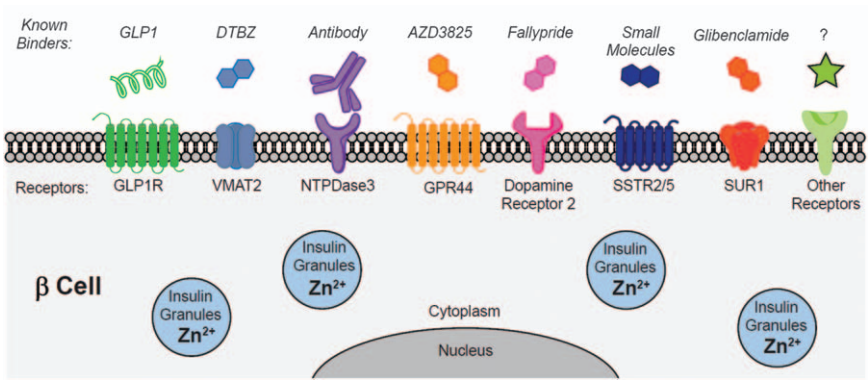

Figure 1.3 Cell schematic of the beta cell, with promising surface markers indicated. The top row lists known binders, and whether they are peptides (green helix), small molecules (double hexagons), or proteins (purple antibody). The bottom row lists the names of the candidate surface receptors.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

Table 1.2 Beta-cell markers that can be leveraged for cell-specific therapeutic delivery. Note that not all markers are surface markers (*e.g.*, zinc).

Marker	Binder	Specificity and possible off-targets	Applications
GLP1 receptor	Peptide GLP-1(7–36)-NH_2	Relatively specific, but can be found in other tissues	Co-delivery of glucagon, amylin, cholecytokinin, gastrin, and nuclear hormones in the form of hybrid peptides, delivery of antisense oligonucleotides, imaging of transplanted islets in humans
VMAT2	DTBZ	Expressed in beta cells and neuroendocrine cells. Not found in rat pancreas	Imaging of endogenous pancreatic beta-cell mass in human, used for delivery of proliferating reagent to mice pancreas with uncleavable linker
Dopamine receptor 2	Fallypride and (+)-4-propyl-9-hydroxynaphthoxazine (PHNO)	Expressed in brain, endocrine tissue, pancreas	[^{18}F]-Fallypride used in imaging of transplanted islets in mice, [^{11}C]-PHNO showed promise in differentiation between T1DM and health subjects
SUR1	Glibenclamide	Pancreatic beta cells and in neurons, astrocytes, oligodendrocytes, endothelial cells	Glibenclamide-based dendrimers were used to label human islets *in vitro*
GPR44	AZD3825	Identified as a beta cell-specific biomarker through a proteomic screen, but its ligand accumulates in small intestines and spleen	Used to stain human pancreas, and *in vivo* in pigs and non-human primates
NTPDase 3	Antibody	Somewhat specific to beta cells, found on delta cells, but not on alpha cells	NTPDase3 antibody enabled sorting of beta cells and *in vivo* imaging
Somatostatin receptor SSTR2/5	Beta-naphthylalanine, several other binders reported	SSTR2 in both alpha and beta cells, SSTR5 expressed in β-cells only	Higher uptake in pancreas demonstrated in mice
LAT	5-Hydroxy-tryptophan, DOPA	Localized to islets, absent in exocrine pancreas. Found in brain, liver, kidney, *etc.*	Estimation of beta-cell mass in humans
GLUT2	Fluorodeoxyglucose	Expressed in liver, endocrine pancreas, and kidneys	Imaging in clinical islet transplantation
VDCC	Manganese-based probes	Ubiquitously expressed in various *cell* types throughout the body	Used to distinguish between non-diabetic and type 2 diabetic patients by MRI
Zinc	Zinc chelators	Functional beta cells have over a million-fold higher zinc ion (Zn^{2+}) concentration than other cell types and plasma	Imaging and sorting of beta cells, more recently, *in vitro* delivery of mitogens

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

target D2; and glibenclamide, which targets the K_{ATP} channel. Radiolabeled probes based on their specific ligands were used for PET imaging of the pancreas in humans. Other markers, such as GRP44, somatostatin receptor SSTR2/5, and NTPDase3, are less studied, but still hold promise. GPR44 was identified as a beta cell-specific marker through a proteomic screen,[246] and its ligand AZD3825 demonstrated ten times higher endocrine-to-exocrine binding ratio than for DTBZ. In contrast to SSTR2, which can be found on the surface of both alpha and beta cells, SSTR5 was shown to be almost exclusively expressed in beta cells.[247] Furthermore, an increased pancreatic uptake of its radiolabeled ligand beta-naphthylalanine was demonstrated in mice.[248] Besides beta-naphthylalanine, several small-molecule binders of SSTR5 were discovered and can be leveraged for the development of beta cell-specific probes.[249–251] Recently, NTPDase3 was identified as a highly specific marker of beta cells.[252] Immunohistochemistry on pancreatic tissue sections from human donors showed high expression of NTPDase3 on the surface of beta cells, some expression on delta cells, but none on the surface of alpha cells. Using NTPDase3 antibody, isolation and *in vivo* imaging of beta cells were performed. In addition to all of these mentioned candidates, voltage-gated calcium channel (VDCC), large neutral amino acid transporter (LAT), and glucose transporter 2 (GLUT2) were reported to be used to image beta cells. For example, the correlation between C-peptide production and ^{11}C-5-hydroxy-tryptophan uptake points to the possibility of LAT exploration in beta cell-targeted delivery.[253]

1.8.2.1 Leveraging GLP-1R for Beta-cell Targeting

As we have discussed, the glucagon-like peptide-1 receptor (GLP-1R) is a GPCR that is highly expressed on beta cells, although there is also some distribution on delta cells, found in the islet, and other cell types.[254] When GLP-1 binds its receptor, it stimulates a cascade that ultimately triggers insulin release. Thus, several peptide-based receptor agonists have been developed to trigger insulin release, but there are practical side-effects to using these in the clinic. More recently, non-peptidic, orally available GLP1-R agonists have been developed, and while clinical development is underway, they can serve now as a potential probe to which to tether cargo.[255] Several groups have already conjugated GLP-1 peptides to cargo and demonstrated their specific delivery to beta cells. These have included ^{125}I-radiolabeled GLP-1 analogs to visualize insulinomas *in vivo*.[256] Following this work, an ^{11}In-labeled exendin analog was developed and used for visualization of beta-cell mass in rats, mice, and recently humans by single photon emission computed tomography.[257–259] Other efforts developed a near-infrared probe based on extendin-4 and VivoTag-680 fluorochrome. Three minutes after intravenous administration of this probe to mice, pancreatic islets were detectable by intravital microscopy and the signal peaked after 20 minutes, with 6 : 1 target-to-background selectivity.[260] Soon after this discovery, the group improved selectivity of imaging up to 64 : 1 using VivoTag-750.[261] Besides applications in beta-cell imaging, GLP-1R was also used for the

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

delivery of therapeutic agents. For example, estrogen conjugated to GLP-1 was used as a targeted therapy to reverse metabolic syndrome in rodents.[262] Further, anti-sense oligonucleotides conjugated to a GLP-1 peptide showed increased uptake in beta cells.[263,264] As an alternative approach, GLP-1 and glucagon co-agonists have been developed, as well as GLP-1 and GIP co-agonists. These examples illustrate how GLP-1R-targeting moieties could be used to deliver active cargo (drugs) to beta cells.

1.8.2.2 Leveraging VMAT2 for Beta-cell Targeting

The first attempt to perform beta cell-specific delivery of mitogens covalently linked the beta-cell proliferating agent aminopyrazine (AP) to the VMAT2 ligand DTBZ.[265] The AP–DTBZ conjugate retained its VMAT2 binding affinity but demonstrated a four-fold reduction in activity to induce proliferation. *In vivo* tissue distribution in Balb/C mice (by LC-MS) demonstrated a promising enrichment of the conjugate in the pancreas, with a tissue-to-plasma ratio of fifteen. However, a significant amount of AP–DTBZ was also found in the liver and kidneys, with tissue-to-plasma ratios of 2.1 and 4.9, respectively. The authors speculated that such low specificity can be attributed to the unsuccessful choice of animal model, since VMAT2 expression in rodent beta cells has been reported to be much lower than in other species. After this publication, there were no follow-up attempts to improve the efficiency of the system or test it in a more suitable model – pig or primate.

1.8.2.3 Leveraging Zinc for Beta-cell Targeting

In addition to cell-surface markers discussed above, beta cells have an incredibly high level of Zn^{2+} ions in their insulin vesicles, with concentrations reaching up to 30 mM in the ''tightly bound'' form and 100 μM in the ''free or loosely bound'' form.[266] By contrast, most other cell types and plasma have no more than 1 nM of Zn^{2+}. Thus, beta cells have over a million-fold higher free Zn^{2+} concentration than other cell types and plasma, allowing Zn^{2+} indicator dyes to be employed in beta-cell sorting and islet transplantation imaging.[267,268] Recently, zinc-based targeting of the beta cell was further extended to specific delivery of proliferation-inducing agents. For example, covalent attachment of the dipicolylamine zinc chelator to the beta-cell mitogen GNF4877 resulted in enhanced accumulation of the hybrid molecule in the beta cell.[269] Treatment of human islets with chelator-modified GNF4877 led to only 1.3-fold selectivity in the induction of proliferation of beta cells *versus* non-beta cells. One of the possible explanations for low specificity is a conjugation-related reduction in the potency of the mitogen. A similar drop in potency (four-fold) was observed when dihydrotetrabenazine (DTBZ) was covalently conjugated to the beta-cell proliferating agent aminopyrazine in the VMAT2-based targeting strategy discussed above.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

The specificity of beta-cell targeting can be further improved with the use of a pro-drug approach, in which an administered compound is converted to an active drug only at the site of action. In this approach, an active molecule can be masked by conjugation with a binder of a beta-cell marker (*e.g.*, Zn^{2+} or VMAT2), which leads to not only the enrichment of drug in beta cells but also unmasking of the active compound following reaction-based activation steps. Zn^{2+} is a powerful Lewis acid, allowing it to be used for the development of highly sensitive and selective reaction-based probes to image Zn^{2+} in tissues.[246,250] In these probes, coordination of the Zn^{2+} ion to its chelator is followed by ligand-directed Zn^{2+}-catalyzed ester/carbamate hydrolysis and release of fluorescent compound. In our proof-of-concept studies, molecules able to release ZP1 and BODIPY selectively in beta cells were designed and applied for beta-cell sorting and islet imaging.[270,271] Furthermore, second-generation conjugates of zinc chelator with proliferating reagents (GNF4877 and harmine) were developed.[271,272] In contrast to the first-generation molecules developed, new hybrid compounds are based on a pro-drug strategy, thus allowing beta cell-specific delivery of unmodified proliferation-inducing agents with preserved potency. There is still much room left for improvement of beta cell-targeting pro-drugs based on the Lewis acidity of zinc, and new assays that could allow rapid screening of known zinc chelators, cargos (mitogens, immunosuppressants, *etc.*), and the nature of chemical bonds between them, need to be developed.

1.8.3 Next Steps Toward Selective Targeting

In addition to the markers mentioned above, there are several underexplored proteins which could be leveraged for the development of beta cell-specific therapies. For example, TMEM27 and its protease BACE2 are enriched in beta cells, and radio- and fluorophore-labeled TMEM27 antibodies were used for beta-cell imaging.[273,274] However, the development of specific small-molecule binders for TMEM27 will be required to increase the therapeutic potential of this target. TAK-875, a binder of another promising target, GRP40, was recently used for the design of novel probes for beta-cell imaging.[275] In the case of zinc-based probes and pro-drugs, specificity can be further improved with the use of novel zinc binders, such as low-affinity hydroxyphenylbenzoxazole or benzimidazole-based ligands.[276] However, despite the incredible potential of these candidate markers, specificity still might be a limiting factor on the way to therapeutic application. Thus far, we have been unable to single out even one marker that does not have off-target expression in pancreas or other tissues. Nevertheless, this limitation can be addressed *via* the careful design of agents with several layers of specificity. For example, one group synthesized a triagonist:GLP-1/GIP/glucagon,[277] and we predict that combinations like these will continue to emerge to compensate for the lack of beta cell-specific therapeutic agents. Furthermore, any of the chemical biology approaches described throughout this chapter (*e.g.*, DELs, PROTACs, CRISPR screening, photo-cross-linking, and target

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

identification) can be used to develop probes or drugs for the understanding and treatment of diabetes.

Abbreviations

CETSA	cellular thermal shift assay
CMap	Connectivity Map
DARTS	drug affinity responsive target stability
DEL	DNA-encoded library
DOS	diversity-oriented synthesis
GIP	glucose-dependent insulinotropic peptide
GLP-1	glucagon-like peptide-1
GPCR	G-protein coupled receptor
GRI	glucose-responsive insulin
GSIS	glucose-stimulated insulin secretion
HTS	high-throughput screening
NOD	non-obese diabetic mouse model
PROTAC	proteolysis targeting chimera
SGLT2	sodium-glucose co-transporter 2
SPROX	stability of proteins from rates of oxidation
T1D	type 1 diabetes
T2D	type 2 diabetes
TPP	thermal proteome profiling
TSA	thermal shift assay
WHO	World Health Organization

References

1. W. H. Herman and P. Zimmet, *Diabetes Care*, 2012, **35**, 943–944.
2. G. Roglic, *Global Report on Diabetes*, World Health Organization, 2016.
3. P. Workman and I. Collins, *Chem. Biol.*, 2010, **17**, 561–577.
4. R. M. Rydzewski, *Real World Drug Discovery: A Chemist's Guide to Biotech and Pharmaceutical Research*, Elsevier, 2008.
5. B. K. Wagner, *Introduction to Chemical Probes*, Royal Socierty of Chemistry, 2021.
6. C. J. Gerry and S. L. Schreiber, *Nat. Rev. Drug Discovery*, 2018, **17**, 333–352.
7. E. S. Lander, L. M. Linton, B. Birren, C. Nusbaum, M. C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, R. Funke, D. Gage, K. Harris, A. Heaford, J. Howland, L. Kann, J. Lehoczky, R. LeVine, P. McEwan, K. McKernan, J. Meldrim, J. P. Mesirov, C. Miranda, W. Morris, J. Naylor, C. Raymond, M. Rosetti, R. Santos, A. Sheridan, C. Sougnez, Y. Stange-Thomann, N. Stojanovic, A. Subramanian, D. Wyman, J. Rogers, J. Sulston, R. Ainscough, S. Beck, D. Bentley, J. Burton, C. Clee, N. Carter, A. Coulson, R. Deadman, P. Deloukas, A. Dunham, I. Dunham, R. Durbin, L. French, D. Grafham, S. Gregory, T. Hubbard, S. Humphray, A. Hunt, M. Jones, C. Lloyd, A. McMurray, L. Matthews, S. Mercer, S. Milne,

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

J. C. Mullikin, A. Mungall, R. Plumb, M. Ross, R. Shownkeen, S. Sims, R. H. Waterston, R. K. Wilson, L. W. Hillier, J. D. McPherson, M. A. Marra, E. R. Mardis, L. A. Fulton, A. T. Chinwalla, K. H. Pepin, W. R. Gish, S. L. Chissoe, M. C. Wendl, K. D. Delehaunty, T. L. Miner, A. Delehaunty, J. B. Kramer, L. L. Cook, R. S. Fulton, D. L. Johnson, P. J. Minx, S. W. Clifton, T. Hawkins, E. Branscomb, P. Predki, P. Richardson, S. Wenning, T. Slezak, N. Doggett, J. F. Cheng, A. Olsen, S. Lucas, C. Elkin, E. Uberbacher, M. Frazier, R. A. Gibbs, D. M. Muzny, S. E. Scherer, J. B. Bouck, E. J. Sodergren, K. C. Worley, C. M. Rives, J. H. Gorrell, M. L. Metzker, S. L. Naylor, R. S. Kucherlapati, D. L. Nelson, G. M. Weinstock, Y. Sakaki, A. Fujiyama, M. Hattori, T. Yada, A. Toyoda, T. Itoh, C. Kawagoe, H. Watanabe, Y. Totoki, T. Taylor, J. Weissenbach, R. Heilig, W. Saurin, F. Artiguenave, P. Brottier, T. Bruls, E. Pelletier, C. Robert, P. Wincker, D. R. Smith, L. Doucette-Stamm, M. Rubenfield, K. Weinstock, H. M. Lee, J. Dubois, A. Rosenthal, M. Platzer, G. Nyakatura, S. Taudien, A. Rump, H. Yang, J. Yu, J. Wang, G. Huang, J. Gu, L. Hood, L. Rowen, A. Madan, S. Qin, R. W. Davis, N. A. Federspiel, A. P. Abola, M. J. Proctor, R. M. Myers, J. Schmutz, M. Dickson, J. Grimwood, D. R. Cox, M. V. Olson, R. Kaul, C. Raymond, N. Shimizu, K. Kawasaki, S. Minoshima, G. A. Evans, M. Athanasiou, R. Schultz, B. A. Roe, F. Chen, H. Pan, J. Ramser, H. Lehrach, R. Reinhardt, W. R. McCombie, M. de la Bastide, N. Dedhia, H. Blocker, K. Hornischer, G. Nordsiek, R. Agarwala, L. Aravind, J. A. Bailey, A. Bateman, S. Batzoglou, E. Birney, P. Bork, D. G. Brown, C. B. Burge, L. Cerutti, H. C. Chen, D. Church, M. Clamp, R. R. Copley, T. Doerks, S. R. Eddy, E. E. Eichler, T. S. Furey, J. Galagan, J. G. Gilbert, C. Harmon, Y. Hayashizaki, D. Haussler, H. Hermjakob, K. Hokamp, W. Jang, L. S. Johnson, T. A. Jones, S. Kasif, A. Kaspryzk, S. Kennedy, W. J. Kent, P. Kitts, E. V. Koonin, I. Korf, D. Kulp, D. Lancet, T. M. Lowe, A. McLysaght, T. Mikkelsen, J. V. Moran, N. Mulder, V. J. Pollara, C. P. Ponting, G. Schuler, J. Schultz, G. Slater, A. F. Smit, E. Stupka, J. Szustakowki, D. Thierry-Mieg, J. Thierry-Mieg, L. Wagner, J. Wallis, R. Wheeler, A. Williams, Y. I. Wolf, K. H. Wolfe, S. P. Yang, R. F. Yeh, F. Collins, M. S. Guyer, J. Peterson, A. Felsenfeld, K. A. Wetterstrand, A. Patrinos, M. J. Morgan, P. de Jong, J. J. Catanese, K. Osoegawa, H. Shizuya, S. Choi, Y. J. Chen, J. Szustakowki and International Human Genome Sequencing Consortium, *Nature*, 2001, **409**, 860–921.

8. J. C. Venter, M. D. Adams, E. W. Myers, P. W. Li, R. J. Mural, G. G. Sutton, H. O. Smith, M. Yandell, C. A. Evans, R. A. Holt, J. D. Gocayne, P. Amanatides, R. M. Ballew, D. H. Huson, J. R. Wortman, Q. Zhang, C. D. Kodira, X. H. Zheng, L. Chen, M. Skupski, G. Subramanian, P. D. Thomas, J. Zhang, G. L. Gabor Miklos, C. Nelson, S. Broder, A. G. Clark, J. Nadeau, V. A. McKusick, N. Zinder, A. J. Levine, R. J. Roberts, M. Simon, C. Slayman, M. Hunkapiller, R. Bolanos, A. Delcher, I. Dew, D. Fasulo, M. Flanigan, L. Florea, A. Halpern, S. Hannenhalli, S. Kravitz, S. Levy, C. Mobarry, K. Reinert, K. Remington, J. Abu-Threideh, E. Beasley,

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

K. Biddick, V. Bonazzi, R. Brandon, M. Cargill, I. Chandramouliswaran, R. Charlab, K. Chaturvedi, Z. Deng, V. Di Francesco, P. Dunn, K. Eilbeck, C. Evangelista, A. E. Gabrielian, W. Gan, W. Ge, F. Gong, Z. Gu, P. Guan, T. J. Heiman, M. E. Higgins, R. R. Ji, Z. Ke, K. A. Ketchum, Z. Lai, Y. Lei, Z. Li, J. Li, Y. Liang, X. Lin, F. Lu, G. V. Merkulov, N. Milshina, H. M. Moore, A. K. Naik, V. A. Narayan, B. Neelam, D. Nusskern, D. B. Rusch, S. Salzberg, W. Shao, B. Shue, J. Sun, Z. Wang, A. Wang, X. Wang, J. Wang, M. Wei, R. Wides, C. Xiao, C. Yan, A. Yao, J. Ye, M. Zhan, W. Zhang, H. Zhang, Q. Zhao, L. Zheng, F. Zhong, W. Zhong, S. Zhu, S. Zhao, D. Gilbert, S. Baumhueter, G. Spier, C. Carter, A. Cravchik, T. Woodage, F. Ali, H. An, A. Awe, D. Baldwin, H. Baden, M. Barnstead, I. Barrow, K. Beeson, D. Busam, A. Carver, A. Center, M. L. Cheng, L. Curry, S. Danaher, L. Davenport, R. Desilets, S. Dietz, K. Dodson, L. Doup, S. Ferriera, N. Garg, A. Gluecksmann, B. Hart, J. Haynes, C. Haynes, C. Heiner, S. Hladun, D. Hostin, J. Houck, T. Howland, C. Ibegwam, J. Johnson, F. Kalush, L. Kline, S. Koduru, A. Love, F. Mann, D. May, S. McCawley, T. McIntosh, I. McMullen, M. Moy, L. Moy, B. Murphy, K. Nelson, C. Pfannkoch, E. Pratts, V. Puri, H. Qureshi, M. Reardon, R. Rodriguez, Y. H. Rogers, D. Romblad, B. Ruhfel, R. Scott, C. Sitter, M. Smallwood, E. Stewart, R. Strong, E. Suh, R. Thomas, N. N. Tint, S. Tse, C. Vech, G. Wang, J. Wetter, S. Williams, M. Williams, S. Windsor, E. Winn-Deen, K. Wolfe, J. Zaveri, K. Zaveri, J. F. Abril, R. Guigo, M. J. Campbell, K. V. Sjolander, B. Karlak, A. Kejariwal, H. Mi, B. Lazareva, T. Hatton, A. Narechania, K. Diemer, A. Muruganujan, N. Guo, S. Sato, V. Bafna, S. Istrail, R. Lippert, R. Schwartz, B. Walenz, S. Yooseph, D. Allen, A. Basu, J. Baxendale, L. Blick, M. Caminha, J. Carnes-Stine, P. Caulk, Y. H. Chiang, M. Coyne, C. Dahlke, A. Mays, M. Dombroski, M. Donnelly, D. Ely, S. Esparham, C. Fosler, H. Gire, S. Glanowski, K. Glasser, A. Glodek, M. Gorokhov, K. Graham, B. Gropman, M. Harris, J. Heil, S. Henderson, J. Hoover, D. Jennings, C. Jordan, J. Jordan, J. Kasha, L. Kagan, C. Kraft, A. Levitsky, M. Lewis, X. Liu, J. Lopez, D. Ma, W. Majoros, J. McDaniel, S. Murphy, M. Newman, T. Nguyen, N. Nguyen, M. Nodell, S. Pan, J. Peck, M. Peterson, W. Rowe, R. Sanders, J. Scott, M. Simpson, T. Smith, A. Sprague, T. Stockwell, R. Turner, E. Venter, M. Wang, M. Wen, D. Wu, M. Wu, A. Xia, A. Zandieh and X. Zhu, *Science*, 2001, **291**, 1304–1351.

9. S. L. Schreiber, *Bioorg. Med. Chem.*, 1998, **6**, 1127–1152.
10. Z. A. Knight and K. M. Shokat, *Cell*, 2007, **128**, 425–430.
11. H. Nagumo, Y. Sasaki, Y. Ono, H. Okamoto, M. Seto and Y. Takuwa, *Am. J. Physiol.: Cell Physiol.*, 2000, **278**, C57–65.
12. B. J. Druker and N. B. Lydon, *J. Clin. Invest.*, 2000, **105**, 3–7.
13. M. Ranson, W. Mansoor and G. Jayson, *Expert Rev. Anticancer Ther.*, 2002, **2**, 161–168.
14. K. S. Bhullar, N. O. Lagaron, E. M. McGowan, I. Parmar, A. Jha, B. P. Hubbard and H. P. V. Rupasinghe, *Mol. Cancer*, 2018, **17**, 48.
15. D. C. Swinney, *Clin. Pharmacol. Ther.*, 2013, **93**, 299–301.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

16. S. Campagne, S. Boigner, S. Rudisser, A. Moursy, L. Gillioz, A. Knorlein, J. Hall, H. Ratni, A. Clery and F. H. Allain, *Nat. Chem. Biol.*, 2019, **15**, 1191–1198.
17. M. P. Boyle and K. De Boeck, *Lancet Respir. Med.*, 2013, **1**, 158–163.
18. J. A. Lemm, D. O'Boyle, 2nd, M. Liu, P. T. Nower, R. Colonno, M. S. Deshpande, L. B. Snyder, S. W. Martin, D. R. St Laurent, M. H. Serrano-Wu, J. L. Romine, N. A. Meanwell and M. Gao, *J. Virol.*, 2010, **84**, 482–491.
19. B. K. Wagner, *Phenotypic Screening: Methods and Protocols*, Springer, 2018.
20. F. Vincent, P. M. Loria, A. D. Weston, C. M. Steppan, R. Doyonnas, Y. M. Wang, K. L. Rockwell and M. C. Peakman, *Cell Chem. Biol.*, 2020, **27**, 1332–1346.
21. C. H. Arrowsmith, J. E. Audia, C. Austin, J. Baell, J. Bennett, J. Blagg, C. Bountra, P. E. Brennan, P. J. Brown, M. E. Bunnage, C. Buser-Doepner, R. M. Campbell, A. J. Carter, P. Cohen, R. A. Copeland, B. Cravatt, J. L. Dahlin, D. Dhanak, A. M. Edwards, M. Frederiksen, S. V. Frye, N. Gray, C. E. Grimshaw, D. Hepworth, T. Howe, K. V. Huber, J. Jin, S. Knapp, J. D. Kotz, R. G. Kruger, D. Lowe, M. M. Mader, B. Marsden, A. Mueller-Fahrnow, S. Muller, R. C. O'Hagan, J. P. Overington, D. R. Owen, S. H. Rosenberg, B. Roth, R. Ross, M. Schapira, S. L. Schreiber, B. Shoichet, M. Sundstrom, G. Superti-Furga, J. Taunton, L. Toledo-Sherman, C. Walpole, M. A. Walters, T. M. Willson, P. Workman, R. N. Young and W. J. Zuercher, *Nat. Chem. Biol.*, 2015, **11**, 536–541.
22. J. L. Dahlin, D. S. Auld, I. Rothenaigner, S. Haney, J. Z. Sexton, J. W. M. Nissink, J. Walsh, J. A. Lee, J. M. Strelow, F. S. Willard, L. Ferrins, J. B. Baell, M. A. Walters, B. K. Hua, K. Hadian and B. K. Wagner, *Cell Chem. Biol.*, 2021, **28**, 356–370.
23. J. L. Dahlin, K. M. Nelson, J. M. Strasser, D. Barsyte-Lovejoy, M. M. Szewczyk, S. Organ, M. Cuellar, G. Singh, J. H. Shrimp, N. Nguyen, J. L. Meier, C. H. Arrowsmith, P. J. Brown, J. B. Baell and M. A. Walters, *Nat. Commun.*, 2017, **8**, 1527.
24. J. L. Dahlin, J. W. Nissink, J. M. Strasser, S. Francis, L. Higgins, H. Zhou, Z. Zhang and M. A. Walters, *J. Med. Chem.*, 2015, **58**, 2091–2113.
25. B. Dasgeb, D. Kornreich, K. McGuinn, L. Okon, I. Brownell and D. L. Sackett, *Br. J. Dermatol.*, 2018, **178**, 350–356.
26. J. F. Borel, C. Feurer, H. U. Gubler and H. Stahelin, *Agents Actions*, 1976, **6**, 468–475.
27. T. Goto, T. Kino, H. Hatanaka, M. Nishiyama, M. Okuhara, M. Kohsaka, H. Aoki and H. Imanaka, *Transplant. Proc.*, 1987, **19**, 4–8.
28. E. J. Brown, M. W. Albers, T. B. Shin, K. Ichikawa, C. T. Keith, W. S. Lane and S. L. Schreiber, *Nature*, 1994, **369**, 756–758.
29. D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2020, **83**, 770–803.
30. C. J. O'Connor, L. Laraia and D. R. Spring, *Chem. Soc. Rev.*, 2011, **40**, 4332–4345.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

31. D. R. Spring, *Chem. Soc. Rev.*, 2005, **34**, 472–482.
32. A. J. Carter, O. Kraemer, M. Zwick, A. Mueller-Fahrnow, C. H. Arrowsmith and A. M. Edwards, *Drug Discovery Today*, 2019, **24**, 2111–2115.
33. M. D. Burke and S. L. Schreiber, *Angew. Chem., Int. Ed.*, 2004, **43**, 46–58.
34. S. L. Schreiber, *Science*, 2000, **287**, 1964–1969.
35. C. J. Gerry and S. L. Schreiber, *Curr. Opin. Chem. Biol.*, 2020, **56**, 1–9.
36. N. Kato, E. Comer, T. Sakata-Kato, A. Sharma, M. Sharma, M. Maetani, J. Bastien, N. M. Brancucci, J. A. Bittker, V. Corey, D. Clarke, E. R. Derbyshire, G. L. Dornan, S. Duffy, S. Eckley, M. A. Itoe, K. M. Koolen, T. A. Lewis, P. S. Lui, A. K. Lukens, E. Lund, S. March, E. Meibalan, B. C. Meier, J. A. McPhail, B. Mitasev, E. L. Moss, M. Sayes, Y. Van Gessel, M. J. Wawer, T. Yoshinaga, A. M. Zeeman, V. M. Avery, S. N. Bhatia, J. E. Burke, F. Catteruccia, J. C. Clardy, P. A. Clemons, K. J. Dechering, J. R. Duvall, M. A. Foley, F. Gusovsky, C. H. Kocken, M. Marti, M. L. Morningstar, B. Munoz, D. E. Neafsey, A. Sharma, E. A. Winzeler, D. F. Wirth, C. A. Scherer and S. L. Schreiber, *Nature*, 2016, **538**, 344–349.
37. D. H. Chou, A. Vetere, A. Choudhary, S. S. Scully, M. Schenone, A. Tang, R. Gomez, S. M. Burns, M. Lundh, T. Vital, E. Comer, P. W. Faloon, V. Dancik, C. Ciarlo, J. Paulk, M. Dai, C. Reddy, H. Sun, M. Young, N. Donato, J. Jaffe, P. A. Clemons, M. Palmer, S. A. Carr, S. L. Schreiber and B. K. Wagner, *J. Am. Chem. Soc.*, 2015, **137**, 7929–7934.
38. J. P. Maianti, G. A. Tan, A. Vetere, A. J. Welsh, B. K. Wagner, M. A. Seeliger and D. R. Liu, *Nat. Chem. Biol.*, 2019, **15**, 565–574.
39. B. Maji, S. A. Gangopadhyay, M. Lee, M. Shi, P. Wu, R. Heler, B. Mok, D. Lim, S. U. Siriwardena, B. Paul, V. Dancik, A. Vetere, M. F. Mesleh, L. A. Marraffini, D. R. Liu, P. A. Clemons, B. K. Wagner and A. Choudhary, *Cell*, 2019, **177**, 1067–1079 e1019.
40. S. M. Corsello, J. A. Bittker, Z. Liu, J. Gould, P. McCarren, J. E. Hirschman, S. E. Johnston, A. Vrcic, B. Wong, M. Khan, J. Asiedu, R. Narayan, C. C. Mader, A. Subramanian and T. R. Golub, *Nat. Med.*, 2017, **23**, 405–408.
41. J. Janes, M. E. Young, E. Chen, N. H. Rogers, S. Burgstaller-Muehlbacher, L. D. Hughes, M. S. Love, M. V. Hull, K. L. Kuhen, A. K. Woods, S. B. Joseph, H. M. Petrassi, C. W. McNamara, M. S. Tremblay, A. I. Su, P. G. Schultz and A. K. Chatterjee, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, 10750–10755.
42. Y. Cha, T. Erez, I. J. Reynolds, D. Kumar, J. Ross, G. Koytiger, R. Kusko, B. Zeskind, S. Risso, E. Kagan, S. Papapetropoulos, I. Grossman and D. Laifenfeld, *Br. J. Pharmacol.*, 2018, **175**, 168–180.
43. S. Brenner and R. A. Lerner, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 5381–5383.
44. R. A. Goodnow, Jr., C. E. Dumelin and A. D. Keefe, *Nat. Rev. Drug Discovery*, 2017, **16**, 131–147.
45. D. Neri and R. A. Lerner, *Annu. Rev. Biochem.*, 2018, **87**, 479–502.
46. P. Dickson and T. Kodadek, *Org. Biomol. Chem.*, 2019, **17**, 4676–4688.
47. R. M. Franzini and C. Randolph, *J. Med. Chem.*, 2016, **59**, 6629–6644.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

48. C. J. Gerry, M. J. Wawer, P. A. Clemons and S. L. Schreiber, *J. Am. Chem. Soc.*, 2019, **141**, 10225–10235.
49. C. J. Gerry, Z. Yang, M. Stasi and S. L. Schreiber, *Org. Lett.*, 2019, **21**, 1325–1330.
50. S. L. Schreiber and G. R. Crabtree, *Immunol. Today*, 1992, **13**, 136–142.
51. S. L. Schreiber, *Cell*, 2021, **184**, 3–9.
52. B. Z. Stanton, E. J. Chory and G. R. Crabtree, *Science*, 2018, **359**, eaao5902.
53. K. M. Sakamoto, K. B. Kim, A. Kumagai, F. Mercurio, C. M. Crews and R. J. Deshaies, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 8554–8559.
54. A. C. Lai and C. M. Crews, *Nat. Rev. Drug Discovery*, 2017, **16**, 101–114.
55. D. L. Buckley, J. L. Gustafson, I. Van Molle, A. G. Roth, H. S. Tae, P. C. Gareiss, W. L. Jorgensen, A. Ciulli and C. M. Crews, *Angew. Chem., Int. Ed.*, 2012, **51**, 11463–11467.
56. D. L. Buckley, I. Van Molle, P. C. Gareiss, H. S. Tae, J. Michel, D. J. Noblin, W. L. Jorgensen, A. Ciulli and C. M. Crews, *J. Am. Chem. Soc.*, 2012, **134**, 4465–4468.
57. G. E. Winter, D. L. Buckley, J. Paulk, J. M. Roberts, A. Souza, S. Dhe-Paganon and J. E. Bradner, *Science*, 2015, **348**, 1376–1381.
58. D. L. Buckley, K. Raina, N. Darricarrere, J. Hines, J. L. Gustafson, I. E. Smith, A. H. Miah, J. D. Harling and C. M. Crews, *ACS Chem. Biol.*, 2015, **10**, 1831–1837.
59. D. P. Bondeson, A. Mares, I. E. Smith, E. Ko, S. Campos, A. H. Miah, K. E. Mulholland, N. Routly, D. L. Buckley, J. L. Gustafson, N. Zinn, P. Grandi, S. Shimamura, G. Bergamini, M. Faelth-Savitski, M. Bantscheff, C. Cox, D. A. Gordon, R. R. Willard, J. J. Flanagan, L. N. Casillas, B. J. Votta, W. den Besten, K. Famm, L. Kruidenier, P. S. Carter, J. D. Harling, I. Churcher and C. M. Crews, *Nat. Chem. Biol.*, 2015, **11**, 611–617.
60. S. Ziegler, V. Pries, C. Hedberg and H. Waldmann, *Angew. Chem., Int. Ed.*, 2013, **52**, 2744–2792.
61. M. Schenone, V. Dancik, B. K. Wagner and P. A. Clemons, *Nat. Chem. Biol.*, 2013, **9**, 232–240.
62. K. Kubota, M. Funabashi and Y. Ogura, *Biochim. Biophys. Acta, Proteins Proteomics*, 2019, **1867**, 22–27.
63. H. Park, J. Ha and S. B. Park, *Curr. Opin. Chem. Biol.*, 2019, **50**, 66–72.
64. Q. T. L. Pasquer, I. A. Tsakoumagkos and S. Hoogendoorn, *Molecules*, 2020, **25**, 5702.
65. B. J. Leslie and P. J. Hergenrother, *Chem. Soc. Rev.*, 2008, **37**, 1347–1360.
66. U. Rix and G. Superti-Furga, *Nat. Chem. Biol.*, 2009, **5**, 616–624.
67. X. Chen and Y. W. Wu, *Org. Biomol. Chem.*, 2016, **14**, 5417–5439.
68. L. I. Willems, W. A. van der Linden, N. Li, K. Y. Li, N. Liu, S. Hoogendoorn, G. A. van der Marel, B. I. Florea and H. S. Overkleeft, *Acc. Chem. Res.*, 2011, **44**, 718–729.
69. B. F. Cravatt, A. T. Wright and J. W. Kozarich, *Annu. Rev. Biochem.*, 2008, **77**, 383–414.
70. D. S. Hewings, J. A. Flygare, M. Bogyo and I. E. Wertz, *FEBS J.*, 2017, **284**, 1555–1576.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

71. H. A. Flaxman, D. K. Miyamoto and C. M. Woo, *Curr. Protoc. Chem. Biol.*, 2019, **11**, e75.
72. E. Smith and I. Collins, *Future Med. Chem.*, 2015, **7**, 159–183.
73. L. P. Conway, W. Li and C. G. Parker, *Cell Chem. Biol.*, 2021, **28**, 371–393.
74. B. Lomenick, R. Hao, N. Jonai, R. M. Chin, M. Aghajan, S. Warburton, J. Wang, R. P. Wu, F. Gomez, J. A. Loo, J. A. Wohlschlegel, T. M. Vondriska, J. Pelletier, H. R. Herschman, J. Clardy, C. F. Clarke and J. Huang, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 21984–21989.
75. E. C. Strickland, M. A. Geer, D. T. Tran, J. Adhikari, G. M. West, P. D. DeArmond, Y. Xu and M. C. Fitzgerald, *Nat. Protoc.*, 2013, **8**, 148–161.
76. D. Martinez Molina, R. Jafari, M. Ignatushchenko, T. Seki, E. A. Larsson, C. Dan, L. Sreekumar, Y. Cao and P. Nordlund, *Science*, 2013, **341**, 84–87.
77. F. H. Niesen, H. Berglund and M. Vedadi, *Nat. Protoc.*, 2007, **2**, 2212–2221.
78. R. Jafari, H. Almqvist, H. Axelsson, M. Ignatushchenko, T. Lundback, P. Nordlund and D. Martinez Molina, *Nat. Protoc.*, 2014, **9**, 2100–2122.
79. D. Martinez Molina and P. Nordlund, *Annu. Rev. Pharmacol. Toxicol.*, 2016, **56**, 141–161.
80. M. M. Savitski, F. B. Reinhard, H. Franken, T. Werner, M. F. Savitski, D. Eberhard, D. Martinez Molina, R. Jafari, R. B. Dovega, S. Klaeger, B. Kuster, P. Nordlund, M. Bantscheff and G. Drewes, *Science*, 2014, **346**, 1255784.
81. J. M. Dziekan, H. Yu, D. Chen, L. Dai, G. Wirjanata, A. Larsson, N. Prabhu, R. M. Sobota, Z. Bozdech and P. Nordlund, *Sci. Transl. Med.*, 2019, **11**, eaau3174.
82. M. Kitagawa, P. J. Liao, K. H. Lee, J. Wong, S. C. Shang, N. Minami, O. Sampetrean, H. Saya, D. Lingyun, N. Prabhu, G. K. Diam, R. Sobota, A. Larsson, P. Nordlund, F. McCormick, S. Ghosh, D. M. Epstein, B. W. Dymock and S. H. Lee, *Nat. Commun.*, 2017, **8**, 2200.
83. S. Vartanian, T. P. Ma, J. Lee, P. M. Haverty, D. S. Kirkpatrick, K. Yu and D. Stokoe, *Mol. Cell. Proteomics*, 2016, **15**, 1220–1231.
84. P. D. Hsu, E. S. Lander and F. Zhang, *Cell*, 2014, **157**, 1262–1278.
85. H. Wang, M. La Russa and L. S. Qi, *Annu. Rev. Biochem.*, 2016, **85**, 227–264.
86. A. V. Wright, J. K. Nunez and J. A. Doudna, *Cell*, 2016, **164**, 29–44.
87. M. Jost and J. S. Weissman, *ACS Chem. Biol.*, 2018, **13**, 366–375.
88. C. Kasap, O. Elemento and T. M. Kapoor, *Nat. Chem. Biol.*, 2014, **10**, 626–628.
89. J. E. Neggers, B. Kwanten, T. Dierckx, H. Noguchi, A. Voet, L. Bral, K. Minner, B. Massant, N. Kint, M. Delforge, T. Vercruysse, E. Baloglu, W. Senapedis, M. Jacquemyn and D. Daelemans, *Nat. Commun.*, 2018, **9**, 502.
90. T. Katsila, G. A. Spyroulias, G. P. Patrinos and M. T. Matsoukas, *Comput. Struct. Biotechnol. J.*, 2016, **14**, 177–184.
91. G. B. Li, Z. J. Yu, S. Liu, L. Y. Huang, L. L. Yang, C. T. Lohans and S. Y. Yang, *J. Chem. Inf. Model.*, 2017, **57**, 1640–1651.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

92. N. S. Madhukar, P. K. Khade, L. Huang, K. Gayvert, G. Galletti, M. Stogniew, J. E. Allen, P. Giannakakou and O. Elemento, *Nat. Commun.*, 2019, **10**, 5221.
93. S. M. Gustafsdottir, V. Ljosa, K. L. Sokolnicki, J. Anthony Wilson, D. Walpita, M. M. Kemp, K. Petri Seiler, H. A. Carrel, T. R. Golub, S. L. Schreiber, P. A. Clemons, A. E. Carpenter and A. F. Shamji, *PLoS One*, 2013, **8**, e80999.
94. T. Schneidewind, A. Brause, A. Pahl, A. Burhop, T. Mejuch, S. Sievers, H. Waldmann and S. Ziegler, *ChemBioChem*, 2020, **21**, 3197–3207.
95. J. Lamb, E. D. Crawford, D. Peck, J. W. Modell, I. C. Blat, M. J. Wrobel, J. Lerner, J. P. Brunet, A. Subramanian, K. N. Ross, M. Reich, H. Hieronymus, G. Wei, S. A. Armstrong, S. J. Haggarty, P. A. Clemons, R. Wei, S. A. Carr, E. S. Lander and T. R. Golub, *Science*, 2006, **313**, 1929–1935.
96. A. Subramanian, R. Narayan, S. M. Corsello, D. D. Peck, T. E. Natoli, X. Lu, J. Gould, J. F. Davis, A. A. Tubelli, J. K. Asiedu, D. L. Lahr, J. E. Hirschman, Z. Liu, M. Donahue, B. Julian, M. Khan, D. Wadden, I. C. Smith, D. Lam, A. Liberzon, C. Toder, M. Bagul, M. Orzechowski, O. M. Enache, F. Piccioni, S. A. Johnson, N. J. Lyons, A. H. Berger, A. F. Shamji, A. N. Brooks, A. Vrcic, C. Flynn, J. Rosains, D. Y. Takeda, R. Hu, D. Davison, J. Lamb, K. Ardlie, L. Hogstrom, P. Greenside, N. S. Gray, P. A. Clemons, S. Silver, X. Wu, W. N. Zhao, W. Read-Button, X. Wu, S. J. Haggarty, L. V. Ronco, J. S. Boehm, S. L. Schreiber, J. G. Doench, J. A. Bittker, D. E. Root, B. Wong and T. R. Golub, *Cell*, 2017, **171**, 1437–1452 e1417.
97. A. C. Ravindranath, N. Perualila-Tan, A. Kasim, G. Drakakis, S. Liggi, S. C. Brewerton, D. Mason, M. J. Bodkin, D. A. Evans, A. Bhagwat, W. Talloen, H. W. Gohlmann, Z. Shkedy, A. Bender and Q. Consortium, *Mol. BioSyst.*, 2015, **11**, 86–96.
98. M. Karamanou, A. Protogerou, G. Tsoucalas, G. Androutsos and E. Poulakou-Rebelakou, *World J. Diabetes*, 2016, **7**, 1–7.
99. R. A. DeFronzo, *Diabetes*, 1988, **37**, 667–687.
100. R. A. Defronzo, *Diabetes*, 2009, **58**, 773–795.
101. R. Lupi, F. Dotta, L. Marselli, S. Del Guerra, M. Masini, C. Santangelo, G. Patane, U. Boggi, S. Piro, M. Anello, E. Bergamini, F. Mosca, U. Di Mario, S. Del Prato and P. Marchetti, *Diabetes*, 2002, **51**, 1437–1442.
102. D. J. Drucker, *Cell Metab.*, 2006, **3**, 153–165.
103. M. A. Nauck, I. Vardarli, C. F. Deacon, J. J. Holst and J. J. Meier, *Diabetologia*, 2011, **54**, 10–18.
104. J. E. Gerich, *Diabetes Med.*, 2010, **27**, 136–142.
105. M. W. Schwartz, S. C. Woods, D. Porte, Jr., R. J. Seeley and D. G. Baskin, *Nature*, 2000, **404**, 661–671.
106. A. D. Baron, L. Schaeffer, P. Shragg and O. G. Kolterman, *Diabetes*, 1987, **36**, 274–283.
107. A. American Diabetes, *Diabetes Care*, 2020, **43**, S14–S31.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

108. J. S. Skyler, G. L. Bakris, E. Bonifacio, T. Darsow, R. H. Eckel, L. Groop, P. H. Groop, Y. Handelsman, R. A. Insel, C. Mathieu, A. T. McElvaine, J. P. Palmer, A. Pugliese, D. A. Schatz, J. M. Sosenko, J. P. Wilding and R. E. Ratner, *Diabetes*, 2017, **66**, 241–255.
109. A. Petrelli, M. A. Atkinson, M. Pietropaolo and N. Giannoukakis, *Diabetes*, 2021, **70**, 313–322.
110. C. Bender, S. Rajendran and M. G. von Herrath, *Front. Endocrinol.*, 2020, **11**, 606434.
111. M. R. Rickels, A. W. Norris and R. L. Hull, *Diabetologia*, 2020, **63**, 2030–2039.
112. S. J. Richardson, T. Rodriguez-Calvo, I. C. Gerling, C. E. Mathews, J. S. Kaddis, M. A. Russell, M. Zeissler, P. Leete, L. Krogvold, K. Dahl-Jorgensen, M. von Herrath, A. Pugliese, M. A. Atkinson and N. G. Morgan, *Diabetologia*, 2016, **59**, 2448–2458.
113. M. Lundberg, L. Krogvold, E. Kuric, K. Dahl-Jorgensen and O. Skog, *Diabetes*, 2016, **65**, 3104–3110.
114. L. Marroqui, R. S. Dos Santos, A. Op de Beeck, A. Coomans de Brachene, L. Marselli, P. Marchetti and D. L. Eizirik, *Diabetologia*, 2017, **60**, 656–667.
115. M. A. Russell, S. D. Redick, D. M. Blodgett, S. J. Richardson, P. Leete, L. Krogvold, K. Dahl-Jorgensen, R. Bottino, M. Brissova, J. M. Spaeth, J. A. B. Babon, R. Haliyur, A. C. Powers, C. Yang, S. C. Kent, A. G. Derr, A. Kucukural, M. G. Garber, N. G. Morgan and D. M. Harlan, *Diabetes*, 2019, **68**, 988–1001.
116. F. Pociot, B. Akolkar, P. Concannon, H. A. Erlich, C. Julier, G. Morahan, C. R. Nierras, J. A. Todd, S. S. Rich and J. Nerup, *Diabetes*, 2010, **59**, 1561–1571.
117. M. J. Redondo, A. K. Steck and A. Pugliese, *Pediatr. Diabetes*, 2018, **19**, 346–353.
118. J. F. Bach and L. Chatenoud, *Cold Spring Harb. Perspect. Med.*, 2012, **2**, a007799.
119. L. Adorini, G. Penna, N. Giarratana and M. Uskokovic, *J. Cell. Biochem.*, 2003, **88**, 227–233.
120. P. Leete, R. A. Oram, T. J. McDonald, B. M. Shields, C. Ziller, T. s. team, A. T. Hattersley, S. J. Richardson and N. G. Morgan, *Diabetologia*, 2020, **63**, 1258–1267.
121. E. K. Sims, F. Syed, J. Nyalwidhe, H. T. Bahnson, L. Haataja, C. Speake, M. A. Morris, A. N. Balamurugan, R. G. Mirmira, J. Nadler, T. L. Mastracci, P. Arvan, C. J. Greenbaum and C. Evans-Molina, *Transl. Res.*, 2019, **213**, 90–99.
122. P. J. Thompson, A. Shah, V. Ntranos, F. Van Gool, M. Atkinson and A. Bhushan, *Cell Metab.*, 2019, **29**, 1045–1060 e1010.
123. H. Nomoto, L. Pei, C. Montemurro, M. Rosenberger, A. Furterer, G. Coppola, B. Nadel, M. Pellegrini, T. Gurlo, P. C. Butler and S. Tudzarova, *Diabetologia*, 2020, **63**, 149–161.
124. G. F. Bottazzo, *Diabetes Med.*, 1986, **3**, 119–130.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

125. S. A. Soleimanpour and D. A. Stoffers, *Trends Endocrinol. Metab.*, 2013, **24**, 324–331.
126. S. A. Tersey, Y. Nishiki, A. T. Templin, S. M. Cabrera, N. D. Stull, S. C. Colvin, C. Evans-Molina, J. L. Rickus, B. Maier and R. G. Mirmira, *Diabetes*, 2012, **61**, 818–827.
127. S. Morita, S. A. Villalta, H. C. Feldman, A. C. Register, W. Rosenthal, I. T. Hoffmann-Petersen, M. Mehdizadeh, R. Ghosh, L. Wang, K. Colon-Negron, R. Meza-Acevedo, B. J. Backes, D. J. Maly, J. A. Bluestone and F. R. Papa, *Cell Metab.*, 2017, **25**, 1207.
128. H. Lee, Y. S. Lee, Q. Harenda, S. Pietrzak, H. Z. Oktay, S. Schreiber, Y. Liao, S. Sonthalia, A. E. Ciecko, Y. G. Chen, S. Keles, R. Sridharan and F. Engin, *Cell Metab.*, 2020, **31**, 822–836 e825.
129. P. M. Trivedi, K. L. Graham, N. A. Scott, M. R. Jenkins, S. Majaw, R. M. Sutherland, S. Fynch, A. M. Lew, C. J. Burns, B. Krishnamurthy, T. C. Brodnicki, S. I. Mannering, T. W. Kay and H. E. Thomas, *Diabetes*, 2017, **66**, 1650–1660.
130. F. Engin, A. Yermalovich, T. Nguyen, S. Hummasti, W. Fu, D. L. Eizirik, D. Mathis and G. S. Hotamisligil, *Sci. Transl. Med.*, 2013, **5**, 211ra156.
131. F. M. Ashcroft and P. Rorsman, *Cell*, 2012, **148**, 1160–1171.
132. Y. S. Oh, G. D. Bae, D. J. Baek, E. Y. Park and H. S. Jun, *Front. Endocrinol.*, 2018, **9**, 384.
133. N. Kaiser, G. Leibowitz and R. Nesher, *J. Pediatr. Endocrinol. Metab.*, 2003, **16**, 5–22.
134. A. T. Hattersley and J. E. Tooke, *Lancet*, 1999, **353**, 1789–1792.
135. C. S. Yajnik, *Nutr. Rev.*, 2001, **59**, 1–9.
136. D. Walpita, T. Hasaka, J. Spoonamore, A. Vetere, K. K. Takane, D. Fomina-Yadlin, N. Fiaschi-Taesch, A. Shamji, P. A. Clemons, A. F. Stewart, S. L. Schreiber and B. K. Wagner, *J. Biomol. Screen*, 2012, **17**, 509–518.
137. D. Walpita and B. K. Wagner, *Curr. Protoc. Chem. Biol.*, 2014, **6**, 157–168.
138. D. Balboa, R. B. Prasad, L. Groop and T. Otonkoski, *Diabetologia*, 2019, **62**, 1329–1336.
139. K. G. Maxwell and J. R. Millman, *Cell Rep. Med.*, 2021, **2**, 100238.
140. P. Ravassard, Y. Hazhouz, S. Pechberty, E. Bricout-Neveu, M. Armanet, P. Czernichow and R. Scharfmann, *J. Clin. Invest.*, 2011, **121**, 3589–3597.
141. R. Scharfmann, S. Pechberty, Y. Hazhouz, M. von Bulow, E. Bricout-Neveu, M. Grenier-Godard, F. Guez, L. Rachdi, M. Lohmann, P. Czernichow and P. Ravassard, *J. Clin. Invest.*, 2014, **124**, 2087–2098.
142. B. Hastoy, M. Godazgar, A. Clark, V. Nylander, I. Spiliotis, M. van de Bunt, M. V. Chibalina, A. Barrett, C. Burrows, A. I. Tarasov, R. Scharfmann, A. L. Gloyn and P. Rorsman, *Sci. Rep.*, 2018, **8**, 16994.
143. M. Benazra, M. J. Lecomte, C. Colace, A. Muller, C. Machado, S. Pechberty, E. Bricout-Neveu, M. Grenier-Godard, M. Solimena, R. Scharfmann, P. Czernichow and P. Ravassard, *Mol. Metab.*, 2015, **4**, 916–925.
144. M. Asfari, D. Janjic, P. Meda, G. Li, P. A. Halban and C. B. Wollheim, *Endocrinology*, 1992, **130**, 167–178.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

145. A. Merglen, S. Theander, B. Rubi, G. Chaffard, C. B. Wollheim and P. Maechler, *Endocrinology*, 2004, **145**, 667–678.
146. H. E. Hohmeier, H. Mulder, G. Chen, R. Henkel-Rieger, M. Prentki and C. B. Newgard, *Diabetes*, 2000, **49**, 424–430.
147. J. Miyazaki, K. Araki, E. Yamato, H. Ikegami, T. Asano, Y. Shibasaki, Y. Oka and K. Yamamura, *Endocrinology*, 1990, **127**, 126–132.
148. H. Ishihara, T. Asano, K. Tsukuda, H. Katagiri, K. Inukai, M. Anai, M. Kikuchi, Y. Yazaki, J. I. Miyazaki and Y. Oka, *Diabetologia*, 1993, **36**, 1139–1145.
149. V. Poitout, L. E. Stout, M. B. Armstrong, T. F. Walseth, R. L. Sorenson and R. P. Robertson, *Diabetes*, 1995, **44**, 306–313.
150. S. M. Burns, A. Vetere, D. Walpita, V. Dancik, C. Khodier, J. Perez, P. A. Clemons, B. K. Wagner and D. Altshuler, *Cell Metab.*, 2015, **21**, 126–137.
151. J. Huey, K. Keutler and C. Schultz, *Cell Chem. Biol.*, 2020, **27**, 1015–1031.
152. J. E. Campbell and C. B. Newgard, *Nat. Rev. Mol. Cell Biol.*, 2021, **22**, 142–158.
153. P. Proks, F. Reimann, N. Green, F. Gribble and F. Ashcroft, *Diabetes*, 2002, **51**(Suppl 3), S368–376.
154. F. R. Brown, Jr, R. W. Friskey, L. Grindle, L. W. Kinsell and S. Splitter, *Calif. Med.*, 1956, **85**, 285–288.
155. J. H. Hoofnagle, *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*, Bethesda, MD, 2012.
156. P. Rorsman, L. Eliasson, E. Renstrom, J. Gromada, S. Barg and S. Gopel, *News Physiol. Sci.*, 2000, **15**, 72–77.
157. J. E. Gerich, *Diabetes*, 2002, **51**(Suppl 1), S117–121.
158. G. G. Graham, J. Punt, M. Arora, R. O. Day, M. P. Doogue, J. K. Duong, T. J. Furlong, J. R. Greenfield, L. C. Greenup, C. M. Kirkpatrick, J. E. Ray, P. Timmins and K. M. Williams, *Clin. Pharmacokinet.*, 2011, **50**, 81–98.
159. R. S. Hundal, M. Krssak, S. Dufour, D. Laurent, V. Lebon, V. Chandramouli, S. E. Inzucchi, W. C. Schumann, K. F. Petersen, B. R. Landau and G. I. Shulman, *Diabetes*, 2000, **49**, 2063–2069.
160. A. Klip and L. A. Leiter, *Diabetes Care*, 1990, **13**, 696–704.
161. J. Flory and K. Lipska, *JAMA*, 2019, **321**, 1926–1927.
162. A. Golay, *Int. J. Obes.*, 2008, **32**, 61–72.
163. L. G. Fryer, A. Parbu-Patel and D. Carling, *J. Biol. Chem.*, 2002, **277**, 25226–25232.
164. M. J. Nanjan, M. Mohammed, B. R. Prashantha Kumar and M. J. N. Chandrasekar, *Bioorg. Chem.*, 2018, **77**, 548–567.
165. V. Dubois, J. Eeckhoute, P. Lefebvre and B. Staels, *J. Clin. Invest.*, 2017, **127**, 1202–1214.
166. J. H. Hoofnagle, *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury*, Bethesda, MD, 2012.
167. A. H. Barnett, *Vasc. Health Risk Manag.*, 2009, **5**, 141–151.
168. S. E. Nissen and K. Wolski, *N. Engl. J. Med.*, 2007, **356**, 2457–2471.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

169. J. J. Holst, *Front. Endocrinol.*, 2019, **10**, 260.
170. J. J. Holst, *Physiol. Rev.*, 2007, **87**, 1409–1439.
171. S. R. Hoare, *Drug Discovery Today*, 2005, **10**, 417–427.
172. X. Deng, M. S. Tavallaie, R. Sun, J. Wang, Q. Cai, J. Shen, S. Lei, L. Fu and F. Jiang, *Bioorg. Chem.*, 2020, **99**, 103810.
173. L. L. Baggio and D. J. Drucker, *Mol. Metab.*, 2021, **46**, 101090.
174. M. A. Nauck and J. J. Meier, *Eur. J. Endocrinol.*, 2019, **181**, R211–R234.
175. K. Aston-Mourney, J. Proietto, G. Morahan and S. Andrikopoulos, *Diabetologia*, 2008, **51**, 540–545.
176. E. M. Wright, D. D. Loo and B. A. Hirayama, *Physiol. Rev.*, 2011, **91**, 733–794.
177. R. Santer and J. Calado, *Clin. J. Am. Soc. Nephrol.*, 2010, **5**, 133–141.
178. R. F. Rosenwasser, S. Sultan, D. Sutton, R. Choksi and B. J. Epstein, *Diabetes Metab. Syndr. Obes.*, 2013, **6**, 453–467.
179. D. K. McGuire, W. J. Shih, F. Cosentino, B. Charbonnel, D. Z. I. Cherney, S. Dagogo-Jack, R. Pratley, M. Greenberg, S. Wang, S. Huyck, I. Gantz, S. G. Terra, U. Masiukiewicz and C. P. Cannon, *JAMA Cardiol.*, 2021, **6**, 148–158.
180. W. Wu, J. Shang, Y. Feng, C. M. Thompson, S. Horwitz, J. R. Thompson, E. D. MacIntyre, N. A. Thornberry, K. Chapman, Y. P. Zhou, A. D. Howard and J. Li, *J. Biomol. Screen.*, 2008, **13**, 128–134.
181. J. E. de Leeuw van Weenen, E. T. Parlevliet, P. Maechler, L. M. Havekes, J. A. Romijn, D. M. Ouwens, H. Pijl and B. Guigas, *Biochem. Pharmacol.*, 2010, **79**, 1827–1836.
182. I. Garcia-Tornadu, A. M. Ornstein, A. Chamson-Reig, M. B. Wheeler, D. J. Hill, E. Arany, M. Rubinstein and D. Becu-Villalobos, *Endocrinology*, 2010, **151**, 1441–1450.
183. E. Ferrannini, *Cell Metab.*, 2010, **11**, 349–352.
184. H. A. Keenan, J. K. Sun, J. Levine, A. Doria, L. P. Aiello, G. Eisenbarth, S. Bonner-Weir and G. L. King, *Diabetes*, 2010, **59**, 2846–2853.
185. R. A. Oram, T. J. McDonald, B. M. Shields, M. M. Hudson, M. H. Shepherd, S. Hammersley, E. R. Pearson, A. T. Hattersley and U. Team, *Diabetes Care*, 2015, **38**, 323–328.
186. J. L. Sherr, T. Ghazi, A. Wurtz, L. Rink and K. C. Herold, *Diabetes Metab. Res. Rev.*, 2014, **30**, 154–162.
187. X. Ma and S. Zhu, *Acta Biochim. Biophys. Sin*, 2017, **49**, 289–301.
188. S. P. Singh and N. Ninov, *Int. J. Dev. Biol.*, 2018, **62**, 453–464.
189. A. Vetere, A. Choudhary, S. M. Burns and B. K. Wagner, *Nat. Rev. Drug Discovery*, 2014, **13**, 278–289.
190. E. Dirice, D. Walpita, A. Vetere, B. C. Meier, S. Kahraman, J. Hu, V. Dancik, S. M. Burns, T. J. Gilbert, D. E. Olson, P. A. Clemons, R. N. Kulkarni and B. K. Wagner, *Diabetes*, 2016, **65**, 1660–1671.
191. P. Wang, J. C. Alvarez-Perez, D. P. Felsenfeld, H. Liu, S. Sivendran, A. Bender, A. Kumar, R. Sanchez, D. K. Scott, A. Garcia-Ocana and A. F. Stewart, *Nat. Med.*, 2015, **21**, 383–388.
192. W. Shen, B. Taylor, Q. Jin, V. Nguyen-Tran, S. Meeusen, Y. Q. Zhang, A. Kamireddy, A. Swafford, A. F. Powers, J. Walker, J. Lamb,

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

B. Bursalaya, M. DiDonato, G. Harb, M. Qiu, C. M. Filippi, L. Deaton, C. N. Turk, W. L. Suarez-Pinzon, Y. Liu, X. Hao, T. Mo, S. Yan, J. Li, A. E. Herman, B. J. Hering, T. Wu, H. Martin Seidel, P. McNamara, R. Glynne and B. Laffitte, *Nat. Commun.*, 2015, **6**, 8372.
193. H. Liu, M. S. Remedi, K. L. Pappan, G. Kwon, N. Rohatgi, C. A. Marshall and M. L. McDaniel, *Diabetes*, 2009, **58**, 663–672.
194. R. Mussmann, M. Geese, F. Harder, S. Kegel, U. Andag, A. Lomow, U. Burk, D. Onichtchouk, C. Dohrmann and M. Austen, *J. Biol. Chem.*, 2007, **282**, 12030–12037.
195. C. Ackeifi, E. Swartz, K. Kumar, H. Liu, S. Chalada, E. Karakose, D. K. Scott, A. Garcia-Ocana, R. Sanchez, R. J. DeVita, A. F. Stewart and P. Wang, *JCI Insight*, 2020, **5**, e132594.
196. P. Wang, E. Karakose, H. Liu, E. Swartz, C. Ackeifi, V. Zlatanic, J. Wilson, B. J. Gonzalez, A. Bender, K. K. Takane, L. Ye, G. Harb, F. Pagliuca, D. Homann, D. Egli, C. Argmann, D. K. Scott, A. Garcia-Ocana and A. F. Stewart, *Cell Metab.*, 2019, **29**, 638–652 e635.
197. C. Ackeifi, P. Wang, E. Karakose, J. E. Manning Fox, B. J. Gonzalez, H. Liu, J. Wilson, E. Swartz, C. Berrouet, Y. Li, K. Kumar, P. E. MacDonald, R. Sanchez, B. Thorens, R. DeVita, D. Homann, D. Egli, D. K. Scott, A. Garcia-Ocana and A. F. Stewart, *Sci. Transl. Med.*, 2020, **12**, eaaw9996.
198. S. Dhawan, E. Dirice, R. N. Kulkarni and A. Bhushan, *Diabetes*, 2016, **65**, 1208–1218.
199. H. E. Hohmeier, L. Zhang, B. Taylor, S. Stephens, D. Lu, P. McNamara, B. Laffitte and C. B. Newgard, *PLoS One*, 2020, **15**, e0224344.
200. E. Nguidjoe, S. Sokolow, S. Bigabwa, N. Pachera, E. D'Amico, F. Allagnat, J. M. Vanderwinden, A. Sener, M. Manto, M. Depreter, J. Mast, G. Joanny, E. Montanya, J. Rahier, A. K. Cardozo, D. L. Eizirik, S. Schurmans and A. Herchuelz, *Diabetes*, 2011, **60**, 2076–2085.
201. J. Papin, F. P. Zummo, N. Pachera, C. Guay, R. Regazzi, A. K. Cardozo and A. Herchuelz, *J. Endocr. Soc.*, 2018, **2**, 631–645.
202. Z. Assefa, S. Akbib, A. Lavens, G. Stange, Z. Ling, K. H. Hellemans and D. Pipeleers, *Am. J. Physiol.: Endocrinol. Metab.*, 2016, **311**, E698–E705.
203. C. Beaupere, A. Liboz, B. Feve, B. Blondeau and G. Guillemain, *Int. J. Mol. Sci.*, 2021, **22**, 623.
204. M. Cnop, N. Welsh, J. C. Jonas, A. Jorns, S. Lenzen and D. L. Eizirik, *Diabetes*, 2005, **54**(Suppl 2), S97–107.
205. D. L. Eizirik and T. Mandrup-Poulsen, *Diabetologia*, 2001, **44**, 2115–2133.
206. M. I. Darville and D. L. Eizirik, *Diabetologia*, 1998, **41**, 1101–1108.
207. H. I. Callewaert, C. A. Gysemans, L. Ladriere, W. D'Hertog, J. Hagenbrock, L. Overbergh, D. L. Eizirik and C. Mathieu, *Diabetes*, 2007, **56**, 2169–2173.
208. C. A. Gysemans, L. Ladriere, H. Callewaert, J. Rasschaert, D. Flamez, D. E. Levy, P. Matthys, D. L. Eizirik and C. Mathieu, *Diabetes*, 2005, **54**, 2396–2403.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

209. L. G. Grunnet, R. Aikin, M. F. Tonnesen, S. Paraskevas, L. Blaabjerg, J. Storling, L. Rosenberg, N. Billestrup, D. Maysinger and T. Mandrup-Poulsen, *Diabetes*, 2009, **58**, 1807–1815.
210. C. M. Larsen, M. Faulenbach, A. Vaag, A. Volund, J. A. Ehses, B. Seifert, T. Mandrup-Poulsen and M. Y. Donath, *N. Engl. J. Med.*, 2007, **356**, 1517–1526.
211. D. Lim, V. Sreekanth, K. J. Cox, B. K. Law, B. K. Wagner, J. M. Karp and A. Choudhary, *Nat. Commun.*, 2020, **11**, 4043.
212. E. K. Kim, K. B. Kwon, M. J. Han, M. Y. Song, J. H. Lee, N. Lv, K. B. Choi, D. G. Ryu, K. S. Kim, J. W. Park and B. H. Park, *Int. J. Mol. Med.*, 2007, **19**, 535–540.
213. M. Menegazzi, M. Novelli, P. Beffy, V. D'Aleo, E. Tedeschi, R. Lupi, E. Zoratti, P. Marchetti, H. Suzuki and P. Masiello, *Int. J. Biochem. Cell Biol.*, 2008, **40**, 1509–1521.
214. T. Matsuda, K. Ferreri, I. Todorov, Y. Kuroda, C. V. Smith, F. Kandeel and Y. Mullen, *Endocrinology*, 2005, **146**, 175–185.
215. M. Y. Song, E. K. Kim, W. S. Moon, J. W. Park, H. J. Kim, H. S. So, R. Park, K. B. Kwon and B. H. Park, *Toxicol. Appl. Pharmacol.*, 2009, **235**, 57–67.
216. D. H. Chou, J. R. Duvall, B. Gerard, H. Liu, B. A. Pandya, B. C. Suh, E. M. Forbeck, P. Faloon, B. K. Wagner and L. A. Marcaurelle, *ACS Med. Chem. Lett.*, 2011, **2**, 698–702.
217. S. Nagafuchi, Y. Kamada-Hibio, K. Hirakawa, N. Tsutsu, M. Minami, A. Okada, K. Kai, M. Teshima, A. Moroishi, Y. Murakami, Y. Umeno, Y. Yokogawa, K. Kogawa, K. Izumi, K. Anzai, R. Iwakiri, K. Hamaguchi, N. Sasaki, S. Nohara, E. Yoshida, M. Harada, K. Akashi, T. Yanase, J. Ono, T. Okeda, R. Fujimoto, K. Ihara, T. Hara, Y. Kikuchi, M. Iwase, T. Kitazono, F. Kojima, S. Kono, H. Kurisaki, S. Kondo and H. Katsuta, *EBioMedicine*, 2015, **2**, 744–749.
218. A. Coomans de Brachene, A. Castela, A. Op de Beeck, R. G. Mirmira, L. Marselli, P. Marchetti, C. Masse, W. Miao, S. Leit, C. Evans-Molina and D. L. Eizirik, *Diabetes, Obes. Metab.*, 2020, **22**, 1827–1836.
219. S. H. Lee, D. Cunha, C. Piermarocchi, G. Paternostro, A. Pinkerton, L. Ladriere, P. Marchetti, D. L. Eizirik, M. Cnop and F. Levine, *Biochem. Pharmacol.*, 2017, **138**, 140–149.
220. J. Vogel, J. Yin, L. Su, S. X. Wang, R. Zessis, S. Fowler, C. H. Chiu, A. C. Wilson, A. Chen, F. Zecri, G. Turner, T. M. Smith, B. DeChristopher, H. Xing, D. M. Rothman, X. Cai and A. Berdichevsky, *Diabetes*, 2020, **69**, 1032–1041.
221. A. Shalev, C. A. Pise-Masison, M. Radonovich, S. C. Hoffmann, B. Hirshberg, J. N. Brady and D. M. Harlan, *Endocrinology*, 2002, **143**, 3695–3698.
222. L. A. Thielen, J. Chen, G. Jing, O. Moukha-Chafiq, G. Xu, S. Jo, T. B. Grayson, B. Lu, P. Li, C. E. Augelli-Szafran, M. J. Suto, M. Kanke, P. Sethupathy, J. K. Kim and A. Shalev, *Cell Metab.*, 2020, **32**, 353–365 e358.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

223. H. S. Spijker, R. B. Ravelli, A. M. Mommaas-Kienhuis, A. A. van Apeldoorn, M. A. Engelse, A. Zaldumbide, S. Bonner-Weir, T. J. Rabelink, R. C. Hoeben, H. Clevers, C. L. Mummery, F. Carlotti and E. J. de Koning, *Diabetes*, 2013, **62**, 2471–2480.
224. P. Collombat, A. Mansouri, J. Hecksher-Sorensen, P. Serup, J. Krull, G. Gradwohl and P. Gruss, *Genes Dev.*, 2003, **17**, 2591–2603.
225. D. Fomina-Yadlin, S. Kubicek, D. Walpita, V. Dancik, J. Hecksher-Sorensen, J. A. Bittker, T. Sharifnia, A. Shamji, P. A. Clemons, B. K. Wagner and S. L. Schreiber, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 15099–15104.
226. N. Ben-Othman, A. Vieira, M. Courtney, F. Record, E. Gjernes, F. Avolio, B. Hadzic, N. Druelle, T. Napolitano, S. Navarro-Sanz, S. Silvano, K. Al-Hasani, A. Pfeifer, S. Lacas-Gervais, G. Leuckx, L. Marroqui, J. Thevenet, O. D. Madsen, D. L. Eizirik, H. Heimberg, J. Kerr-Conte, F. Pattou, A. Mansouri and P. Collombat, *Cell*, 2017, **168**, 73–85 e11.
227. J. Li, T. Casteels, T. Frogne, C. Ingvorsen, C. Honore, M. Courtney, K. V. M. Huber, N. Schmitner, R. A. Kimmel, R. A. Romanov, C. Sturtzel, C. H. Lardeau, J. Klughammer, M. Farlik, S. Sdelci, A. Vieira, F. Avolio, F. Briand, I. Baburin, P. Majek, F. M. Pauler, T. Penz, A. Stukalov, M. Gridling, K. Parapatics, C. Barbieux, E. Berishvili, A. Spittler, J. Colinge, K. L. Bennett, S. Hering, T. Sulpice, C. Bock, M. Distel, T. Harkany, D. Meyer, G. Superti-Furga, P. Collombat, J. Hecksher-Sorensen and S. Kubicek, *Cell*, 2017, **168**, 86–100 e115.
228. A. M. Ackermann, N. G. Moss and K. H. Kaestner, *Cell Metab.*, 2018, **28**, 787–792 e783.
229. A. Granger and J. A. Kushner, *J. Intern. Med.*, 2009, **266**, 325–338.
230. Y. Yuan, K. Hartland, Z. Boskovic, Y. Wang, D. Walpita, P. A. Lysy, C. Zhong, D. W. Young, Y. K. Kim, N. J. Tolliday, E. M. Sokal, S. L. Schreiber and B. K. Wagner, *Chem. Biol.*, 2013, **20**, 1513–1522.
231. N. Musi and L. J. Goodyear, *Acta Physiol. Scand.*, 2003, **178**, 337–345.
232. P. Steneberg, E. Lindahl, U. Dahl, E. Lidh, J. Straseviciene, F. Backlund, E. Kjellkvist, E. Berggren, I. Lundberg, I. Bergqvist, M. Ericsson, B. Eriksson, K. Linde, J. Westman, T. Edlund and H. Edlund, *JCI Insight*, 2018, **3**, e99114.
233. W. Farris, S. Mansourian, Y. Chang, L. Lindsley, E. A. Eckman, M. P. Frosch, C. B. Eckman, R. E. Tanzi, D. J. Selkoe and S. Guenette, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 4162–4167.
234. I. A. Mirsky and G. Perisutti, *Science*, 1955, **122**, 559–560.
235. J. P. Maianti, A. McFedries, Z. H. Foda, R. E. Kleiner, X. Q. Du, M. A. Leissring, W. J. Tang, M. J. Charron, M. A. Seeliger, A. Saghatelian and D. R. Liu, *Nature*, 2014, **511**, 94–98.
236. M. Brownlee and A. Cerami, *Science*, 1979, **206**, 1190–1191.
237. N. K. Rege, N. F. B. Phillips and M. A. Weiss, *Curr. Opin. Endocrinol., Diabetes Obes.*, 2017, **24**, 267–278.
238. Y. Abdolazimi, Z. Zhao, S. Lee, H. Xu, P. Allegretti, T. M. Horton, B. Yeh, H. P. Moeller, R. J. Nichols, D. McCutcheon, A. Shalizi, M. Smith, N. A. Armstrong and J. P. Annes, *Endocrinology*, 2018, **159**, 3143–3157.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

239. M. Debdab, F. Carreaux, S. Renault, M. Soundararajan, O. Fedorov, P. Filippakopoulos, O. Lozach, L. Babault, T. Tahtouh, B. Baratte, Y. Ogawa, M. Hagiwara, A. Eisenreich, U. Rauch, S. Knapp, L. Meijer and J. P. Bazureau, *J. Med. Chem.*, 2011, **54**, 4172–4186.
240. H. Falke, A. Chaikuad, A. Becker, N. Loaëc, O. Lozach, S. Abu Jhaisha, W. Becker, P. G. Jones, L. Preu, K. Baumann, S. Knapp, L. Meijer and C. Kunick, *J. Med. Chem.*, 2015, **58**, 3131–3143.
241. Y. A. Liu, Q. Jin, Y. Zou, Q. Ding, S. Yan, Z. Wang, X. Hao, B. Nguyen, X. Zhang, J. Pan, T. Mo, K. Jacobsen, T. Lam, T. Y. Wu, H. M. Petrassi, B. Bursulaya, M. DiDonato, W. P. Gordon, B. Liu, J. Baaten, R. Hill, V. Nguyen-Tran, M. Qiu, Y. Q. Zhang, A. Kamireddy, S. Espinola, L. Deaton, S. Ha, G. Harb, Y. Jia, J. Li, W. Shen, A. M. Schumacher, K. Colman, R. Glynne, S. Pan, P. McNamara, B. Laffitte, S. Meeusen, V. Molteni and J. Loren, *J. Med. Chem.*, 2020, **63**, 2958–2973.
242. K. Kumar, C. Suebsuwong, P. Wang, A. Garcia-Ocana, A. F. Stewart and R. J. DeVita, *J. Med. Chem.*, 2021, **64**, 2901–2922.
243. K. Kumar, P. Wang, A. S. E. S. Khamrui, C. Secor, B. L. M. R. F. S. A. Sanchez and R. J. DeVita, *Molecules*, 2020, **25**, 1983.
244. K. Kumar, P. Wang, R. Sanchez, E. A. Swartz, A. F. Stewart and R. J. DeVita, *J. Med. Chem.*, 2018, **61**, 7687–7699.
245. T. Tahtouh, J. M. Elkins, P. Filippakopoulos, M. Soundararajan, G. Burgy, E. Durieu, C. Cochet, R. S. Schmid, D. C. Lo, F. Delhommel, A. E. Oberholzer, L. H. Pearl, F. Carreaux, J. P. Bazureau, S. Knapp and L. Meijer, *J. Med. Chem.*, 2012, **55**, 9312–9330.
246. E. Hellström-Lindahl, A. Danielsson, F. Ponten, P. Czernichow, O. Korsgren, L. Johansson and O. Eriksson, *Acta Diabetol.*, 2016, **53**, 413–421.
247. M. Braun, *Vitam. Horm.*, 2014, **95**, 165–193.
248. J. K. Amartey, C. Esguerra, I. Al-Jammaz, R. S. Parhar and B. Al-Otaibi, *Appl. Radiat. Isot.*, 2006, **64**, 769–777.
249. R. E. Martin, L. G. Green, W. Guba, N. Kratochwil and A. Christ, *J. Med. Chem.*, 2007, **50**, 6291–6294.
250. H. Hirose, T. Yamasaki, M. Ogino, R. Mizojiri, Y. Tamura-Okano, H. Yashiro, Y. Muraki, Y. Nakano, J. Sugama, A. Hata, S. Iwasaki, M. Watanabe, T. Maekawa and S. Kasai, *Bioorg. Med. Chem.*, 2017, **25**, 4175–4193.
251. A. Negi, J. Zhou, S. Sweeney and P. V. Murphy, *Eur. J. Med. Chem.*, 2019, **163**, 148–159.
252. D. C. Saunders, M. Brissova, N. Phillips, S. Shrestha, J. T. Walker, R. Aramandla, G. Poffenberger, D. K. Flaherty, K. P. Weller, J. Pelletier, T. Cooper, M. T. Goff, J. Virostko, A. Shostak, E. D. Dean, D. L. Greiner, L. D. Shultz, N. Prasad, S. E. Levy, R. H. Carnahan, C. Dai, J. Sevigny and A. C. Powers, *Cell Metab.*, 2019, **29**, 745–754 e744.
253. O. Eriksson, D. Espes, R. K. Selvaraju, E. Jansson, G. Antoni, J. Sörensen, M. Lubberink, A. R. Biglarnia, J. W. Eriksson, A. Sundin, H. Ahlström, B. Eriksson, L. Johansson, P. O. Carlsson and O. Korsgren, *Diabetes*, 2014, **63**, 3428–3437.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

254. A. Segerstolpe, A. Palasantza, P. Eliasson, E. M. Andersson, A. C. Andreasson, X. Sun, S. Picelli, A. Sabirsh, M. Clausen, M. K. Bjursell, D. M. Smith, M. Kasper, C. Ammala and R. Sandberg, *Cell Metab.*, 2016, **24**, 593–607.
255. H. J. Choe and Y. M. Cho, *Endocrinol. Metab.*, 2021, **36**, 22–29.
256. M. Gotthardt, M. Fischer, I. Naeher, J. B. Holz, H. Jungclas, H. W. Fritsch, M. Béhé, B. Göke, K. Joseph and T. M. Behr, *Eur. J. Nucl. Med. Mol. Imaging*, 2002, **29**, 597–606.
257. M. Brom, W. Woliner-van der Weg, L. Joosten, C. Frielink, T. Bouckenooghe, P. Rijken, K. Andralojc, B. J. Göke, M. de Jong, D. L. Eizirik, M. Béhé, T. Lahoutte, W. J. Oyen, C. J. Tack, M. Janssen, O. C. Boerman and M. Gotthardt, *Diabetologia*, 2014, **57**, 950–959.
258. L. Joosten, M. Brom, H. Peeters, D. Bos, E. Himpe, L. Bouwens, O. Boerman and M. Gotthardt, *Mol. Pharm.*, 2019, **16**, 4024–4030.
259. G. M. Thurber, *J. Nucl. Med.*, 2021, **62**, 745–747.
260. T. Reiner, R. H. Kohler, C. W. Liew, J. A. Hill, J. Gaglia, R. N. Kulkarni and R. Weissleder, *Bioconjug. Chem.*, 2010, **21**, 1362–1368.
261. T. Reiner, G. Thurber, J. Gaglia, C. Vinegoni, C. W. Liew, R. Upadhyay, R. H. Kohler, L. Li, R. N. Kulkarni, C. Benoist, D. Mathis and R. Weissleder, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 12815–12820.
262. B. Finan, B. Yang, N. Ottaway, K. Stemmer, T. D. Muller, C. X. Yi, K. Habegger, S. C. Schriever, C. Garcia-Caceres, D. G. Kabra, J. Hembree, J. Holland, C. Raver, R. J. Seeley, W. Hans, M. Irmler, J. Beckers, M. H. de Angelis, J. P. Tiano, F. Mauvais-Jarvis, D. Perez-Tilve, P. Pfluger, L. Zhang, V. Gelfanov, R. D. DiMarchi and M. H. Tschop, *Nat. Med.*, 2012, **18**, 1847–1856.
263. C. Ämmälä, W. J. Drury, 3rd, L. Knerr, I. Ahlstedt, P. Stillemark-Billton, C. Wennberg-Huldt, E. M. Andersson, E. Valeur, R. Jansson-Löfmark, D. Janzén, L. Sundström, J. Meuller, J. Claesson, P. Andersson, C. Johansson, R. G. Lee, T. P. Prakash, P. P. Seth, B. P. Monia and S. Andersson, *Sci. Adv.*, 2018, **4**, eaat3386.
264. L. Knerr, T. P. Prakash, R. Lee, W. J. Drury Iii, M. Nikan, W. Fu, E. Pirie, L. Maria, E. Valeur, A. Hayen, M. Olwegard-Halvarsson, J. Broddefalk, C. Ammala, M. E. Ostergaard, J. Meuller, L. Sundstrom, P. Andersson, D. Janzen, R. Jansson-Lofmark, P. P. Seth and S. Andersson, *J. Am. Chem. Soc.*, 2021, **143**, 3416–3429.
265. M. K. Schäfer, N. R. Hartwig, N. Kalmbach, M. Klietz, M. Anlauf, L. E. Eiden and E. Weihe, *Diabetologia*, 2013, **56**, 1047–1056.
266. Y. V. Li, *Endocrine*, 2014, **45**, 178–189.
267. H. Ichii, L. Inverardi, A. Pileggi, R. D. Molano, O. Cabrera, A. Caicedo, S. Messinger, Y. Kuroda, P. O. Berggren and C. Ricordi, *Am. J. Transplant.*, 2005, **5**, 1635–1645.
268. B. Lukowiak, B. Vandewalle, R. Riachy, J. Kerr-Conte, V. Gmyr, S. Belaich, J. Lefebvre and F. Pattou, *J. Histochem. Cytochem.*, 2001, **49**, 519–528.
269. T. M. Horton, P. A. Allegretti, S. Lee, H. P. Moeller, M. Smith and J. P. Annes, *Cell Chem. Biol.*, 2019, **26**, 213–222 e216.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023

270. S. Kahraman, D. Manna, E. Dirice, B. Maji, J. Small, B. K. Wagner, A. Choudhary and R. N. Kulkarni, *Life Sci. Alliance*, 2021, **4**, e202000840.
271. M. Lee, B. Maji, D. Manna, S. Kahraman, R. M. Elgamal, J. Small, P. Kokkonda, A. Vetere, J. M. Goldberg, S. J. Lippard, R. N. Kulkarni, B. K. Wagner and A. Choudhary, *J. Am. Chem. Soc.*, 2020, **142**, 6477–6482.
272. K. Yang, M. Lee, P. A. Jones, S. S. Liu, A. Zhou, J. Xu, V. Sreekanth, J. L. Y. Wu, L. Vo, E. A. Lee, R. Pop, Y. Lee, B. K. Wagner, D. A. Melton, A. Choudhary and J. M. Karp, *Sci. Adv.*, 2020, **6**, 33208361.
273. D. Vats, H. Wang, D. Esterhazy, K. Dikaiou, C. Danzer, M. Honer, F. Stuker, H. Matile, C. Migliorini, E. Fischer, J. Ripoll, R. Keist, W. Krek, R. Schibli, M. Stoffel and M. Rudin, *Diabetologia*, 2012, **55**, 2407–2416.
274. D. Esterházy, I. Stützer, H. Wang, M. P. Rechsteiner, J. Beauchamp, H. Döbeli, H. Hilpert, H. Matile, M. Prummer, A. Schmidt, N. Lieske, B. Boehm, L. Marselli, D. Bosco, J. Kerr-Conte, R. Aebersold, G. A. Spinas, H. Moch, C. Migliorini and M. Stoffel, *Cell Metab.*, 2011, **14**, 365–377.
275. R. Bertrand, A. Wolf, Y. Ivashchenko, M. Löhn, M. Schäfer, M. Brönstrup, M. Gotthardt, V. Derdau and O. Plettenburg, *ACS Chem. Biol.*, 2016, **11**, 1745–1754.
276. X. Yan, J. J. Kim, H. S. Jeong, Y. K. Moon, Y. K. Cho, S. Ahn, S. B. Jun, H. Kim and Y. You, *Inorg. Chem.*, 2017, **56**, 4332–4346.
277. T. D. Muller, C. Clemmensen, B. Finan, R. D. DiMarchi and M. H. Tschop, *Pharmacol. Rev.*, 2018, **70**, 712–746.

Downloaded from http://books.rsc.org/books/edited-volume/chapter-pdf/1675194/bk9781839164705-00001.pdf by guest on 03 June 2023