Final Report Summary - BETABAT (Development of novel treatment strategies based on knowledge of cellular dysfunction in diabetes)

Executive Summary:
A reduction in functional pancreatic beta cell mass, caused by progressive loss of beta cell function and apoptosis, is the key feature of both type 1 (T1D) and type 2 diabetes (T2D). Beta cell failure is exacerbated in the context of obesity and insulin resistance, and strategies promoting weight loss and energy dissipation have beneficial effects on beta cell function. Brown adipose tissue (BAT) is a highly metabolic organ that mediates energy dissipation and glucose disposal, and thus contributes to maintain an adequate energy balance and carbohydrate homeostasis. Conversely, there is evidence that BAT dysfunction promotes obesity and impairs carbohydrate metabolism, ultimately resulting in increased functional demand on the beta cells. Hence, we hypothesised that a deleterious metabolic “crosstalk” between beta cells and BAT will perpetuate/aggravate cellular dysfunction in diabetes. We proposed the integrative concept that crosstalk between defective key gene and metabolic networks and/or insufficient protective responses (e.g. particular susceptibility to organelle dysfunction, low antioxidant defences) will increase the functional vulnerability of the beta cell and in severe cases lead to apoptosis. This crosstalk is modulated by the genetic background of the individuals at risk, but it is presently unknown how candidate genes for diabetes affect cell function and survival, and how these susceptible genetic networks interact with environmental agents to trigger disease. Our objective was thus to identify common and specific regulatory pathways involved in the dysfunction and apoptosis of beta cells and BAT leading to diabetes, as well as potential therapeutic approaches that address the problem at its real level of complexity and integration.

To address this challenge, we proposed an original concept, in which a detailed “organelle diagnosis” based on both focused and systems biology approaches provided the scientific rational for the design of specific interventions to boost the capacity of beta cells and brown adipocytes to regain homeostatic control. We suggested that only by understanding the complex molecular mechanisms triggering cellular dysfunction in diabetes, and by integrating this knowledge at the systems level, will it be possible to develop interventional therapies that protect and restore beta cell and BAT function. Crucially, we departed from the known common pathways that trigger general cell dysfunction in order to identify the putative solutions that need to be implemented on an individualized basis. Our ultimate goal was to offer individual therapeutic choices in stark contrast with the more conventional “monotherapeutic” approach usually taken by most scientists. The conventional approach often disregards important inter-individual variations in disease processes and the fact that mechanisms of cell dysfunction may be dynamic and varying as the disease progresses. Taking this into account, we applied a multi-approach strategy to the development of novel treatments, including screening for small molecule chemical probes, testing of novel modifications of known drugs that may affect specific cellular functions, and use of small interfering RNAs (siRNAs), viral vectors and transgenic (TG) animals to genetically validate “druggable” targets.

Project Context and Objectives:
A decrease in functional pancreatic beta cell mass, caused by progressive loss of beta cell function and apoptosis, is the key feature of both type 1 (T1D) and type 2 diabetes (T2D). Beta cell failure is exacerbated in the context of obesity and insulin resistance, and strategies promoting weight loss and energy dissipation have beneficial effects on beta cell function. Brown adipose tissue (BAT) is a highly metabolic organ that mediates energy dissipation and glucose disposal, and thus contributes...
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Against the background described above, the specific S&T objectives of the project BetaBat were:
1. Identification of the pathways responsible for loss of functional beta cells and BAT in the context of diabetes. The characterisation of common or specific therapeutically relevant downstream effector processes of beta cell and/or BAT dysfunction will initially focus on ER stress, mitochondrial dysfunction and apoptosis, and accumulation of oxygen free radicals and toxic lipid species.
2. Identification and characterisation of mechanisms linking candidate genes for diabetes with mechanisms of beta cell and BAT dysfunction and/or death.
3. Characterisation at the whole organism level of the crosstalk between beta cells, BAT and other important metabolic organs (e.g. central nervous system, (CNS), white fat (WAT) etc) using global integrative systems biology approaches.
4. Identification of novel molecular signatures and pathways responsible for loss of functional beta cells and BAT dysfunction with diagnostic, prognostic and therapeutic value. This will be done using advanced transcriptomic, proteomic and metabolomic profiling, integrated by computational approaches.
5. Therapeutic validation of the signalling pathways identified through candidate and systems approaches using small molecule chemical probes, viral vectors and siRNAs. This objective will take advantage of non-biased high throughput screening (HTS), as well as targeted approaches that preferentially modulate ER stress and favour beta cell survival.

Project Results:
In this part we describe the main results obtained during the 4 years of the BetaBat project, divided into the different work packages. In relation to the specific objectives set forward at the start of the project, we are proud to report that nearly all milestones and deliverables have been achieved (see table of deliverables attached). Thus, the level of advance was achieved as predicted, reaching deliverables and milestones on or even ahead of time. The coordinating team has close monitored the work progress in the different work packages.
The number of publications generated by the consortium was particularly impressive, with a total of 140 publications in high impact peer-reviewed international journals such as Nature, Nature Commun, Science, Science Transl Med, Cell, Mol Cell, Cell Metab, Lancet Diabet Endocrinol, Proc Natl Acad Sci USA, J Cell Biol, Nucleic Acid Res, eLIFE, Cell Death Diff, PLoS Genet, Diabetes, Diabetologia etc. Of note, 23 of these publications were collaborative publications between members of the consortium.

WP 1 – Organelle dysfunction – ER stress and redox
The work in WP 1 was designed to characterise the role of endoplasmic reticulum (ER) stress and reactive oxygen species (ROS) in beta cell and BAT dysfunction, and to explore novel approaches to protect these cells against these stress signals. We aimed first to characterise the impact of proinflammatory cytokines (e.g. interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), alone or in combinations) and glucolipotoxicity (mediated by high concentrations of glucose, palmitate and/or oleate) on the three strands of ER stress signalling in beta cells, focusing on newly discovered facets of IRE1 and PERK activation. We intended also to study ER stress signalling associated with BAT differentiation, lipid storage and lipolysis. Unresolved ER stress culminates in mitochondrial apoptosis, where the Bcl-2 protein family plays a crucial role to trigger cytochrome c release and apoptosis assembly. Conversely, Bcl-2-containing complexes operate as stress rheostats that determine the amplitude and kinetics of adaptive responses against ER stress-related injuries. We aimed as well to study the subcellular generation and distribution of ROS and define their toxic potential in light of recent evidence for the crosstalk between the unfolded protein response (UPR) and ROS production. The formation of disulfide bonds in the ER leads to ROS production and can influence the secretory output of beta cells and adipose tissue and potentially contributes to apoptosis.

In the first 18 months of the project (Report Period 1 (RP1)), we have shown for the first time that markers of ER stress are present in islets from patients with type 1 diabetes mellitus. This provides an important “proof of concept” for part of the proposed work in WP1. New observations by members of the consortium linked ER stress in beta cells to the induction of exacerbated local inflammation, contributing to initiation of islet inflammation and diabetes. New IRE1 interacting proteins that function in beta cells have been identified. Their further characterization promises to shed light on cell death and antigen presentation in beta cells.

New tools were developed and validated to selectively manipulate the different strands of the UPR, while new tools to monitor thiol redox in the ER have also been set up. We provided evidence that the H2O2-sensitive HyPer protein, contrary to other cell organelles, is not suited for monitoring H2O2 generation within the ER. New alternatives are now being explored to fulfil this need. Finally, we observed that peroxiredoxin-IV overexpression significantly protects insulin-producing cells against exogenous H2O2, and partially against cytokines / FFAs-mediated toxicity.

In Report Period 2 (RP2; month 18-36) novel findings characterized in detail the crosstalk between ER stress and inflammation induced apoptosis in models of diabetes, showing key roles for NF-xB, IRE1α, Bim and Bcl-xL in the pro-inflammatory and pro-apoptotic role of ER stress. A new protective pathway in lipotoxic conditions, involving the ER stress response and AKT signaling, was elucidated. Thus it was shown that following palmitate exposure, JunB stimulates XBP1 expression via the transcription factor c/EBPδ. The induction of XBP1s by palmitate promotes AKT activation and subsequent inhibition of the pro-apoptotic BH3 only protein BAD. The crosstalk between the ER stress response and AKT signaling constitutes a crucial defense mechanism against lipotoxic beta cell death and may represent an important mechanism for the prevention/treatment of T2D. In a collaborative study with a group from Kyoto, partners from BetaBat have explored the role of the UPR in glucose intolerance and beta cell dysfunction associated with treatment with modern anti-psychotic drugs such as olanzapine. They unmasked a blockage in PERK activity linked to this treatment that may synergize with other factors to promote beta cell apoptosis.

The mechanisms regulating IRE1α activation by pro-inflammatory cytokines were further clarified by BetaBat partners. This was done by combining MAPPIT (MAnmalian Protein-Protein Interaction Trap)-based IRE1α interactome and functional genomic analysis of human and rodent beta cells exposed to pro-inflammatory cytokines, to identify novel cytokine-induced regulators of IRE1α. Based on this approach, they identified N-myc interactor (NMI) as an IRE1α-interacting/modulator protein in rodent and human pancreatic beta cells. An increased expression of NMI was detected in islets from non-obese diabetic (NOD) mice with insulinitis, and in rodent or human beta cells exposed in vitro to the pro-inflammatory cytokines IL-1β and IFN-γ. Detailed mechanistic studies demonstrated that NMI negatively modulates IRE1α-dependent activation of JNK and apoptosis.
in rodent and human pancreatic beta cells. These findings, recently published, identified NMI induction as a novel negative feedback mechanism that decreases IRE1α-dependent activation of JNK and apoptosis in cytokine-exposed beta cells. In Report Period 3 (RP3, month 36-48), BetaBat partners identified a loss-of- function mutation in PPP1R15B, a protein involved in eIF2α dephosphorylation, as causal of a new syndromic form of diabetes. They applied structural and mechanistic approaches to uncover the basis of beta cell demise in this new syndrome.

Interestingly, a collaborative study inside BetaBat discovered the mechanisms explaining why pancreatic beta cells, but not alpha cells, undergo loss of function and eventually apoptosis in the course of metabolic stress. Electron microscopy was used to search for markers of apoptosis and ER stress in alpha and beta cells in islets from T2D or non-diabetic individuals. There was a significant increase in beta cell apoptosis in T2D respect to controls (0.4% apoptosis in non-diabetic controls vs 6.0% in T2D), while no alpha cell apoptosis was detected. There was, however, similar ER stress in alpha and beta cells from T2D patients. Human islets or FACS-purified rat beta and alpha cells exposed in vitro to the saturated free fatty acid palmitate showed a similar response as the T2D islets, i.e. both cell types showed signs of ER stress but only beta cells progressed to apoptosis. Mechanistic experiments indicate that this alpha cell resistance to palmitate-induced apoptosis is explained, at least in part, by abundant expression of the anti-apoptotic protein Bcl-xL, confirming the cross talk between ER stress (WP 1) and mitochondrial pathways of apoptosis (WP2).

Members of BetaBat characterized the presence of basic mechanistic differences between human and rodent beta cells following cytokine-induced ER stress, and identified JNK1 as a key mediator of ER stress-induced human beta cell apoptosis.

WP 2 - Organelle dysfunction – mitochondria and apoptosis signalling

The work in WP2 was highly complementary to WP 1 and aimed to define the Bcl-2 family members regulating pancreatic beta cell dysfunction and apoptosis following exposure to proinflammatory cytokines and glucolipotoxicity. This information will help the individualization of therapies aiming to protect beta cells, one of the goals of the project. Another area addressed by WP2 is the unfolded protein stress response in the mitochondrial matrix (UPRmt) and the crosstalk between this cellular stress response and the nucleus, modulating expression of key chaperones. Mitochondrial integrity is important to beta cell function, and mutations in nuclear- and mitochondrial-encoded genes modulate susceptibility to T1D and T2D. Mitochondrial activity is also crucial for brown adipocytes, and WP 2 aims to define whether mitochondrial dysfunction in BAT in the context of diabetes shares the same mitochondrial pathogenic mechanisms as beta cells, and to develop novel and targeted intervention strategies for improving mitochondrial function in brown adipocytes.

In the first 18 months of the project we have defined the Bcl-2 family members regulating pancreatic beta cell dysfunction and apoptosis in the context of ER stress, lipotoxicity and cytokine-induced apoptosis, reaching ahead of time most parts of milestone MS5. For lipotoxicity, the two key pro-apoptotic BH3-only proteins are DP5 and PUMA, while Bim plays a central role for cytokine-induced apoptosis. Importantly, we have shown that USP-18 regulates candidate gene networks related to T1D, and the candidate gene PTPN2, regulates Bim, a key BH3-only protein involved in pancreatic beta cell apoptosis. This provided a clear link between mechanisms of beta cell apoptosis and candidate genes for diabetes. Regarding the work on BAT, we have identified p38 MAP kinase as an essential link between FGF21 signaling and promotion of mitochondrial function. The required experimental models have been developed to study apoptosis in brown adipocytes, using mitochondrial toxins and ER stressors. This will enable detailed mechanistic studies of the brown adipocyte apoptosis machinery and the pathways mediating BAT involution in the follow up of the project.

In RP2, a finding of particular relevance was the discovery that two candidate genes for T1D, namely Glis3 and Bach 2, regulate beta cell apoptosis via modulation of the Bcl2 family member Bim. In addition, Bach 2 also modulates the expression of the anti apoptotic proteins Bcl2-A1 and Mcl1. This confirms the clinical relevance of the BetaBat-proposed focus on the role for Bcl2 proteins in diabetes. Viral vectors, siRNAs and small chemical models are being developed to modulate these proteins to protect beta cells in diabetes.

In RP3, Bad, DP5 and Bim were identified as the key mediators of frataxin deficiency-induced beta cell apoptosis. cAMP induction prevented the mitochondrial oxidative stress-dependent activation of this apoptotic process.

Regarding BAT, members of the consortium have demonstrated that a mitochondrial retrograde signalling triggers the release of novel batokines, and have also identified a new protein with the potential to induce WAT browning. They also discovered that beta-Klotho expression can be regulated by drugs and hence can be used as a tool to improve
mitochondrial activity of brown adipocytes.

WP 3 - Crosstalk between BAT, beta cells and other key tissues in diabetes

The work in WP3 focused on the mechanisms mediating the improvement in insulin sensitivity and beta cell function observed following increased BAT activation. Evidence from animals and humans suggests that increased BAT activation protects against diabetes: indeed cold-acclimated rats do not succumb to streptozotocin (STZ)-induced diabetes like those housed at room temperature. Whilst much of this improvement can be attributed to increased glucose disposal and lipid uptake by BAT, there is increasing evidence to suggest that BAT possesses a unique adipokine profile different than what is seen in WAT. Using proteomic screening approaches these potential “BATokines” are being characterised. To assess the role of BAT dysfunction in T2D, there is an additional focus on the central mechanisms regulating thermogenesis. Globally, changes observed in key organs in response to altered thermogenic activity will be assessed on genetic, lipidomic and metabolic platforms using a systems approach to identify patterns of allostatic alterations across tissues which predict specific cues for disease progression. This will be integrated with the more cell targeted systems biology approach used in WP 4. Ultimately we aim to identify new mechanisms to enhance the effectiveness of BAT to improve beta cell function, understand how they may elicit changes across multiple tissues to reduce cell stress and thus pinpoint new aetiological targets and time points for disease intervention in diabetes.

In the first 18 months of the project, an in silico approach for identifying batokines was validated, leading to the identification of two new molecules. More batokines are in the pipeline. It was also observed that an existing WAT cytokine may have relevance for BAT function. We have optimised protocols for studying the acute central responses to cold-exposure and high fat feeding to characterise the CNS control of BAT. Furthermore, we have shown that even in a model of severe hypoinsulinaemia BAT retains its capability to remove glucose from the circulation. BAT even appears to adapt to burn more glucose than fat when faced with impaired lipid uptake/storage due to lack of insulin. In an important “proof of concept” for the goals of WP3, it was confirmed that increasing BAT activity improves the metabolic profile of mice with impaired insulin secretion. In other important experiments we have shown that beta-PPAR-gamma2 overexpression leads to changes in many lipid species in the islets of mice receiving high fat diet. The role of these lipids will be further investigated in the context of lipitoxicity and beta cell failure. Finally, we have further developed the lipidomic analysis of adipose tissue: improved sensitivity of the method will facilitate the comparison of lipidomic profiles between the tissues.

In RP2, two important advances have been made by BetaBat partners. First, in silico and RNAseq analysis of BAT plus prediction of secretable proteins has identified 3 additional batokines which are now under analysis. One of them, a new batokine that acts as a thermogenic “brake”, is of particular interest, since it has the potential to be used to manipulate energy dissipation for therapeutic ends. Second, a chemically defined differentiation protocol to generate human BAT from iPSc has been developed, allowing also definition of intermediate precursors.

In RP3, the neurotrophic properties of a new batokine have been characterized, providing strong evidence for this molecule’s role in expansion of the sympathetic nervous network in brown and white adipose tissue in response to cold exposure. The role of Extra Cellular Matrix remodelling, collagen turnover and fibro-inflammation in the impairment of brown cells function/ differentiation was also characterized. Interestingly, it was found that this alters batokine production in the context of obesity and metabolic dysfunction.

WP 4 - A systems biology approach to identify new effectors of cellular dysfunction in diabetes

In this WP we aimed to utilize an integrative systems biology approach to identify molecular signatures associated with beta cell and BAT dysfunction in diabetes. Bayesian dependency network approach is applied to study dependencies between the molecular variables at multiple levels (e.g. transcriptomic, proteomic, metabolomic) and cellular outcomes. The network structures provide information on which “nodes”, e.g. proteins, metabolites, miRNAs, are mutually dependent, which are highly connected as the so-called “network hubs”, and which are independently associated with specific cellular outcomes of interest. Recently developed multi-way multi-source approaches have been adopted to study the dependencies of such networks across tissues in collaboration with WP 3. This analysis helped us understand as well as predict the effects of specific genetic/pharmacological intervention at the systems level. Such information was used to deliver novel target candidates testable in WP 5. The molecular signatures obtained in WP 4 provided correlates for far downstream biological outcomes.
(function and survival of beta cells and BAT) but also for the intermediate outcomes of organelle stress that we hypothesize to have a critical role in the far downstream outcomes.

In the initial 18 months of the project, we have used RNA sequencing (RNA-seq) to identify all transcripts, including splice variants, expressed in human islets of Langerhans under control condition or following exposure to the pro-inflammatory cytokines IL-1β and IFN-γ. A total of 29,776 transcripts were identified as expressed in human islets. Expression of around 20% of these transcripts was modified by pro-inflammatory cytokines, including apoptosis- and inflammation-related genes.

Chemokines were among the transcripts most modified by cytokines, a finding confirmed at the protein level by ELISA. Interestingly, 35% of the genes expressed in human islets undergo alternative splicing as annotated in RefSeq, and cytokines caused substantial changes in spliced transcripts. Nova1, previously considered a brain-specific regulator of mRNA splicing, was shown to be expressed in islets and its knockdown (KD) modified splicing. This study doubles the number of known genes expressed in human islets, and shows that cytokines modify alternative splicing in human islet cells. Importantly, it indicates that more than half of the known T1D candidate genes are expressed in human islets. This, and the production of a large number of chemokines and cytokines by cytokine-exposed islets, reinforces the concept of a dialog between pancreatic islets and the immune system in T1D. RNAseq is currently been performed in pancreatic islets obtained from T2D patients, and human islets exposed in vitro to the free fatty acid palmitate. Regulation of alternative splicing in human islet cells and FACS-purified rat beta cells will be further characterized by knocking down splicing regulators and performing new rounds of RNAseq.

The bioinformatics framework has been set up to enable metabolic modeling at the genome-scale using genomic, proteomic and metabolomics data. Specifically, constraint-based modelling (CBM) provides a framework for describing and predicting metabolic phenotypes. This computational approach utilizes stoichiometric, enzymatic and thermodynamic constraints to determine feasible metabolic fluxes. The release of the global human metabolic network, Recon 1, in 2007 has enabled application of CBM on diseases such as cancer or diabetes. We are utilizing the Recon 1 genome scale human metabolic network together with the COBRA-package to analyze the gene expression data from islet cells. The resulting flux solutions will be further analysed by gene set enrichment to discover statistically significant a priori defined set of genes reflecting enzymatic activity on specific metabolic pathways concordant with differences between different biological states.

In RP2, the use of meta-analysis and network inference to define temporal profiling of cytokine-induced genes, identified several key genes that regulate cytokine effects in human, mouse and rat beta cells. Furthermore, Nova1 was identified as a key regulator of alternative splicing in beta cells.

Of particular relevance, the function of three candidate genes for T1D, namely Glis3, Bach2 and CTSH, has been characterized. Importantly, it was found that these genes regulate different pathways leading to beta cell death via activation of the mitochondrial or intrinsic pathway of apoptosis and local inflammation, providing a key “proof of concept” for the relevance of studying these candidate genes at the beta cell level. Furthermore, members of BetaBat harnessed the potential of DNA and RNA sequencing in human pancreatic islets from 89 deceased donors to identify genes of potential importance in the pathogenesis of T2D. This represents the most complete catalog up to now of genetic variants regulating gene expression (eQTL) and exon use (sQTL) in human islets, including many long noncoding RNAs, which are enriched in known T2D-associated loci. Of 35 eQTL genes, whose expression differed between normoglycemic and hyperglycemic individuals, siRNA-mediated knockdown of tetraspanin 33 (TSPAN33), 5′-nucleotidase, ecto (NTSE), transmembrane emp24 protein transport domain containing 6 (TMED6), and p21 protein activated kinase 7 (PAK7) in INS1 cells resulted in reduced glucose-stimulated insulin secretion. In addition, this study provided a genome-wide catalog of allelic expression imbalance, which is also enriched in known T2D-associated loci. Notably, allelic imbalance in paternally expressed gene 3 (PEG3) was associated with its promoter methylation and T2D status. Taken together, this study provides new insights into the complexity of gene regulation in human pancreatic islets and better understanding of how genetic variation can influence glucose metabolism.

In RP3, the global cell autonomous responses of pancreatic beta cells, as compared to alpha cells, have been defined based on advanced bioinformatics tools. Thus, members of BetaBat described a complex cell autonomous gene network that explains the particular susceptibility of beta cells to viral infections. T1D is an autoimmune disease caused by a progressive loss of pancreatic beta cells via apoptosis while neighbour alpha cells are not killed. The triggering of T1D depends on environmental factors that interact with predisposing genes to induce the autoimmune assault against the beta cells. As shown in WP4, many candidate genes for T1D act at the beta cell level and regulate antiviral responses. Accumulating evidence supports the
implication of viral infections, particularly by enteroviruses (e.g. Coxsackievirus, CVB), as triggers for the development of T1D. Cellular permissiveness to viral infection is modulated by cellular innate antiviral responses, which vary among different tissues or cell types. WP4 described that global gene expression is similar in cytokine-treated and virus-infected human islet cells, with up-regulation of a large number of genes and transcription factors involved in cell autonomous immune responses to viral infections. Since this type of cellular response may determine the cell outcome during a viral infection, we next compared the responses of pancreatic alpha and beta cells to infection by potentially diabetogenic CVB5 and 4. We observed that alpha cells trigger a more efficient antiviral response than beta cells, including higher basal and induced expression of STAT1-regulated genes, and are thus better able to clear viral infections than beta cells. This cannot be explained by different expression of receptors for the virus, since the viral receptors are similarly expressed in alpha and beta cells. Thus, pancreatic alpha and beta cells have different cell autonomous signatures, which may explain their different ability to clear viral infections. These differences may explain why pancreatic beta cells, but not alpha cells, are targeted by an autoimmune response and killed during T1D (published).

WP 5 - Therapeutic approach: testing targeted interventions to revert cellular dysfunction in diabetes

WP5 was a central WP in the project, and aimed to translate the novel information generated by WPs 1-4 into novel approaches to revert beta cell and BAT dysfunction in diabetes, leading to (pre)clinical trials of new compounds. This work progressed along several complementary lines, starting first by investigating whether the efficacy of GLP-1 can be improved by increasing its positive impact on beta cell mass and function. GLP-1 analogues were tested for their specific effects on the diverse branches of the ER stress response and in vivo for their glucagon suppressing capability. In a second line of investigation, sirtuins 3 and 4 were tested as possible candidates to improve beta cell and BAT mitochondrial function. In a third line of investigation, novel targets, generated through studies in WPs 1-4, were investigated and validated using overexpression or knockdown approaches supported by the SME Sirion, specialized in the production of recombinant adeno- and adeno-associated viruses (AAVs). In the fourth line of activities, assay development and HTS were performed for the identification of the small molecules that improve cell survival and function in diabetes. In the fifth line of activities, Groop and colleagues validated the use of particular candidate genes for selecting individual therapeutic strategies to treat diabetes. Specifically, Groop and colleagues discovered that a variant of the adrenergic receptor A2-AR increases susceptibility to diabetes and proposes that a specific antagonist of this receptor may help to treat the diabetic carriers of this gene variant. This pilot clinical trial, integrated in BetaBat, represented the first successful individualized therapeutic strategy for T2D and proposes that a specific antagonist of this receptor may help to treat the diabetic carriers of this gene variant.

Pathway analysis of candidate genes expressed in human islets identified a central role for interferon-regulated pathways and the tyrosine kinase TYK2. Polymorphisms in the TYK2 gene predicted to decrease function are associated with a decreased risk of developing type 1 diabetes. TYK2-silenced human beta cells exposed to polyinosinic-polycitidilic acid (PIC; a mimick of double stranded RNA produced during viral infection) showed less type I interferon pathway activation and lower production of IFNs and CXCL10. These cells had also decreased expression of MHC class I proteins, a hallmark of early beta cell inflammation in T1D. Importantly, TYK2 inhibition prevented PIC-induced beta cell apoptosis via the mitochondrial pathway of cell death. These novel findings suggest that TYK2 regulates apoptotic and pro-inflammatory pathways in pancreatic beta cells via modulation of IFNα signaling, subsequent increase in MHC class I protein, and modulation of chemokines such as CXCL10 that are important for recruitment of T cells to the islets.
neurological dysfunction and diabetes. The mechanisms by which these drugs improve beta cell survival are presently being explored. The importance of the mitochondrial sirtuin, SIRT3, in mediating thermogenesis by brown adipose tissue (BAT) has been demonstrated, and data have been obtained showing that FGF21 can improve the BAT differentiation of precursor cells and that transduction of SIRT3 can also improve this differentiation process. In collaboration with WP2, the lipotoxicity-dependent induction of PUMA and DP5 was demonstrated and the role of DP5 in apoptosis was elucidated using siRNA-mediated knockdown experiments and DP5 knockout mice. Separately, a small molecule antagonist of the transcription HNF4α has been identified by a high-throughput screening and shown to induce beta cell replication in mice and rabbits. HNF4α agonists have also been identified, and are being tested in models of T2D. Finally, the first intervention trial has been initiated to test yohimbine-increased insulin secretion in patients with at risk allele of the ADRA2 gene, previously shown to reduce insulin secretion capacity in beta cells. The study was completed and published in RP3, and the results support the viability of genotype-based stratified treatment.

In RP2, new studies have shown that glutamine-induced IGF2 secretion is regulated at the translational level both in insulinoma and primary beta cells. Glutamine-induced IGF2 secretion leads to increased Akt phosphorylation and reduced cytokine-induced apoptosis. Using βIGF-2KO mice members of BetaBat further showed that IGF2 is required to preserve normal GSIS in old female mice and to protect against beta cell loss in high fat diet fed mice; IGF2 is also required for normal estrogen-induced beta cell mass expansion in pregnancy and account for ~30% of increased beta cell mass in response to pharmacologically-induced insulin resistance.

In an important “proof of concept” experiment, based on mechanistic observations made in WP1, it was shown that chemical chaperones (modulators of ER stress) protect two mouse models of T1D against development of hyperglycaemia. This may lead to clinical trials in the coming years.

Novel screens for compounds that prevent beta cell apoptosis are under way, as are specific interventions based on the genotype of non-diabetic and T2D patients carrying homozygous risk alleles.

In RP3, GLP-1 and GIP agonists have been shown to protect beta cells and neurons in frataxin-deficient beta cells through mitochondrial mechanisms. This may have therapeutic potential in the neurodegenerative disease Friedreich’s ataxia, and a pilot clinical trial will start in the coming months. Clic4 was identified as a now protein that sensitizes beta cells to cytokine and glucolipotoxicity-induced apoptosis making this protein an interesting target for protecting beta cells against death. A high throughput screen for molecules that could reverse lipotoxicity effects, using the insulin promoter in a screening assay, yielded two interesting hits that are being pursued further.

WP 6 - Project management, coordination and training

The WP 6 provided management, administrative support and training for the scientific WPs in the consortium. The training activities focused on enabling all scientists from the partner laboratories to learn and apply the novel techniques and tools gathered in the consortium, to harmonise the work in and between the laboratories and to facilitate crosstalk between disciplines. The management part of WP 6 ensured an efficient management and administration of the consortium and a smooth communication with the European Commission (EC). WP 6 also provided the educational and organisational backbone for sharing information and fostering collaborations with related projects in FP7.

During the first 18 months management of the project developed smoothly, with a successful focus in promoting interactions between partners and follow-up the work. For this purpose, the kick-off meeting on December 2011 in Brussels was a key event: clear objectives were defined for the first year of the project by each PI, and their execution was closely followed by the Coordinator via frequent phone calls and meetings with the WP leaders and PIs. Furthermore, at the second annual meeting of the project, on November 2012 in Barcelona, it became clear that the settled goals of the project were been fulfilled. BetaBat also organized on this occasion, in collaboration with the University of Barcelona, an open symposium for the scientific community, entitled “From whole body metabolism to cell dysfunction in diabetes”, attended by a large number of scientists (see announcement of the meeting in annex). Great effort was put in getting the website (http://betabat.ulb.ac.be/) up and running, which was successfully achieved by month 1. This website is kept up to date, in order to promote the work performed within BetaBat, but also to give internal feedback to partners on the different activities of the consortium. Next to the website, intense communication exists between partners via frequent phone-conferences and one-on-one meetings between.

Nevertheless, particular attention is put on broad meetings between partners. In order to save money, often these face-to-face
meetings are combined with international conferences where several partners are present. Administrative issues are few and when problems are present, they are resolved smoothly. In year one there was the substitution of two PIs in two partners (Sandord/Burham Medical Research Institute and DNAvision), but the changes were smooth and the new PIs fully integrated in the project. Major attention has been given to communication and bringing the results from the consortium and the BetaBat project to the public. Press events were organized in Belgium, with links with the Juvenile Diabetes Research Foundation, international press releases on findings from the project (the RNAseq of human islets, described above in WP4, was the subject of a Press Release in collaboration with the JDRF, generating much interest). A lot of Press releases are still available on Internet with the help of the External Relations Department, Research Communication, ULB, Belgium.

In RP2, an overview of the activities of the consortium was presented to the International Scientific Advisory Board for scientific review and guidance. This was organized at the BetaBat 3rd Internal Meeting in Cambridge (December 2013), and the Advisory considered the evolution of BetaBat excellent.

During the second reporting period the participation of Benef 7 (VTT) was terminated, and a new partner, Steno Diabetes Center (SDC), added to BetaBat. This was due to accommodate the fact that Prof. Oresic, leader of WP4, moved to the SDC. These administrative changes did not delay the project or WP 4 – both moved ahead as planned, with minimal or no delays.

During the second reporting period an open symposium entitled “Brown fat and beta cells: examining inter-organ crosstalk and cellular dysfunction in metabolic disease” was organized in Cambridge in December 10-11, 2013. Major attention was given to communication and bringing the results from the BetaBat project to the public. Several press events were organized focusing on novel findings from the project and important awards received by partners of the consortium.

In RP3 the focus was on further stimulating collaboration, completing the ongoing collaborative research projects and fulfill the planned deliverables and milestones. This was successful, leading to several articles in process of submission or completion and assuring that nearly all deliverables and milestones were achieved. During the third reporting period an open symposium entitled “Beta cells and adipose tissue – partners in crime for the development of T2D” was organized in Malmo, Sweden, in December 12, 2014. Particular attention was given to communication and bringing the results from the BetaBat project to the public. Several press events were organized focusing on novel findings from the project. Furthermore, there were detailed discussions on how to continue the collaborative work between partners beyond the end of BetaBat, which was instrumental in the preparation of a new project, “T2DSystems”, recently funded by Horizon2020 (to start in 01/2016), and for the IMI Project Rhapsody, presently under evaluation.

Potential Impact:
The potential impact of the present project is great, both on a scientific and a therapeutic level. We have managed to execute this work with great energy, with all partners contributing substantially to the goals of the project. The identification of basic disease mechanisms, the development of tools to modulate these mechanisms, and the performance of high throughput analysis to identify new molecules to protect beta cells and brown adipose cells hold great promise. Research has progressed well in all work packages, and the first steps to the clinic have been made in WP5. Many interactions have also taken place between our BetaBat consortium and other FP7 consortia dealing with diabetes, particularly NAIMIT and BETAIMAGE, where the coordinator of BetaBat also participated as a partner. BetaBat also interacted with the new NIH initiative, the HIRN.

In the dissemination arena, up to date, the internet page has attracted an important public. We have made professional contacts through the information on the web page. Also the number of scientific publications was outstanding, with a total of 140 publications over a period of 4 years in high impact peer-reviewed international journals such as Nature, Nature Commun, Science, Science Transl Med, Cell, Mol Cell, Cell Metab, Lancet Diabet Endocrinol, Proc Natl Acad Sci USA, J Cell Biol, Nucleic Acid Res, eLIFE, Cell Death Diff, PLoS Genet, Diabetes, Diabetologia etc. Of note, 23 of these publications were collaborative publications between members of the consortium. The BetaBat consortium not only performed high quality research, but also disseminated the results both in peer-reviewed media, and via press releases and interviews in Europe and in the USA.

Impact:
Researchers in the diabetes field and members of the press perceive BetaBat as a well run consortium of high-level researchers in the field of diabetes, with a strong multidisciplinary approach to understand disease at an holistic level. BetaBat has taken major steps in the path to a better understanding of the pathogenesis of diabetes and towards finding novel
treatments based on knowledge of cellular dysfunction in key organs affected by the disease, namely the beta cells and adipose tissue. Impact has been relevant for researchers and society as a whole.

Impact on researchers: BetaBat has brought together scientists working on beta cells, brown and white adipose tissue and other organs that contribute for the pathogenesis of diabetes. This created a unique and highly interdisciplinary collaborative network of researchers in Europe, allowing these researchers to grow and, through collaborations and exchanges, to increase their impact in the field. Many of the researchers involved in BetaBat succeeded in leveraging the knowledge obtained within the project to attract additional funding. Of particular relevance in this context is the new and recently funded Horizon 2020 project T2DSystems, in many ways a follow up project of BetaBat, and new funding obtained by European partners of BetaBat at the NIH and IMI levels. Long-lasting collaborations were developed, with joint PhD projects between partners, exchanges of personnel, sharing of techniques and research tools. Of particular relevance, partners who have never before performed functional genomics analysis based on RNA sequencing and consequent bioinformatics, or used high throughput screening to identify novel and promising agents to treat diabetes, become familiar with these techniques. This allowed them to obtain novel and very relevant data, and increased the quality of their scientific output.

Impact on society as a whole: Diabetes is a major public health problem, affecting more than 380 million individuals worldwide. In Europe, 55 million citizens suffer from diabetes and an additional 66 million have impaired glucose tolerance (IGT), causing 630,000 deaths each year. By 2030 the European prevalence of diabetes is projected to reach 150 million, meaning that every 5th European will have diabetes or IGT. As a result, around 28% of European healthcare budgets are assigned to diabetes care, costing at least 78 billion Euros a year. Diabetes is a multifactorial disease with many contributing risk factors, including age, obesity and a family history of diabetes. The rise in T2D prevalence is in part due to ageing; 30% of the European population is currently over 50 years of age, and this will increase to more than 40% by 2030. The increasing obesity prevalence also contributes to the T2D epidemic, with overweight and obesity rates currently ranging between 55-62% in men and 48-58% in women in Central, Eastern and Western Europe. The prevalence of T1D, a disease affecting mostly children and teenagers, is also expected to double in Europe in the coming decades.

Through the findings and results achieved within BetaBat, we hope to fundamentally change the way diabetes is understood at the cellular and molecular level, opening the way for “logical” clinical interventions that target disease pathogenesis and not only its signals, i.e. hyperglycemia. Of particular relevance where the first pilot trials performed by partners on BetaBat based on specific candidate genes present in diabetic patients. Success in these ongoing trials will open the way for truly personalized medicine, a goal that will be pursued in the follow up Horizon 2020 project T2DSystems. Importantly, we have devoted a lot of effort in sharing our results in an understandable manner with non-scientists since the society needs to be informed of the research progress to understand the relevance of EC-sponsored research. Thus, we have established an accessible website, and participated in a series of press releases and interviews with the lay press to “spread the news” about BetaBat findings.

The project website (http://betabat.ulb.ac.be/) has been available since the start of the project. It shows the logo of the European Commission, FP7 and BetaBat and contains an open access section. This is aimed at researchers, patients and all interested members of society, presenting a summary of the project (including background information, aims of BetaBat, information on the participants, BetaBat open scientific meetings etc). Our website has been visited by 7821 unique visitors during the period of the project. A password-protected section for BetaBat researchers holds confidential information. This was supplemented by a systematic distribution of novel publications by partners of BetaBat by email, these articles were distributed to all partners at the proof stage, a simple approach that much stimulated collaboration.

Another way of disseminating the results obtained within BetaBat was via publications in top scientific journals. BetaBat scientists published a total of 140 publications over a period of 4 years in high impact peer-reviewed international journals such as Nature, Nature Commun, Science, Science Transl Med, Cell, Mol Cell, Cell Metab, Lancet Diabet Endocrinol, Proc Natl Acad Sci USA, J Cell Biol, Nucleic Acid Res, eLIFE, Cell Death Diff, PLoS Genet, Diabetes, Diabetologia etc. Of note, 23 of these publications were collaborative publications between members of the consortium. Other dissemination activities include publication of articles in the popular press, for a broader, non-scientific audience. BetaBat has also received a lot of attention by the lay press. Different press releases have been launched, both at the start of the project and as a consequence of
particularly relevant publications (see list of disseminations) at key occasions during the project.

Exploitation of results:
Most of the research performed in BetaBat was preclinical, paving the way for future clinical work to be pursued in T2DSystems and other follow up projects. Of particular relevance was the discovery of novel small molecules, based on high throughput screening (presently under validation), and of novel pathways to stimulate BAT, which may become relevant therapies to treat obesity.
For the SMEs the results obtained within NAIMIT increased their visibility and access to new customers. Thus, Syrion established itself as a reliable provider of novel and sophisticated viral vectors, and is a partner in T2DSystems. DNAVision, in charge of the RNA sequencing, evolved from a mostly Walloon company into a company well know across Europe, and is now performing RNA sequencing to several new customers.

List of Websites:
http://betabat.ulb.ac.be

Informazioni correlate

| Risultato in breve | The relation between brown fat and pancreatic beta cells |

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