

## Role of ZAC1 in transient neonatal diabetes mellitus and glucose metabolism

Anke Hoffmann, Dietmar Spengler

Anke Hoffmann, Dietmar Spengler, Max Planck Institute of Psychiatry, Translational Research, 80804 Munich, Germany

Author contributions: Hoffmann A and Spengler D jointly contributed to this work.

Supported by Max Planck Institute of Psychiatry.

Conflict-of-interest statement: The authors declare no conflict of interest.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

Correspondence to: Dietmar Spengler, MD, Max Planck Institute of Psychiatry, Translational Research, Kraepelinstrasse 2-10, 80804 Munich, Germany. [spengler@psych.mpg.de](mailto:spengler@psych.mpg.de)  
Telephone: +49-89-30622587  
Fax: +49-89-30622605

Received: April 20, 2015

Peer-review started: April 24, 2015

First decision: June 3, 2015

Revised: June 19, 2015

Accepted: July 11, 2015

Article in press: July 14, 2015

Published online: August 26, 2015

### Abstract

Transient neonatal diabetes mellitus 1 (TNDM1) is a rare genetic disorder representing with severe neonatal hyperglycaemia followed by remission within one and a

half year and adolescent relapse with type 2 diabetes in half of the patients. Genetic defects in TNDM1 comprise uniparental isodisomy of chromosome 6, duplication of the minimal TNDM1 locus at 6q24, or relaxation of genomically imprinted *ZAC1/HYMAI*. Whereas the function of HYMAI, a non-coding mRNA, is still unidentified, biochemical and molecular studies show that zinc finger protein 1 regulating apoptosis and cell cycle arrest (ZAC1) behaves as a factor with versatile transcriptional functions dependent on binding to specific GC-rich DNA motives and interconnected regulation of recruited coactivator activities. Genome-wide expression profiling enabled the isolation of a number of *Zac1* target genes known to regulate different aspects of  $\beta$ -cell function and peripheral insulin sensitivity. Among these, upregulation of *Ppar $\gamma$*  and *Tcf4* impairs insulin-secretion and  $\beta$ -cell proliferation. Similarly, *Zac1*-mediated upregulation of *Socs3* may attenuate  $\beta$ -cell proliferation and survival by inhibition of growth factor signalling. Additionally, *Zac1* directly represses *Pac1* and *Rasgrf1* with roles in insulin secretion and  $\beta$ -cell proliferation. Collectively, concerted dysregulation of these target genes could contribute to the onset and course of TNDM1. Interestingly, *Zac1* overexpression in  $\beta$ -cells spares the effects of stimulatory G-protein signaling on insulin secretion and raises the prospect for tailored treatments in relapsed TNDM1 patients. Overall, these results suggest that progress on the molecular and cellular foundations of monogenetic forms of diabetes can advance personalized therapy in addition to deepening the understanding of insulin and glucose metabolism in general.

**Key words:** Diabetes mellitus; *ZAC1*; Transient neonatal diabetes mellitus 1; Genomic imprinting; Insulin; Glucose; Target genes; Tailored therapy

© **The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** Accidents of nature leading to rare genetic diseases can provide important insights into the molecular and cellular foundations of related common diseases. Various genetic anomalies at chromosome 6q24 manifest with life-threatening transient neonatal diabetes mellitus (TNDM1). All of these genetic defects share overexpression of the maternally imprinted transcriptional regulator *ZAC1*. Genome-wide expression profiling identified a number of downstream target genes sharing a critical role in insulin secretion,  $\beta$ -cell proliferation, and survival. Importantly, *Zac1* overexpression in  $\beta$ -cells spares the effects of G-protein signaling on insulin secretion opening the prospect for tailored therapy in TNDM1 patients.

Hoffmann A, Spengler D. Role of ZAC1 in transient neonatal diabetes mellitus and glucose metabolism. *World J Biol Chem* 2015; 6(3): 95-109 Available from: URL: <http://www.wjgnet.com/1949-8454/full/v6/i3/95.htm> DOI: <http://dx.doi.org/10.4331/wjbc.v6.i3.95>

## INTRODUCTION

The individual, societal, and economic burden of the diabetes epidemic has raised steadily over the last decades despite continuous refinements in dietary and pharmacological therapies<sup>[1]</sup>. On current trends, at least one in 10 people alive today are at risk to suffer from diabetes at some stage during their lifespan. At the same time, the total number of individuals being at risk to develop diabetes is predicted to double in a generation from 150 million in 2000 to 300 million by 2025. Existing medical treatments frequently ameliorate diabetic conditions without reinstating normal metabolism and thus leave many patients exposed to debilitating, or even life-threatening, impairments<sup>[2]</sup>. In conjunction with genetic predisposition (particularly in some ethnicities) the epidemic derives mainly from type 2 diabetes (T2D) and associated conditions termed "diabesity" and "metabolic syndrome". These states encompass various risk factors such as obesity, overly rich nutrition, and a sedentary lifestyle<sup>[3]</sup>. Although lifestyle modifications (balanced diet, more exercise, and weight reduction) are well-recognized to delay, or even prevent, T2D in persons at risk, such cautionary measures are notoriously hard to sustain<sup>[4]</sup>.

A large number of epidemiological and experimental studies have aimed to detect the genetic and environmental factors underlying the onset, progression, and treatment response of diabetes over the last decades. Contrary to the well-established damage of insulin-producing  $\beta$ -cells by autoimmune processes in type 1 diabetes (T1D), the pathophysiological basis of T2D is still incompletely understood and involves multiple cellular and physiological systems influencing each other's set points, thresholds, and metabolic

functions<sup>[5]</sup>. Insulin resistance typically evolves during the course of T2D as result of a diminished sensitivity to insulin's metabolic regulatory functions in several peripheral tissues (fat, muscle, and liver) aggravated by reduced exercise, aging, and obesity. T2D represents a relative, rather than an absolute insulin deficiency, as a result of a progressive malfunction of  $\beta$ -cells to secrete sufficient amounts of insulin for sustaining efficient carbohydrate and lipid homeostasis<sup>[6]</sup>. Conclusively, refined insight into the pathways that underlie  $\beta$ -cell dysfunction at a physiological and molecular scale remains desirable to advance personalized treatment of T2D.

How can we examine human  $\beta$ -cells when these are barely accessible? Single gene defects encoding severe  $\beta$ -cell dysfunction with manifest diabetes provide unique inroads to disease mechanisms if the causative gene can be isolated. In this regard, positional cloning enabled the identification of heterozygous mutations in the hepatic transcription factors HNF1A and HNF4A as the cause of early-onset diabetes<sup>[7,8]</sup>. This important discovery drove forth the subsequent discovery of a transcription factor network underpinning normal  $\beta$ -cell development<sup>[9]</sup> as well as susceptibility to T2D<sup>[10,11]</sup>. Hence, monogenetic diseases can guide biological studies aimed to unlock the cellular and molecular basis of the specific condition under study, but may have also important implications for related multifactorial diseases.

Based on this perspective, we will discuss in this review recent progress in the molecular genetics of transient neonatal diabetes mellitus (TNDM) and how these new findings advance insight into  $\beta$ -cell development and physiology. Specifically, we will focus on the transcriptional regulator ZAC1 (zinc-finger protein 1 regulating apoptosis and cell cycle arrest), which is encoded by a genomically imprinted gene at 6q24 in human. TNDM is characterized by a unique clinical course manifesting prenatally with T1D and an increased risk for T2D in adolescence. Importantly, the identification of various target genes over the last years eventually enabled insight into ZAC1's role in pancreatic  $\beta$ -cell development and maturation and potential pharmacological treatments of newborns suffering from TNDM.

## NEONATAL DIABETES MELLITUS

Neonatal diabetes mellitus (NDM), also recognized as "early-onset" diabetes mellitus, manifests unregulated hyperglycemia during the first 6 mo of life and is a rare disorder developing uniformly in all races and ethnic groups. Typically, NDM presents with intrauterine growth retardation (IUGR), reduced subcutaneous fat, failure to thrive, and low or undetectable C-peptide levels (a peptide of 31 amino acids connecting the A-chain and the B-chain of proinsulin)<sup>[12]</sup>. NDM commonly arises from mutations in genes that play a

crucial role in pancreas development,  $\beta$ -cell apoptosis, insulin processing, and the regulation of insulin release<sup>[13]</sup>. NDM has an incidence of 1 in 300000 to 500000 live births, whereby current data indicate that approximately over half (57%) of the cases are transient (TNDM), depend on initial insulin application, and resolve spontaneously in less than one and a half year, only to relapse in adolescence.

Different genetic defects have been identified as the source of TNDM; these include mutations in the potassium channel genes *ABCC8* (TNDM2; OMIM 310374) and *KCNJ11* (TNDM3; OMIM 610582)<sup>[14]</sup>, *insulin*<sup>[15]</sup>, and occasionally *HNF1B*<sup>[16]</sup>. About two-third of all cases of TNDM derive from genetic mutations or loss of genomic imprinting at a locus on 6q24 (TNDM1; OMIM 603044)<sup>[13]</sup> encoding *ZAC1* (alias *PLAGL1*), which is in the focus of the remainder of this review, and *HYMAI* (hydatiform mole associated and imprinted transcript) whose biological function remains unknown so far.

## TNDM1

### **Clinical picture and course**

TNDM1 was firstly described as a clinical entity in 1926<sup>[17]</sup>. Similar to NDM, newborns suffering from TNDM1 represent with perinatal hyperglycemia, undetectable or very low insulin levels, and severe dehydration. Altogether, this life threatening condition requires immediate insulin therapy. IUGR develops in > 95% of TNDM1 cases during the third trimester and concurs with severely reduced subcutaneous fat formation in neonates<sup>[18]</sup>. Consistent with insulin's hypothesized role in growth stimulation, postnatal substitution initiates a fast catch-up growth and normalizes TNDM1 patients' height and weight by the age of 2 years.

Additionally, TNDM1 patients can display a number of symptoms varying in penetrance and unrelated to diabetes such as macroglossia, umbilical hernia, cardiac anomalies, brain malformation, and mental impairments in lately diagnosed newborns. These features are shared among TNDM1 infants irrespective of the underlying genetic anomalies<sup>[18]</sup>.

It is important to note, that insulin dependency declines within a few months postnatally (median treatment duration 3 mo) and rarely extends for up to one and a half year. Following on, TNDM1 remits and patients represent as normoglycemic, free of glycemic compromise, and sustain normal growth<sup>[19]</sup>. This reversible course seems compatible with a temporary rather than a persistent defect in the control of insulin synthesis and/or secretion. Yet, approximately one half of all TNDM1 patients develop in adolescence or early adulthood signs of T2D<sup>[20]</sup> and require dietetic as well as pharmacological treatments (sulfonylurea and insulin) to ameliorate hyperglycemia. Puberty-related hormonal changes and resumption of somatic growth are known to impose an increased demand on insulin's

availability, may compromise pancreatic functions, and ultimately, lead to relapse with T2D in persons at risk.

Alternatively, the same genetic defect underlying impaired insulin secretion, and consequently IUGR, may predispose to relapse in later life<sup>[21]</sup>. Epigenetic mechanisms, particularly DNA-methylation, are thought to mediate epigenetic programming in response to early life conditions<sup>[22,23]</sup> and are increasingly recognized to interact with genetic risk factors.

Collectively, TNDM1 represents with neonatal hyperglycemia, infant remission, and adolescent relapse. IUGR and hyperglycemia suggest a decline in available insulin due to a deficit in  $\beta$ -cell number and/or compromised insulin secretion.

### **Molecular and clinical genetics of TNDM1**

Paternal uniparental disomy of chromosome 6 (UPD6) was firstly discovered in two TNDM1 patients in 1995<sup>[24]</sup>. The same report presciently suggested that TNDM1 is a genomic imprinting disorder<sup>[25]</sup> - a hypothesis that has been confirmed as early as five years later<sup>[26,27]</sup>. Expression at the TNDM1 locus on chromosome 6q24 is confined to the paternally derived allele with the maternal allele being silenced. This process, termed genomic imprinting, is initiated in the respective parental germ cells and involves DNA methylation as a central mechanism<sup>[28]</sup>. As a result, the expressed paternal *ZAC1* allele is unmethylated at a confined region of 1 kb in size, the so called differentially methylated region (DMR), while the silenced maternal allele is methylated. The DMR straddles the shared promoter of *ZAC1* and *HYMAI* establishing monoallelic expression in healthy individuals. Contrarily, a defect in DMR methylation results in relaxation of *ZAC1/HYMAI* imprinting with biallelic expression in TNDM1<sup>[29]</sup>. Although it has not been formally proven whether one or both of these genes underlie TNDM1, it appears decent to assign a critical role to *ZAC1* based on its molecular and cellular properties (see below). In contrast, the non-coding RNA *HYMAI* is transcribed from the same imprinted promoter and in the same orientation as *ZAC1* and is ubiquitously expressed throughout development and maturation although its function remains still unresolved<sup>[27,30]</sup>. Notwithstanding the limited information on *HYMAI*, further studies are looked for with respect to the critical role of non-coding RNAs in the control of imprinted gene expression<sup>[28]</sup>.

In addition to the proximal promoter, *ZAC1* contains a second promoter some 50 kb upstream, which is devoid of genomic imprinting, drives biallelic *ZAC1* expression, and predominates in blood cells<sup>[31]</sup>. Although biallelic expression may prevail in some tissues or across defined periods, monoallelic expression from the imprinted promoter is preserved during mouse development and seems to fulfil a particular role in tissues regulating glucose metabolism and/or showing dynamic changes in perinatal *Zac1* expression (see below). Still, the possibility of postnatal switches in

promoter usage<sup>[32]</sup> during postnatal remission and/or relapse with T2D should not be dismissed and requires further analysis.

Although half of the TNDM1 patients relapse with T2D in early adulthood, *ZAC1* seems not to encode a T2D susceptibility locus. Whereas the chromosomal region 6q22-25 has been initially captured in some genome-wide association studies, particularly in the Pima Indian population<sup>[33]</sup>, this finding was refuted in a recent large case-control study in which not a single *ZAC1* nucleotide polymorphism (SNP) associated with T2D<sup>[34]</sup>. This study lacked however sufficient power to address a parental impact on SNP effects - an important issue since investigations in the Iceland population evidenced several imprinted loci among regions with a disease association dependent on parental origin<sup>[35]</sup>.

In addition to the original discovery of UPD6, duplication of the TNDM1 region, and maternal hypomethylation at the DMR have been identified subsequently as a genetic cause of TNDM1<sup>[36]</sup>.

Children suffering from UPD6 (40%) can display additional symptoms unmasked by uniparental disomy (*e.g.*, recessive mutations). Since UPD6 is the result of a stochastic reproductive error, it is non-hereditary. In contrast, duplication of 6q in TNDM1 patients (32%) comprises a critical region of approximately 160 kb and can be passed to offspring with manifestation in males<sup>[37]</sup>. The majority of these duplications are submicroscopic, differ in breakpoints, but share a similar phenotype<sup>[36]</sup>.

Hypomethylation at the DMR in TNDM1 patients (28%) leads to erasure of all maternal methylated CpG residues and suggests that normal *ZAC1* gene dosage has to be severely disrupted to manifest TNDM1. It is important to note, that approximately 50% of TNDM1 patients with *ZAC1* DMR hypomethylation suffer from hypomethylation at other imprinted loci as well<sup>[38]</sup>. This raises the possibility that defects in the imprinting machinery (*e.g.*, loss of ZFP57, a zinc-finger transcription factor containing a KRAB domain<sup>[39]</sup>) or imprinted gene networks (IGN) can give rise to TNDM1<sup>[32]</sup>.

In sum, TNDM1 patients show genetic defects leading to overexpression of *ZAC1* from the imprinted region of chromosome 6q24. The underlying chromosomal anomalies comprise paternal UPD6, paternally derived duplication of 6q24, and maternal methylation defects at the DMR<sup>[20,29]</sup>.

the inheritance in human, hyperglycemia in neonate mice was confined to offspring derived from paternal transmission of the overexpressed PAC.

Interestingly, the effects from *ZAC1* overexpression in mice largely recapitulated human TNDM1 with neonate hyperglycemia, juvenile remission, and adult glucose intolerance<sup>[40]</sup>. Yet, neonate growth was not retarded at birth nor were aged mice overt diabetic. Histological examination of pancreata from TNDM1 mice suggested a reduction in the development and existence of all endocrine cell types including  $\beta$ -cells concomitantly with a downregulation of the important developmental transcription factors Ngn3, Pax6, and Pdx1. Puzzlingly, in late pregnancy and postnatally transgenic  $\beta$ -cells assumed a rapid catch-up growth as a result of increased proliferation and/or decreased apoptosis and surpassed in numbers their normal counterparts. Irrespective of this increase in neonatal  $\beta$ -cell numbers, insulin content and secretion were diminished and resulted in postnatal hyperglycemia. Still, an ongoing increase in  $\beta$ -cell numbers enabled their duplication and reinstatement of normal glucose tolerance in juvenile mice. This compensatory increase was, however, not sustained in aged mice, which suffered from an accelerated decline in  $\beta$ -cell numbers and compromised insulin secretory capacity when compared to controls.

Together, these findings in mice corroborate *ZAC1/HYMAI*'s maternal imprinting status and evidence the impact of these genes on pancreatic  $\beta$ -cell proliferation, differentiation, and insulin secretion from late gestation through adulthood. Consistent with these data, *ZAC1* overexpression in rodent  $\beta$ -cell lines<sup>[41,42]</sup> reduces glucose-stimulated insulin secretion (GSIS) as will be discussed in more detail in the section on target genes.

In contrast to *ZAC1* overexpression in pancreatic  $\beta$ -cells, results from *Zac1* deficient mice (*Zac1*<sup>+/-pat</sup>) evidenced no obvious effects on  $\beta$ -cell proliferation or insulin secretion<sup>[43]</sup>. *Zac1*<sup>+/-pat</sup> mice represented with IUGR, altered bone formation, impaired pulmonary respiration, hydrocephaly, disturbed neural cell numbers, and neonatal lethality<sup>[43,44]</sup>. Notably the same authors<sup>[43]</sup> assigned to *Zac1* a critical role in the regulation of an IGN, which on its own may affect  $\beta$ -cell function and confound *Zac1* defects.

All in all, these studies support a role of *ZAC1* overexpression in TNDM1 and prompt the question through which molecular and cellular mechanisms *ZAC1* contributes to the clinical picture and course of TNDM1.

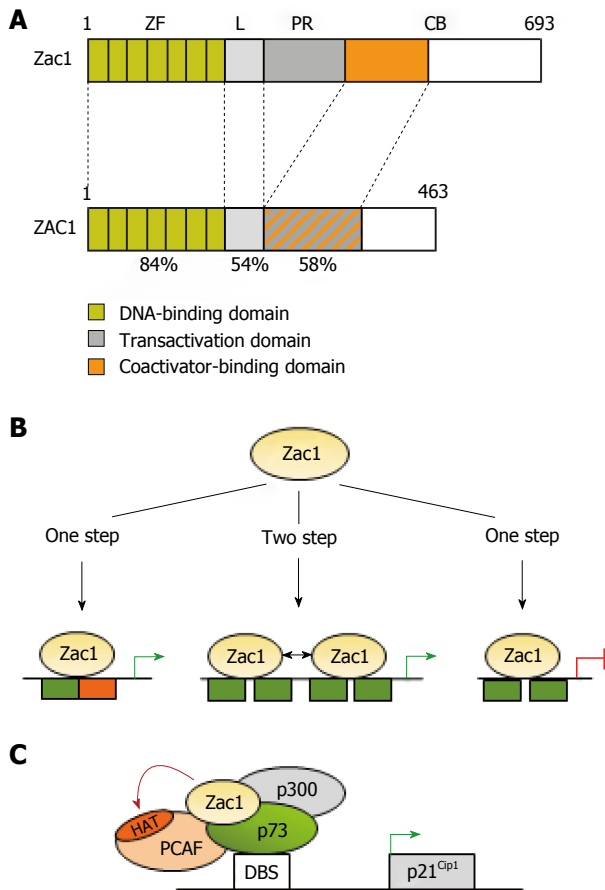
## TRANSGENIC MOUSE MODELS OF TNDM1

*ZAC1* overexpression in TNDM1 stimulated the creation of suitable mouse models to analyze the underlying pathology. For this purpose, a P1-derived artificial chromosome (PAC) of 175 kb comprising the entire *ZAC1* and *HYMAI* loci was integrated with multiple copies into the mouse genome<sup>[40]</sup>. In accord with

## TRANSCRIPTIONAL REGULATOR ZAC1

The zinc-finger protein *Zac1* was originally isolated from clonal corticotroph tumor cells derived from mice<sup>[45]</sup> and shortly afterwards from malignantly transformed ovary surface epithelial cells<sup>[46]</sup>. *Zac1* overexpression studies together with appropriate knock-down experiments





**Figure 1 Transcriptional activities of Zac1.** A: Schematic drawing of Zac1 proteins. Domains are boxed and numbers refer to amino acids and percentage of homology between mouse and human. The N-terminal DNA binding domain is highly conserved between human and mice and comprises seven canonical C2H2-type zinc fingers (ZF). The linker domain (L) in conjunction with the proline-rich region (PR) confers transactivation in mice, which is further enhanced through the C-terminus's coactivator binding (CB) domain. Contrarily, transactivation and coactivator recruitment jointly localize to ZAC1's C-terminus; B: Zac1 recognizes different DNA-elements to confer transcriptional regulation. Monomeric Zac1 binding to GC-rich palindromes (left) or dimerization at G/C rich direct repeat elements (middle) results in transactivation. On the other hand, monomeric Zac1 binding to G/C rich half sites causes repression (right); C: Zac1 coactivation of p73. Following recognition of its DNA-binding site (DBS), the transcription factor p73 recruits Zac1 together with the general coactivator p300 and PCAF to the  $p21^{Cip1}$  promoter during early neural differentiation. Due to its scaffolding function, Zac1's zinc fingers stabilize this interaction and enhance additionally PCAF's histone acetyltransferase (HAT) activity. This event enhances histone acetylation at the  $p21^{Cip1}$  promoter and subsequent transcription.

evidenced Zac1's role in the regulation of apoptosis and cell cycle arrest and stimulated its name-giving<sup>[45,47,48]</sup>. In agreement with these observations, human ZAC1<sup>[48]</sup>, undergoes methylation-sensitive silencing in various tumors including ovary and mammary cancer<sup>[46,49]</sup>.

Human and mouse ZAC1 proteins consist of 463 and 693 amino acids, respectively, and share a highly conserved amino-terminal DNA-binding domain enclosing seven zinc fingers conforming with the classical C2H2-type (Figure 1A). Apart from that, a central and C-terminal domain enriched in proline and glutamic acid residues, respectively, exists solely

in mice<sup>[48]</sup>. The region adjacent to the DNA-binding domain, termed linker-region, together with the central proline-rich region confers transactivation in mice, which is enhanced by the C-terminus' interaction with the general coactivators p300/CBP<sup>[50]</sup>. On the other side, transactivation and coactivator recruitment are merged in ZAC1's C-terminus and illustrate how different protein motives are used species-specifically in transactivation (Figure 1A).

Monomeric Zac1 can bind to palindromic GC-rich DNA elements to confer transactivation (Figure 1B). Similarly, Zac1 dimerization at direct and reverse G/C-rich repeat DNA elements confers transactivation whereas monomeric binding to single half sites leads to repression<sup>[51]</sup> (Figure 1B). In addition to DNA binding, zinc finger 6 and 7 interact with p300 to increase (palindromes and repeats) or decrease (half sites) its catalytic activities<sup>[50]</sup>. This interrelationship between Zac1's DNA address code and enzymatic regulation of transcription is thought to support efficient regulation of target genes.

Moreover, Zac1 can coregulate the activity of various members of the nuclear receptor and p53 families without binding on its own to DNA due to its scaffolding function and concomitant coactivator regulation<sup>[52-54]</sup> (Figure 1C). The p53 family comprises the eponym p53 and the relatives p63 and p73, all of which share critical roles in cell renewal and proliferation, cell fate commitment and differentiation, and ultimately apoptosis<sup>[55]</sup>. As an example, the transcription factor p73 tethers the coactivators PCAF/p300 and Zac1 jointly to the  $p21^{Cip1}$  (*Cdkn1a*) promoter in a model of early neuronal differentiation. Consistent with its scaffolding function Zac1 strengthens *via* its zinc fingers the interaction between PCAF and p300 and enhances at the same time PCAF's catalytic activity (Figure 1C)<sup>[52]</sup>. This finding evidences an authentic Zac1 coactivator function and suggests that similar mechanisms apply to Zac1-dependent nuclear receptor coregulation<sup>[53]</sup>.

In summary, Zac1 transcription factor activities are determined by sequence-specific DNA binding and interconnected regulation of coactivator activities. Additionally, Zac1 can act as coregulator for unrelated transcription factors by stabilizing complex formation with recruited coactivators and enhancement of their catalytic functions.

## ZAC1 TARGET GENES IN THE PANCREAS

Zac1's role as transcription factor and coregulator offers the opportunity to gain insight into its function in TNDM1 *via* the identification of downstream target genes. In the context of this review we will pay particular attention to those targets, which are expressed in pancreatic  $\beta$ -cell islets and are likely to share a role in insulin and glucose metabolism. Readers interested in further Zac1 functions are referred to recent reviews<sup>[32,56]</sup>.

In support of ZAC1's potential role in TNDM1, strong immunoreactive signals were detected in the nuclei of insulin positive cells, whereas the exocrine pancreas was only sparsely stained in adult human<sup>[42]</sup>. Refined immunohistochemistry in mice evidenced strong Zac1 signals at prenatal and neonatal stages [(embryonic day 18 (E18) and postnatal day 1 (P1)] in the endocrine pancreas of both insulin-expressing and non-expressing cells. Moreover, sparse immunoreactivity was spread throughout the exocrine pancreas. Postnatally, Zac1 immunoreactivity rapidly declined in either pancreas compartment (P4 to P12) in accord with previous reports<sup>[40,57]</sup>. Subsequently, Zac1 expression was maintained at lower levels in insulin-positive cells in early adult mice (P42) but was largely absent in the parenchyma consistent with the findings in human<sup>[42]</sup>.

Together, these findings corroborate ZAC1 expression in insulin-positive cells in human and mice and reveal dynamic changes in perinatal expression levels.

### PAC1

PAC1 (*alias ADCYAP1R1*) encodes a G-protein coupled receptor (GPCR) for the neuropeptide pituitary adenylate cyclase activating polypeptide (PACAP) and was the first Zac1 target gene to be identified<sup>[45,58,59]</sup>. Zac1 regulates *Pac1* gene expression and related promoter reporter constructs in a cell type-specific fashion as evidenced by activation in cerebellar granule cells, epithelial kidney cells, and osteosarcoma cells vs inhibition in pituitary gonadotroph cells and pancreatic  $\beta$ -cells<sup>[58,60,61]</sup> (Hoffmann A, Spengler D. Unpublished findings. 2012). The neuropeptide PACAP was firstly purified from sheep hypothalamus<sup>[62]</sup> and has multiple functions on the neuroendocrine, endocrine, and nervous system<sup>[63]</sup>. Pancreatic islets are richly innervated *via* pancreatic ganglia that derive from the vagus dorsal motor nucleus. Both ganglia and islets contain PACAP as well as the related vasointestinal peptide (VIP) among other neurotransmitters. This distribution suggests that PACAP can act at the same time as neurotransmitter and islet endocrine peptide<sup>[64]</sup>.

The VIP/PACAP receptor family comprises PAC1 (specific for PACAP), VPAC1, and VPAC2 (both with equal affinity for VIP and PACAP)<sup>[65]</sup>. Pancreatic islets express all of these receptors, which couple to stimulation of adenylate cyclase<sup>[66,67]</sup>. Increases in intracellular levels of cAMP activate protein kinase A (PKA) and guanine nucleotide exchange factors of the Epac family that enhance insulin secretion through distinct pathways involving  $K_{ATP}$  channels together with the exocytotic machinery<sup>[68]</sup>.

Intracellular glucose metabolism presents the major stimulus for GSIS. This process commences with the uptake of glucose into  $\beta$ -cells *via* the glucose transporter protein 2 (Glut-2). Breakdown of glucose leads to ATP production and an enhanced ATP/ADP ratio followed by closing of  $K_{ATP}$  channels. Subsequently, plasma membrane depolarization and

$Ca^{2+}$  influx stimulate the exocytosis of insulin.

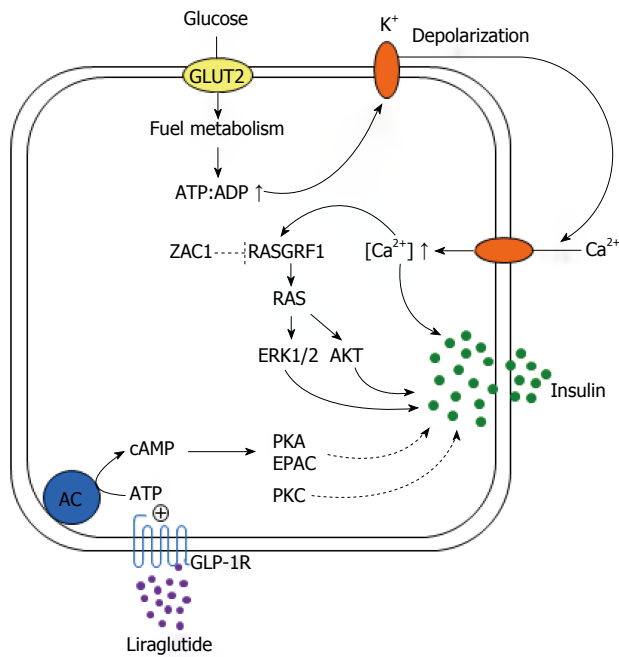
PACAP and VIP are thought to potentiate glucose-stimulated, but barely resting, insulin secretion by increasing intracellular  $Ca^{2+}$  concentrations<sup>[64]</sup>. In accord with this hypothesis, *Pac1* null mice develop apparently normal and represent with inconspicuous resting plasma glucose and insulin levels<sup>[69]</sup>. On the other side, the response to PACAP on GSIS was reduced by half in isolated islets from *Pac1* null mice. Moreover, these mice showed reduced GSIS following intravenous or oral glucose administration. Hence, *Pac1* seems to control the insulin-secretory response to insulin itself in addition to its potentiation. Consistent with this possibility, *Pac1* null islets showed a diminished insulin response to glucose indicating *Pac1*'s critical role for optimal GSIS during food intake<sup>[69]</sup>. A possible explanation for this finding is that intracellular PACAP, independently of neural release, triggers PKA activation necessary for optimal calcium influx in the context of GSIS.

In contrast to PACAP's role for insulin secretion, its effects on long-term  $\beta$ -cell mass have been less studied. Islets commonly decline during the course of T2D and aggravate the increased demand for insulin. Interestingly, PACAP overexpression in  $\beta$ -cells ameliorated the effects of streptozotocin-induced cell death as evidenced by lower plasma glucose levels in transgenic mice when compared to controls<sup>[70]</sup>. Morphometric analysis showed a tendency towards increased  $\beta$ -cell mass suggestive of a role for PACAP in the regulation of islet cell proliferation or differentiation. Compatible with this view, PACAP enhances the growth of pancreatic cancer cells through cAMP/PKA-dependent stimulation of the MAP kinase pathway together with the upregulation of nuclear oncogenes<sup>[71]</sup>. Relatedly, other studies suggest that intracellular PACAP protects  $\beta$ -cell mass by preventing harmful exogenous insults on the  $\beta$ -cell<sup>[72,73]</sup>.

All in all, these studies raise the possibility that altered ZAC1 dosage in TNDM1 reduces PAC1 expression in  $\beta$ -cells, GSIS, the insulin-secretory response itself, and  $\beta$ -cell proliferation or survival on the long term.

### PPAR $\gamma$

Genome-wide expression analysis<sup>[74]</sup> led to the identification of *Ppar $\gamma$*  (peroxisome proliferator-activated receptor) as a Zac1 target gene. PPAR $\gamma$  belongs to the nuclear hormone receptor family with an important function in adipogenesis, lipid and glucose metabolism, differentiation, antiproliferation, apoptosis, and inflammation<sup>[75]</sup>. The 5' end of PPAR $\gamma$  is subject to alternative splicing that produces two protein isoforms differing in 30 amino acids present in the PPAR $\gamma$ 2, but absent in the PPAR $\gamma$ 1, amino terminus<sup>[76]</sup>. PPAR $\gamma$ 2 expression prevails in adipose tissue, whereas PPAR $\gamma$ 1 is more widely expressed comprising heart and skeletal muscle, kidney, liver, spleen, colon, pituitary, brain, and pancreas. ZAC1 binds *in vitro* and *in vivo* to the



**Figure 2** Roles for RAS protein-specific guanine nucleotide-releasing factor 1 in insulin secretion and transient neonatal diabetes mellitus type 1. Glucose is the main stimulus for insulin secretion. Once transported into pancreatic  $\beta$ -cells by glucose transporter 2 (GLUT2), metabolism of glucose results in the production of ATP. A subsequent rise in the ATP:ADP ratio drives closure of ATP-regulated  $K^+$  channels and accumulation of intracellular  $K^+$ . Heightened  $K^+$  concentrations lead to depolarization of the plasma membrane and promote opening of voltage-dependent  $Ca^{2+}$  channels with subsequent influx of  $Ca^{2+}$  and increases in free cytoplasmic  $Ca^{2+}$ . This event stimulates exocytosis of insulin from the insulin-storing secretory granules through different routes including activation of the  $Ca^{2+}$ -calmodulin activated RAS protein-specific guanine nucleotide-releasing factor 1 (RASGRF1), a direct ZAC1 target gene. In its activated state the small G-protein RAS couples to ERK1/2 and PI3K/AKT signaling among other downstream effectors, which jointly enhance the exocytotic process of insulin secretion. ZAC1 overexpression in transient neonatal diabetes mellitus type 1 (TNDM1) is predicted to directly repress RASGRF1 and consequently GSIS. Contrarily, PKA signaling is undisturbed in TNDM1 as well as the potentiating effects of the GLP-1R agonist liraglutide on GSIS.

proximal promoter region of *PPAR $\gamma$ 1* in various cell types (neural cells, pituitary cells, and colon cells among others) derived from rodent or men<sup>[74]</sup>. Functionally, ZAC1 antiproliferation partly depends on *PPAR $\gamma$ 1* expression and associates with the upregulation of *PPAR $\gamma$*  target genes contributing to differentiation (*KER20* and *TSC22*), apoptosis (*POX*) or growth inhibition (*PTEN*).

Notably, *PPAR $\gamma$*  overexpression has been suggested to attenuate glucose- and potassium-induced stimulated insulin secretion<sup>[77,78]</sup>. This effect is thought to relate to *PPAR $\gamma$* 's upregulation of uncoupling protein-2 (*UCP-2*), which reduces the availability of ATP needed for the energy-consuming exocytotic process of insulin secretion.

On the contrary,  $\beta$ -cell specific deletion of *Ppar $\gamma$*  causes islet hyperplasia with unaltered glucose homeostasis<sup>[75]</sup>. A plausible *Ppar $\gamma$*  target gene contributing to these effects is *Pten* (phosphatase and tensin homologue

deleted on chromosome 10), which diminishes intracellular levels of phosphatidylinositol *via* inhibition of phosphatidylinositol-3-kinase (PI3K). As a result, deletion of *Pten* enhances cell proliferation and survival without disrupting differentiation<sup>[79]</sup>.

Interestingly, *Pten*'s effects on  $\beta$ -cell proliferation extend to models of T2D and deteriorated insulin secretion in aged mice. In this regard, continuous  $\beta$ -cell specific deletion of *Pten* protects against T2D ensuing from high-fat diet or deficient leptin-signaling due to a gain in  $\beta$ -cell mass under basal and diabetic circumstances<sup>[80]</sup>. Similarly, a knockout of *Pten* at adult age manifests an enhanced  $\beta$ -cell mass and proliferation as result of a sustained response to mitogenic stimulation during aging<sup>[81]</sup>.

In summary, these findings support that enhanced ZAC1 expression may impair insulin secretion and  $\beta$ -cell proliferation *via* induction of *PPAR $\gamma$*  and its downstream targets *UCP-2* and *PTEN*. Because this pathway has not formally been proven to exist in pancreatic  $\beta$ -cells, further studies on this transcriptional network are looked for.

### Rasgrf1

The small G-protein Ras integrates multiple upstream signaling pathways and connects them to different downstream effectors comprising the Raf-MEK-Erk kinase cascade, the PI3K-Akt pathway, and RaIGDS family members among others<sup>[82]</sup>. Ras alternates between an active GTP-bound and inactive GDP-bound state. This balance is tightly regulated by GTPase-activating proteins (GAPs) and guanine nucleotide-exchange factors (GEFs), respectively. Various GEF proteins have been isolated; among these RAS protein-specific guanine nucleotide-releasing factor 1 (RASGRF1) and RASGRF2 are stimulated by the G-protein subunits  $\beta$  and  $\gamma$  or by calmodulin-mediated  $Ca^{2+}$  influxes (Figure 2).

Genome-wide expression profiling for *Zac1* target genes in neural cells evidenced downregulation of *Rasgrf1*<sup>[74]</sup>. Originally thought to be confined to postnatal brain, *Rasgrf1* expression has been also detected in adult mice islets<sup>[83]</sup>. Consistent with this report, cytoplasmic RASGRF1 expression colocalizes with nuclear ZAC1 expression in adult human insulin-positive cells with weak expression in the parenchyma<sup>[42]</sup>. A similar expression pattern exists in mice with *Rasgrf1* and *Zac1* mRNA and protein expression correlating inversely with each other pre- and postnatally. Accordingly, *Rasgrf1* was weakly expressed at E18 when *Zac1* expression is high and rapidly increased postnatally when *Zac1* expression declined. Expression of both *Zac1* and *Rasgrf1* in two mouse  $\beta$ -cell models (Min6 and R7T1) was similar to P4 neonate islets from mice with a strong decrease of *Rasgrf1* expression following *Zac1* transfection. Chromatin-immunoprecipitation (ChIP)<sup>[74]</sup> analysis evidenced that *Zac1* bound at multiple predicted DNA-binding sites at the *Rasgrf1* promoter

and associated with the presence of repressive histone marks<sup>[42]</sup>.

R7T1  $\beta$ -cells were stably transfected to express double amounts of *Zac1* as an *in vitro* model to simulate the effects of biallelic ZAC1 expression in TNDM1. *Zac1* overexpressing  $\beta$ -cells showed moderately delayed differentiation with unaltered cell proliferation. Importantly, *Zac1* overexpression impaired glucose-, insulin-, and potassium-induced depolarization and the subsequent activation of MAPK and PI3K pathways driving insulin secretion (Figure 2). Consistent with a crucial function of *Rasgrf1* in *Zac1*-dependent insulin secretion, reinstatement of *Rasgrf1* expression in *Zac1* overexpressing R7T1  $\beta$ -cells normalized insulin secretion<sup>[42]</sup>. Moreover, subcutaneous transplantation of parent, but not of *Zac1* overexpressing R7T1  $\beta$ -cells ameliorated hyperglycemia in streptozotocin-treated diabetic mice.

Notably, activated Ras may additionally switch on the GEF function of *RalGEF* and *Tiam1*. Hereby, *Tiam1* facilitates the action of *Rac1* with a regulatory role in GSIS<sup>[84]</sup> and *RalGEF* enhances the activity of the small G-protein *Ral* with a regulatory role in different steps of insulin exocytosis<sup>[85,86]</sup>. Hence, *Zac1*-dependent *Rasgrf1* repression may integrate additional pathways beyond canonical MAPK and PI3K signaling, which overall result in an impaired GSIS.

Activation of GPCRs leading to an increase in intracellular levels of cAMP (see above), are known to optimize the insulinotropic effects of glucose. In this regard, the glucagon-like peptide 1 receptor (GLP-1R) stimulates insulin secretion independent of Ras pathways. Indeed, liraglutide, a clinically used GLP-1 analog, undistinguishably stimulated MAPK and PI3K pathways and downstream insulin secretion in stable mock- and *Zac1*-transfected R7T1  $\beta$ -cells and strongly reduced hyperglycemia following their subcutaneous transplantation in experimental diabetic mice.

Interestingly, the oral or intravenous glucose tolerance tests display a subnormal insulin response in adolescent TNDM1 patients whilst glucagon-stimulated insulin secretion is preserved<sup>[87]</sup>. Hence,  $\beta$ -cells in TNDM1 patients seem to sustain the capability to secrete insulin in response to stimulatory GPCR pathways, but not to glucose stimulation.

In summary, this work evidences a critical role for the regulatory Ras/*Rasgrf1* dyad in GSIS. *Zac1*-dependent repression of *Rasgrf1* diminishes the activation of Ras' downstream effectors comprising canonical and non-canonical pathways in insulin secretion. On the contrary, GPCR's capability to potentiate GSIS seems to be spared from *Zac1* overexpression in accord with findings from adolescent TNDM1 patients (Figure 2).

### Socs3

The neural stem cell line (C17.2) has been used for additional genome-wide expression profiling and led to the identification of *Socs3* (suppressor of cytokine

signaling) as potential *Zac1* target gene<sup>[88]</sup>. SOCS proteins comprise a group of eight members (SOCS1 through SOCS7 and CIS) all of which encompass a COOH-terminal SOCS box and a characteristic central Src homology-2 (SH2) domain. Cytokine stimulation leads to the activation and nuclear translocation of the signal transducer and activator of transcription (STAT) proteins that in turn upregulate the expression of SOCS genes among others. These transcription factors recognize their DNA address code at regulatory regions of the SOCS genes, and once induced, CIS, SOCS1, SOCS2, and SOCS3 form a canonical negative feedback loop that serves to attenuate further cytokine signaling<sup>[89]</sup>. Similarly, SOCS family members might also inhibit tyrosine kinase signaling, particularly by the insulin-like growth factor-1 (IGF-1) and insulin receptors (see below).

The *Socs3* gene contains multiple predicted *Zac1* DNA-binding sites clustered at the regulatory upstream region and the neighboring first intron. Complementary experiments including different *Socs3* promoter reporter assays in conjunction with various *Zac1* expression constructs as well as ChIP studies evidenced that *Zac1* confers transactivation in an additive manner following binding to the *Socs3* promoter and first intron. Similarly, ZAC1 potently induced SOCS3 in human neuroblastoma cells indicating that regulation of this target gene is preserved in human cells<sup>[88]</sup>. Functionally, *Zac1* transiently induces *Socs3* in NSCs during the course of astroglial, but not of neural, differentiation. *Zac1*-dependent *Socs3* expression peaks at the astroglial transition to maturation and has been suggested to prevent precocious astroglial differentiation by inhibition of related signaling pathways independent of *Zac1*'s cell cycle arrest function. In accord with this hypothesis, *Zac1* knock-down advanced astroglial differentiation. Conversely, knock-down of *Socs3* in *Zac1* overexpressing cells reinstated timely astroglial differentiation irrespective of a *Zac1*-driven sustained G1 arrest.

In sum, *Zac1* transactivates in a lineage-specific manner *Socs3* in NSCs and thus fine tunes astroglial differentiation independent of *Zac1*'s cell cycle arrest function<sup>[88]</sup>. Moreover, these findings raise the possibility of a *Zac1* role for *Socs3* regulation in TNDM1, particularly in pancreatic islets. Although there are no experimental data available yet, we will discuss in the following part the rationale for future studies to explore this topic.

Originally viewed as negative regulators of cytokine signaling, SOCS proteins have been more recently also suggested to attenuate insulin receptor signaling<sup>[90]</sup>. Contrarily to cytokine receptors, insulin and IGF-1 receptors contain tyrosine kinase activity on their own. Once bound by its receptor, insulin triggers autophosphorylation and catalytic activation leading to the phosphorylation of various intracellular targets, including insulin receptor substrate (IRS) proteins. In



their phosphorylated state, IRSs associate with multiple SH2 domain-containing proteins and enable that multiple signaling proteins are subsequently recruited to the plasma membrane where they regulate various signal transduction pathways controlling translocation of glucose transporters from intracellular vesicles to the plasma membrane, protein and glycogen synthesis, and ultimately cell proliferation and survival<sup>[90]</sup>.

Insulin resistance denotes an impaired tissue response to insulin and presents an important pathogenic determinant in the manifestation of T2D. Heightened concentrations of proinflammatory cytokines comprising tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), interleukins (IL-1 $\beta$  and IL-6), and growth hormone (GH) during injury, obesity, or infection, are considered to advance the onset of insulin resistance in peripheral tissues<sup>[6]</sup>. In this context it is important to note that SOCS1 and SOCS3 proteins have been shown to disrupt signaling complex formation at insulin or IGF-1 receptors as a result of an enhanced degradation of the adaptor proteins IRS-1 and IRS-2. Consequently, insulin signaling declines in disease-relevant organs such as liver and muscle as well as in adipose tissue<sup>[90]</sup>. Because both ZAC1 and SOCS3 are expressed in these tissues, ZAC1 overexpression in TNDM1 patients may contribute to relapse with T2D in adults *via* sustained upregulation of SOCS3 and subsequent impairments in insulin signaling.

In addition to an effect on peripheral insulin signaling, SOCS3 appears also to affect pancreatic  $\beta$ -cells themselves<sup>[91]</sup>. Tyrosine phosphorylation of the insulin receptor and associated IRS proteins in response to insulin binding is inhibited by IL-1 $\beta$  *via* upregulation of SOCS3 expression and the formation of SOCS3/insulin receptor complexes. Together, these events result in reduced PI3K signaling and impaired long term survival of  $\beta$ -cells<sup>[91]</sup>. Relatedly, SOCS3 has been reported to attenuate growth factor signaling important to  $\beta$ -cell proliferation and cell mass<sup>[92]</sup>. In this respect, overexpression of SOCS3 exclusively in  $\beta$ -cells caused a reduction in  $\beta$ -cell volume by one third in transgenic mice and reduced STAT5 phosphorylation upon GH treatment of transgenic islets when compared to wild-type ones. Similar results were obtained for adenovirus mediated SOCS3 overexpression in primary islets. Notably, SOCS3 overexpression spared the effects of GLP-1, mediated by PKA, on cell proliferation. Collectively, these results suggest that SOCS3 inhibits  $\beta$ -cell proliferation by counteracting growth factor receptor signaling.

On the other side, a number of reports evidenced for SOCS3 a role in  $\beta$ -cell survival following exposure to cytotoxic factors. As far back as 2001, Socs3 has been suggested to protect against interferon- $\gamma$  and IL-1 $\beta$ -mediated  $\beta$ -cell damage by reduced transcription of inducible nitric oxide synthase (iNOS)<sup>[93]</sup>. Similarly, TNF $\alpha$ -induced apoptosis with a possible role in T1D is prevented by Socs3 overexpression in rat INS  $\beta$ -cells<sup>[94]</sup>. In further support of these findings, ciliary

neurotrophic factor signaling (CNTF) increased STAT3 (cytoplasmatic and nuclear phosphorylated forms) as well as Socs3 expression and protected against cytokine- or streptozotocin-induced apoptosis<sup>[95,96]</sup>. At the same time, increased Socs3 expression impaired GSIS in isolated rat neonatal islets.

Collectively, these results suggest that the effects from SOCS3 expression are context-dependent involving growth inhibition as well as improved survival following cytotoxic insults.

Overall, ZAC1-dependent SOCS3 upregulation may contribute to different aspects in TNDM1 by: (1) facilitating development of peripheral insulin resistance in adults; (2) inhibiting prenatal  $\beta$ -cell proliferation; and (3) impairing GSIS. Future experimental studies are looked for to address these topics.

### p57<sup>Kip2</sup>

The cyclin-dependent kinase inhibitor (CKI) p57<sup>KIP2</sup> fulfils a critical task in cell cycle progression and differentiation<sup>[97]</sup>. Cyclin-dependent kinases (CDK) represent an evolutionary conserved group of serine/threonine kinases whose catalytic activities are tightly controlled through the association with various cyclins. The expression levels of cyclins oscillate periodically across the cell cycle and stimulate the sequential formation of different CDK-cyclin complexes that drive the coordinate progression through the cell cycle.

Application of the neuropeptide PACAP (see above) rapidly increases p57<sup>KIP2</sup> expression in embryonic cortical precursors and reduces the activity of CDK2, entry into S-phase, and the synthesis of DNA. At the same time PACAP enhanced the association of p57<sup>KIP2</sup>, but not of p21<sup>Cip1</sup> or p27<sup>Kip1</sup>, with the kinase complex in accord with PACAP's antimitogenic function<sup>[98]</sup>. Hence, Zac1-dependent induction of *Pac1* in neural cells, but not in  $\beta$ -cells where *Pac1* is repressed, may contribute *via* enhanced p57<sup>KIP2</sup> expression to antiproliferation.

Relatedly, Zac1 can induce p57<sup>KIP2</sup> *via* an alternative route involving the basic helix-loop-helix (bHLH) protein Tcf4. The family of bHLH proteins coordinate cell lineage decisions with proliferation and differentiation and the subsequent migration of progenitors during neurogenesis among other functions<sup>[99]</sup>. These proteins share a bHLH domain enabling homo- or heterodimerization upon DNA-binding to E-box (CANNTG) motives. Two major groups can be distinguished: the spatiotemporally expressed specification factors (*e.g.*, NeuroD, neurogenin, Mash, and Math) and the ubiquitously expressed dimerization factors known as E-proteins. The latter group comprises the two splice variants of *E2A*, *E12* and *E47*, *HEB*, and *TCF4* (also called *E2-2*)<sup>[100]</sup>. Among these genes, *Tcf4* has been recently identified as direct Zac1 target gene during early neurogenesis<sup>[101]</sup>. Zac1 recognizes multiple GC-rich DNA-elements at both the promoter and first intron of *Tcf4* to confer synergistic transactivation as shown by reporter assays with various *Tcf4* promoter reporter plasmids and Zac1 constructs.

Moreover, sequential ChIP assays corroborated that Zac1 binding associates with the presence of active histone marks at *Tcf4*.

Interestingly, E-proteins share with Zac1 a cell cycle arrest function, which has been attributed to their induction of  $p57^{Kip2}$  in neuroblastoma cells<sup>[102]</sup>. Compatible with these findings several predicted E-box motifs exist in the distal  $p57^{Kip2}$  promoter and confer Zac1 transactivation *via* Tcf4 upregulation<sup>[101]</sup>.

Collectively, these data suggest that  $p57^{Kip2}$  is an indirect Zac1 target gene *via* transactivation of *Tcf4*. In this regard, a further investigation has pointed to a critical function of  $p57^{Kip2}$  in  $\beta$ -cell replication<sup>[103]</sup>. Hyperinsulinism of infancy presents a complex syndrome in which  $\beta$ -cells fail to suppress the secretion of insulin under hypoglycemic conditions<sup>[104]</sup>. The majority of patients suffer from mutations of either in *KCNJ11* (encoding two subunits of the  $\beta$ -cell ATP-sensitive  $K^+$  channel)<sup>[105]</sup> or *ABCC8* (encoding a sulfonylurea receptor)<sup>[106]</sup>, both of which regulate the secretion of insulin.

Hyperinsulinism of infancy can be histologically distinguished into a diffuse form affecting all  $\beta$ -cells and a focal one in which a single cell population undergoes clonal expansion. Genetically, focal  $\beta$ -cell hyperplasia results from a duplication of the p terminus of paternal chromosome 11 at the cost of the maternal allele. This recombination event results in two mutated alleles at the *ABCC8/KCNJ11* loci and UPD for all genes telomeric to this site<sup>[107]</sup>. The duplicated segment harbors several paternally silenced imprinted genes including  $p57^{KIP2}$  whose expression is extinguished in mutated  $\beta$ -cell descendants<sup>[108]</sup>. To evidence a role in  $\beta$ -cell proliferation, shRNA was used to silence  $p57^{KIP2}$  in islets obtained from deceased donors free of pancreatic dysfunctions<sup>[103]</sup>. Interestingly, such  $p57^{KIP2}$  knock-down  $\beta$ -cells reassumed replication when transplanted under the kidney capsule of immunodeficient hyperglycemic mice and showed a normal glucose-induced calcium influx in support of an intact  $\beta$ -cell function.

In sum, this study identifies biallelic silencing of  $p57^{KIP2}$  as a critical pathogenic factor for focal hyperinsulinism. Functionally,  $p57^{KIP2}$  seems to act as a gatekeeper of mature  $\beta$ -cell replication. In this respect, ZAC1 overexpression in TNDM1 may upregulate  $p57^{KIP2}$  *via* *TCF4* at crucial stages of prenatal and postnatal development. Transgenic ZAC1 mice provide a valuable model to explore this hypothesis in future studies.

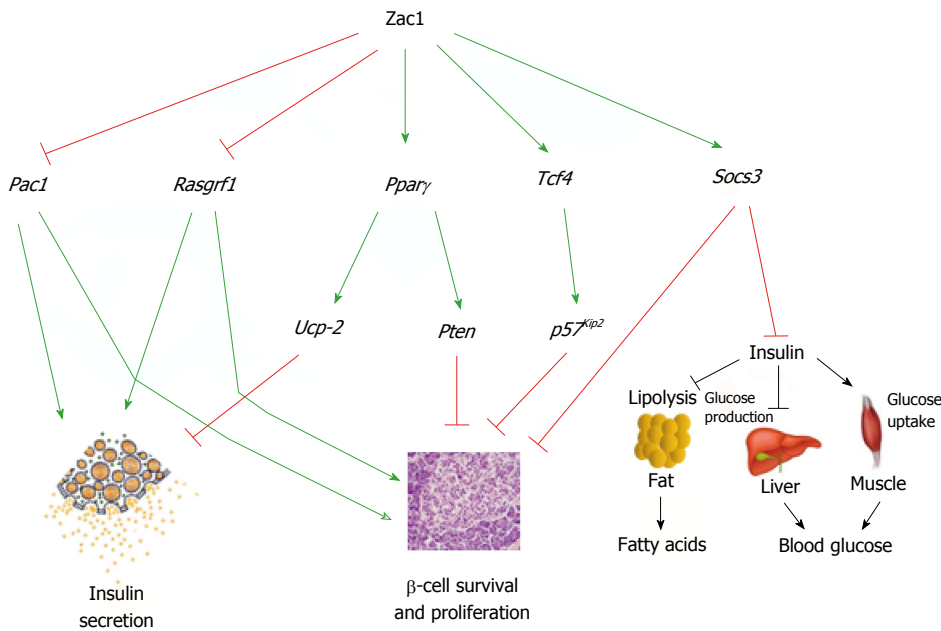
## CONCLUSION

The steady rise of the diabetic epidemic makes further progress on the genetic, cellular, and physiological basis of this multifactorial disease mandatory. Accidents of nature leading to well-defined genetic defects can provide valuable insights into  $\beta$ -cell function and glucose metabolism in general. TNDM1, a rare

monogenetic disease, appears of particular interest in this respect given its variable clinical course representing postnatally with severe hyperglycemia and insulin deficiency, remission within one and a half year, and frequent relapse with T2D in adolescence. Identification of the genetic defects at chromosome 6q24 (UPD6, duplication of the TNDM1 region, relaxation of genomic imprinting) point to altered dosage of monoallelically expressed ZAC1 as important pathogenic factor in TNDM1. Consistent with this view, ZAC1 overexpression in transgenic mice simulated major aspects of the human disease. A set of biochemical and molecular studies showed that Zac1 represents a zinc-finger protein with versatile regulatory functions dependent on DNA-binding to specific GC-rich motives and interconnected regulation of recruited coactivator activities. Additionally, Zac1 can serve as coactivator for the p53 and nuclear receptor families even so this function is presently largely unexplored with respect to TNDM1. Contrarily, the role as canonical transcription factor allowed gaining insight into ZAC1's role in TNDM1 through the identification of downstream target genes. Genome-wide expression profiling enabled the isolation of a set of direct target genes known to regulate various aspects of  $\beta$ -cell function and peripheral insulin sensitivity. Among these, Zac1-dependent upregulation of the transcription factors Ppar $\gamma$  and Tcf4 may translate into an altered expression of downstream targets sharing a role in insulin secretion (*Ucp-2*) and  $\beta$ -cell proliferation (*Pten* and  $p57^{KIP2}$ ) (Figure 3, middle). Similarly, Zac1-mediated transactivation of *Socs3* may inhibit  $\beta$ -cell proliferation and survival by attenuating growth factor signaling. Moreover, altered ZAC1 dosage in TNDM1 may also extend to SOCS3 upregulation in peripheral tissues and underlie impaired insulin signaling in relapsed patients with T2D (Figure 3, right).

Additionally, Zac1-dependent repression of downstream target genes may as well contribute to reduced insulin secretion and  $\beta$ -cell proliferation. In this regard, *Pac1* and *Rasgrf1* were repressed by Zac1 in  $\beta$ -cells, but not in neural cells, indicating that Zac1's transcriptional or coregulatory activities are tissue-specific<sup>[42,53,59,61]</sup>. *Pac1* enhances the insulin-secretory response to insulin, GSIS, and  $\beta$ -cell proliferation (Figure 3, left). Similarly, *Rasgrf1* repression attenuated Ras-mediated activation of canonical and non-canonical pathways in GSIS. Even so, Zac1 overexpression in  $\beta$ -cells spared the effects of GPCR on GSIS. In any case, the concerted regulation of these target genes in  $\beta$ -cells and peripheral tissues may partly explain the clinical course of TNDM1 ranging from fatal neonatal hyperglycemia, subsequent remission, to adolescent relapse with T2D.

Interestingly, relapsed TNDM1 patients show in response to both the oral and intravenous glucose tolerance test a subnormal insulin secretion, whilst glucagon-stimulated insulin secretion is preserved<sup>[87]</sup>. This raises the possibility that insulin secretion *via*



**Figure 3** Integrated model of Zac1's role in  $\beta$ -cell function and glucose metabolism. Zac1 DNA-binding at downstream target genes confers transcriptional activation or repression in a partly tissue-specific manner in mice. In this respect Zac1 binding represses *Pac1* and *Rasgrf1* in pancreatic  $\beta$ -cells leading to an impaired insulin-secretory response and GSIS (left). Additionally, Zac1 upregulation of the transcription factors *Ppar $\gamma$*  and *Tcf4* may translate in enhanced expression of their downstream targets *Ucp-2*, *Pten*, and *p57<sup>Kip2</sup>* with an inhibitory role in GSIS and  $\beta$ -cell proliferation (middle). Moreover, Zac1-dependent transactivation of *Socs3* may attenuate growth factor and insulin signaling in  $\beta$ -cells and peripheral tissues, respectively (right).

stimulatory G-protein signaling is maintained in TNDM1 and thus partly restores  $\beta$ -cell function while being compromised for GSIS. Indeed, treatments targeting the GLP-1 pathway have been lately reported as an interesting therapeutic option instead of insulin substitution in relapsed TNDM1 patients<sup>[109]</sup>. This finding corroborates previous work in experimental diabetic mice, where liraglutide treatment ameliorated hyperglycemia following subcutaneous transplantation of either parent or Zac1 overexpressing  $\beta$ -cells<sup>[42]</sup>.

Overall, these results demonstrate that elucidation of the molecular and cellular foundations of rare monogenetic diabetic diseases can substantially advance our understanding of  $\beta$ -cell function and glucose metabolism in general. The advent of precision medicine<sup>[110]</sup> may not only offer access to personalized information we look for to stay healthy but may also lead to tailored treatments as suggested here for TNDM1 following ZAC1 overexpression.

## ACKNOWLEDGMENTS

We are thankful for members of our group for critical discussions and advice on the manuscript.

## REFERENCES

- Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001; **414**: 782-787 [PMID: 11742409 DOI: 10.1038/414782a]
- UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet* 1998; **352**: 837-853 [PMID: 9742976]
- Tuomilehto J, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukkaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343-1350 [PMID: 11333990 DOI: 10.1056/NEJM200105033441801]
- DeFronzo RA, Ferrannini E, Zimmet P, Alberti KGMM. International textbook of diabetes mellitus. 4th ed. New York: Wiley-Interscience, 2015: 1-1240
- Kahn RC, Weir GC, King GL, Moses AC, Smith RJ, Jacobson AM. Joslin's diabetes mellitus. 14th ed. Philadelphia, Pa: Lippincott Williams & Wilkins, 2005: 1-1224
- Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* 2001; **414**: 799-806 [PMID: 11742412 DOI: 10.1038/414799a]
- Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Hansen T, Pedersen O, Polonsky KS, Bell GI. Mutations in the hepatocyte nuclear factor-1 $\alpha$  gene in maturity-onset diabetes of the young (MODY3) *Nature* 1996; **384**: 455-458 [PMID: 8945470 DOI: 10.1038/384455a0]
- Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI. Mutations in the hepatocyte nuclear factor-4 $\alpha$  gene in maturity-onset diabetes of the young (MODY1) *Nature* 1996; **384**: 458-460 [PMID: 8945471 DOI: 10.1038/384458a0]
- Servitja JM, Ferrer J. Transcriptional networks controlling pancreatic development and beta cell function. *Diabetologia* 2004; **47**: 597-613 [PMID: 15298336 DOI: 10.1007/s00125-004-1368-9]
- Love-Gregory LD, Wasson J, Ma J, Jin CH, Glaser B, Suarez BK, Permutt MA. A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4 $\alpha$  gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an ashkenazi jewish population. *Diabetes* 2004; **53**: 1134-1140 [PMID: 15047632]

- 11 **Silander K**, Mohlke KL, Scott LJ, Peck EC, Hollstein P, Skol AD, Jackson AU, Deloukas P, Hunt S, Stavrides G, Chines PS, Erdos MR, Narisu N, Conneely KN, Li C, Fingerlin TE, Dhanjal SK, Valle TT, Bergman RN, Tuomilehto J, Watanabe RM, Boehnke M, Collins FS. Genetic variation near the hepatocyte nuclear factor-4 alpha gene predicts susceptibility to type 2 diabetes. *Diabetes* 2004; **53**: 1141-1149 [PMID: 15047633]
- 12 **Fösel S**. Transient and permanent neonatal diabetes. *Eur J Pediatr* 1995; **154**: 944-948 [PMID: 8801100]
- 13 **Aguilar-Bryan L**, Bryan J. Neonatal diabetes mellitus. *Endocr Rev* 2008; **29**: 265-291 [PMID: 18436707 DOI: 10.1210/er.2007-0029]
- 14 **Flanagan SE**, Patch AM, Mackay DJ, Edghill EL, Gloyn AL, Robinson D, Shield JP, Temple K, Ellard S, Hattersley AT. Mutations in ATP-sensitive K<sup>+</sup> channel genes cause transient neonatal diabetes and permanent diabetes in childhood or adulthood. *Diabetes* 2007; **56**: 1930-1937 [PMID: 17446535 DOI: 10.2337/db07-0043]
- 15 **Edghill EL**, Flanagan SE, Patch AM, Boustred C, Parrish A, Shields B, Shepherd MH, Hussain K, Kapoor RR, Malecki M, MacDonald MJ, Stöy J, Steiner DF, Philipson LH, Bell GI, Hattersley AT, Ellard S. Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. *Diabetes* 2008; **57**: 1034-1042 [PMID: 18162506 DOI: 10.2337/db07-1405]
- 16 **Edghill EL**, Bingham C, Slingerland AS, Minton JA, Noordam C, Ellard S, Hattersley AT. Hepatocyte nuclear factor-1 beta mutations cause neonatal diabetes and intrauterine growth retardation: support for a critical role of HNF-1beta in human pancreatic development. *Diabet Med* 2006; **23**: 1301-1306 [PMID: 17116179 DOI: 10.1111/j.1464-5491.2006.01999.x]
- 17 **Ramsey WR**. Glycosuria of the newborn treated with insulin. *Trans Am Pediatr Soc* 1926; **38**: 100-101
- 18 **Mackay DJ**, Temple IK. Transient neonatal diabetes mellitus type 1. *Am J Med Genet C Semin Med Genet* 2010; **154C**: 335-342 [PMID: 20803656 DOI: 10.1002/ajmg.c.30272]
- 19 **Shield JP**, Temple IK, Sabin M, Mackay D, Robinson DO, Betts PR, Carson DJ, Cavé H, Chevenne D, Polak M. An assessment of pancreatic endocrine function and insulin sensitivity in patients with transient neonatal diabetes in remission. *Arch Dis Child Fetal Neonatal Ed* 2004; **89**: F341-F343 [PMID: 15210671 DOI: 10.1136/adc.2003.030502]
- 20 **Temple IK**, Gardner RJ, Mackay DJ, Barber JC, Robinson DO, Shield JP. Transient neonatal diabetes: widening the understanding of the etiopathogenesis of diabetes. *Diabetes* 2000; **49**: 1359-1366 [PMID: 10923638]
- 21 **Hattersley AT**, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. *Lancet* 1999; **353**: 1789-1792 [PMID: 10348008 DOI: 10.1016/S0140-6736(98)07546-1]
- 22 **Hoffmann A**, Spengler D. DNA memories of early social life. *Neuroscience* 2014; **264**: 64-75 [PMID: 22575695 DOI: 10.1016/j.neuroscience.2012.04.003]
- 23 **Murgatroyd C**, Spengler D. Epigenetics of early child development. *Front Psychiatry* 2011; **2**: 1-15 [DOI: 10.3389/fpsy.2011.00016]
- 24 **Temple IK**, James RS, Crolla JA, Sitch FL, Jacobs PA, Howell WM, Betts P, Baum JD, Shield JP. An imprinted gene(s) for diabetes? *Nat Genet* 1995; **9**: 110-112 [PMID: 7719335 DOI: 10.1038/ng0295-110]
- 25 **Ferguson-Smith AC**. Genomic imprinting: the emergence of an epigenetic paradigm. *Nat Rev Genet* 2011; **12**: 565-575 [PMID: 21765458 DOI: 10.1038/nrg3032]
- 26 **Gardner RJ**, Mackay DJ, Mungall AJ, Polychronakos C, Siebert R, Shield JP, Temple IK, Robinson DO. An imprinted locus associated with transient neonatal diabetes mellitus. *Hum Mol Genet* 2000; **9**: 589-596 [PMID: 10699182]
- 27 **Kamiya M**, Judson H, Okazaki Y, Kusakabe M, Muramatsu M, Takada S, Takagi N, Arima T, Wake N, Kamimura K, Satomura K, Hermann R, Bonthron DT, Hayashizaki Y. The cell cycle control gene ZAC/PLAGL1 is imprinted--a strong candidate gene for transient neonatal diabetes. *Hum Mol Genet* 2000; **9**: 453-460 [PMID: 10655556]
- 28 **Barlow DP**. Genomic imprinting: a mammalian epigenetic discovery model. *Annu Rev Genet* 2011; **45**: 379-403 [PMID: 21942369 DOI: 10.1146/annurev-genet-110410-132459]
- 29 **Mackay DJ**, Coupe AM, Shield JP, Storr JN, Temple IK, Robinson DO. Relaxation of imprinted expression of ZAC and HYMAI in a patient with transient neonatal diabetes mellitus. *Hum Genet* 2002; **110**: 139-144 [PMID: 11935319 DOI: 10.1007/s00439-001-0671-5]
- 30 **Arima T**, Wake N. Establishment of the primary imprint of the HYMAI/PLAGL1 imprint control region during oogenesis. *Cytogenet Genome Res* 2006; **113**: 247-252 [PMID: 16575187 DOI: 10.1159/000090839]
- 31 **Valley EM**, Cordery SF, Bonthron DT. Tissue-specific imprinting of the ZAC/PLAGL1 tumour suppressor gene results from variable utilization of monoallelic and biallelic promoters. *Hum Mol Genet* 2007; **16**: 972-981 [PMID: 17341487 DOI: 10.1093/hmg/ddm041]
- 32 **Hoffmann A**, Daniel G, Schmidt-Edelkraut U, Spengler D. Roles of imprinted genes in neural stem cells. *Epigenomics* 2014; **6**: 515-532 [PMID: 25431944 DOI: 10.2217/epi.14.42]
- 33 **Lindsay RS**, Kobes S, Knowler WC, Bennett PH, Hanson RL. Genome-wide linkage analysis assessing parent-of-origin effects in the inheritance of type 2 diabetes and BMI in Pima Indians. *Diabetes* 2001; **50**: 2850-2857 [PMID: 11723070]
- 34 **Gloyn AL**, Mackay DJ, Weedon MN, McCarthy MI, Walker M, Hitman G, Knight BA, Owen KR, Hattersley AT, Frayling TM. Assessment of the role of common genetic variation in the transient neonatal diabetes mellitus (TNDM) region in type 2 diabetes: a comparative genomic and tagging single nucleotide polymorphism approach. *Diabetes* 2006; **55**: 2272-2276 [PMID: 16873690 DOI: 10.2337/db06-0216]
- 35 **Kong A**, Steinthorsdottir V, Masson G, Thorleifsson G, Sulem P, Besenbacher S, Jonasdottir A, Sigurdsson A, Kristinsson KT, Jonasdottir A, Frigge ML, Gylfason A, Olason PI, Gudjonsson SA, Sverrisson S, Stacey SN, Sigurgeirsson B, Benediktsson KR, Sigurdsson H, Jonsson T, Benediktsson R, Olafsson JH, Johannsson OT, Hreidarsson AB, Sigurdsson G, Ferguson-Smith AC, Gudbjartsson DF, Thorsteinsdottir U, Stefansson K. Parental origin of sequence variants associated with complex diseases. *Nature* 2009; **462**: 868-874 [PMID: 20016592 DOI: 10.1038/nature08625]
- 36 **Docherty LE**, Kabwama S, Lehmann A, Hawke E, Harrison L, Flanagan SE, Ellard S, Hattersley AT, Shield JP, Ennis S, Mackay DJ, Temple IK. Clinical presentation of 6q24 transient neonatal diabetes mellitus (6q24 TNDM) and genotype-phenotype correlation in an international cohort of patients. *Diabetologia* 2013; **56**: 758-762 [PMID: 23385738 DOI: 10.1007/s00125-013-2832-1]
- 37 **Docherty LE**, Poole RL, Mattocks CJ, Lehmann A, Temple IK, Mackay DJ. Further refinement of the critical minimal genetic region for the imprinting disorder 6q24 transient neonatal diabetes. *Diabetologia* 2010; **53**: 2347-2351 [PMID: 20668833 DOI: 10.1007/s00125-010-1853-2]
- 38 **Mackay DJ**, Boonen SE, Clayton-Smith J, Goodship J, Hahnemann JM, Kant SG, Njølstad PR, Robin NH, Robinson DO, Siebert R, Shield JP, White HE, Temple IK. A maternal hypomethylation syndrome presenting as transient neonatal diabetes mellitus. *Hum Genet* 2006; **120**: 262-269 [PMID: 16816970 DOI: 10.1007/s00439-006-0205-2]
- 39 **Mackay DJ**, Callaway JL, Marks SM, White HE, Acerini CL, Boonen SE, Dayanikli P, Firth HV, Goodship JA, Haemers AP, Hahnemann JM, Kordonouri O, Masoud AF, Oestergaard E, Storr J, Ellard S, Hattersley AT, Robinson DO, Temple IK. Hypomethylation of multiple imprinted loci in individuals with transient neonatal diabetes is associated with mutations in ZFP57. *Nat Genet* 2008; **40**: 949-951 [PMID: 18622393 DOI: 10.1038/ng.187]
- 40 **Ma D**, Shield JP, Dean W, Leclerc I, Knauf C, Burcelin R Ré, Rutter GA, Kelsey G. Impaired glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes locus, TNDM. *J Clin Invest* 2004; **114**: 339-348 [PMID: 15286800 DOI: 10.1172/JCI19876]
- 41 **Du X**, Ounissi-Benkhalha H, Loder MK, Rutter GA, Polychronakos



- C. Overexpression of ZAC impairs glucose-stimulated insulin translation and secretion in clonal pancreatic beta-cells. *Diabetes Metab Res Rev* 2012; **28**: 645-653 [PMID: 22865650 DOI: 10.1002/dmrr.2325]
- 42 **Hoffmann A**, Spengler D. Transient neonatal diabetes mellitus gene *Zac1* impairs insulin secretion in mice through *Rasgrf1*. *Mol Cell Biol* 2012; **32**: 2549-2560 [PMID: 22547676 DOI: 10.1128/MCB.06637-11]
- 43 **Varrault A**, Gueydan C, Delalbre A, Bellmann A, Houssami S, Aknin C, Severac D, Chotard L, Kahli M, Le Digarcher A, Pavlidis P, Journot L. *Zac1* regulates an imprinted gene network critically involved in the control of embryonic growth. *Dev Cell* 2006; **11**: 711-722 [PMID: 17084362 DOI: 10.1016/j.devcel.2006.09.003]
- 44 **Valente T**. Expressió de la *Zac1* durant el desenvolupament de ratolí. Paper de la *Zac1* en el sistema nerviós central. 2005. [cited 2005 Apr 15]. Available from: URL: <http://www.tdx.cat/handle/10803/840>
- 45 **Spengler D**, Villalba M, Hoffmann A, Pantaloni C, Houssami S, Bockaert J, Journot L. Regulation of apoptosis and cell cycle arrest by *Zac1*, a novel zinc finger protein expressed in the pituitary gland and the brain. *EMBO J* 1997; **16**: 2814-2825 [PMID: 9184226 DOI: 10.1093/emboj/16.10.2814]
- 46 **Abdollahi A**, Godwin AK, Miller PD, Getts LA, Schultz DC, Taguchi T, Testa JR, Hamilton TC. Identification of a gene containing zinc-finger motifs based on lost expression in malignant transformed rat ovarian surface epithelial cells. *Cancer Res* 1997; **57**: 2029-2034 [PMID: 9158001]
- 47 **Pagotto U**, Arzberger T, Ciani E, Lezoualc'h F, Pilon C, Journot L, Spengler D, Stalla GK. Inhibition of *Zac1*, a new gene differentially expressed in the anterior pituitary, increases cell proliferation. *Endocrinology* 1999; **140**: 987-996 [PMID: 9927333 DOI: 10.1210/endo.140.2.6532]
- 48 **Varrault A**, Ciani E, Apiou F, Bilanges B, Hoffmann A, Pantaloni C, Bockaert J, Spengler D, Journot L. *hZAC* encodes a zinc finger protein with antiproliferative properties and maps to a chromosomal region frequently lost in cancer. *Proc Natl Acad Sci USA* 1998; **95**: 8835-8840 [PMID: 9671765]
- 49 **Bilanges B**, Varrault A, Basyuk E, Rodriguez C, Mazumdar A, Pantaloni C, Bockaert J, Theillet C, Spengler D, Journot L. Loss of expression of the candidate tumor suppressor gene *ZAC* in breast cancer cell lines and primary tumors. *Oncogene* 1999; **18**: 3979-3988 [PMID: 10435621 DOI: 10.1038/sj.onc.1202933]
- 50 **Hoffmann A**, Barz T, Spengler D. Multitasking C2H2 zinc fingers link *Zac* DNA binding to coordinated regulation of p300-histone acetyltransferase activity. *Mol Cell Biol* 2006; **26**: 5544-5557 [PMID: 16809786 DOI: 10.1128/MCB.02270-05]
- 51 **Hoffmann A**, Ciani E, Boeckardt J, Holsboer F, Journot L, Spengler D. Transcriptional activities of the zinc finger protein *Zac* are differentially controlled by DNA binding. *Mol Cell Biol* 2003; **23**: 988-1003 [PMID: 12529403]
- 52 **Hoffmann A**, Spengler D. A new coactivator function for *Zac1*'s C2H2 zinc finger DNA-binding domain in selectively controlling PCAF activity. *Mol Cell Biol* 2008; **28**: 6078-6093 [PMID: 18663001 DOI: 10.1128/MCB.00842-08]
- 53 **Huang SM**, Stallcup MR. Mouse *Zac1*, a transcriptional coactivator and repressor for nuclear receptors. *Mol Cell Biol* 2000; **20**: 1855-1867 [PMID: 10669760]
- 54 **Huang SM**, Schönthal AH, Stallcup MR. Enhancement of p53-dependent gene activation by the transcriptional coactivator *Zac1*. *Oncogene* 2001; **20**: 2134-2143 [PMID: 11360197 DOI: 10.1038/sj.onc.1204298]
- 55 **Vousden KH**, Lane DP. p53 in health and disease. *Nat Rev Mol Cell Biol* 2007; **8**: 275-283 [PMID: 17380161 DOI: 10.1038/nrm2147]
- 56 **Daniel G**, Schmidt-Edelkraut U, Spengler D, Hoffmann A. Imprinted *Zac1* in neural stem cells. *World J Stem Cells* 2015; **7**: 300-314 [PMID: 25815116 DOI: 10.4252/wjsc.v7.i2.300]
- 57 **Du X**, Rousseau M, Ounissi-Benkhalha H, Marchand L, Jetha A, Paraskevas S, Goodyer C, Polychronakos C. Differential expression pattern of *ZAC* in developing mouse and human pancreas. *J Mol Histol* 2011; **42**: 129-136 [PMID: 21305342 DOI: 10.1007/s10735-011-9315-9]
- 58 **Ciani E**, Hoffmann A, Schmidt P, Journot L, Spengler D. Induction of the *PAC1-R* (*PACAP*-type I receptor) gene by p53 and *Zac*. *Brain Res Mol Brain Res* 1999; **69**: 290-294 [PMID: 10366751]
- 59 **Hoffmann A**, Ciani E, Houssami S, Brabet P, Journot L, Spengler D. Induction of type I *PACAP* receptor expression by the new zinc finger protein *Zac1* and p53. *Ann N Y Acad Sci* 1998; **865**: 49-58 [PMID: 9927996]
- 60 **Contestabile A**, Fila T, Bartesaghi R, Ciani E. Cyclic AMP-mediated regulation of transcription factor *Lot1* expression in cerebellar granule cells. *J Biol Chem* 2005; **280**: 33541-33551 [PMID: 16061485 DOI: 10.1074/jbc.M413323200]
- 61 **Rodríguez-Henche N**, Jamen F, Leroy C, Bockaert J, Brabet P. Transcription of the mouse *PAC1* receptor gene: cell-specific expression and regulation by *Zac1*. *Biochim Biophys Acta* 2002; **1576**: 157-162 [PMID: 12031496]
- 62 **Miyata A**, Arimura A, Dahl RR, Minamino N, Uehara A, Jiang L, Culler MD, Coy DH. Isolation of a novel 38 residue-hypothalamic polypeptide which stimulates adenylate cyclase in pituitary cells. *Biochem Biophys Res Commun* 1989; **164**: 567-574 [PMID: 2803320]
- 63 **Arimura A**. Perspectives on pituitary adenylate cyclase activating polypeptide (*PACAP*) in the neuroendocrine, endocrine, and nervous systems. *Jpn J Physiol* 1998; **48**: 301-331 [PMID: 9852340]
- 64 **Sakurai Y**, Shintani N, Hayata A, Hashimoto H, Baba A. Trophic effects of *PACAP* on pancreatic islets: a mini-review. *J Mol Neurosci* 2011; **43**: 3-7 [PMID: 20645023 DOI: 10.1007/s12031-010-9424-z]
- 65 **Vaudry D**, Gonzalez BJ, Basille M, Yon L, Fournier A, Vaudry H. Pituitary adenylate cyclase-activating polypeptide and its receptors: from structure to functions. *Pharmacol Rev* 2000; **52**: 269-324 [PMID: 10835102]
- 66 **Borboni P**, Porzio O, Pierucci D, Cicconi S, Magnaterra R, Federici M, Sesti G, Lauro D, D'Agata V, Cavallaro S, Marlier LN. Molecular and functional characterization of pituitary adenylate cyclase-activating polypeptide (*PACAP*-38)/vasoactive intestinal polypeptide receptors in pancreatic beta-cells and effects of *PACAP*-38 on components of the insulin secretory system. *Endocrinology* 1999; **140**: 5530-5537 [PMID: 10579316 DOI: 10.1210/endo.140.12.7208]
- 67 **Spengler D**, Waeber C, Pantaloni C, Holsboer F, Bockaert J, Seeburg PH, Journot L. Differential signal transduction by five splice variants of the *PACAP* receptor. *Nature* 1993; **365**: 170-175 [PMID: 8396727 DOI: 10.1038/365170a0]
- 68 **Filipsson K**, Kvist-Reimer M, Ahrén B. The neuropeptide pituitary adenylate cyclase-activating polypeptide and islet function. *Diabetes* 2001; **50**: 1959-1969 [PMID: 11522660]
- 69 **Jamen F**, Persson K, Bertrand G, Rodríguez-Henche N, Puech R, Bockaert J, Ahrén B, Brabet P. *PAC1* receptor-deficient mice display impaired insulinotropic response to glucose and reduced glucose tolerance. *J Clin Invest* 2000; **105**: 1307-1315 [PMID: 10792006 DOI: 10.1172/JCI9387]
- 70 **Tomimoto S**, Hashimoto H, Shintani N, Yamamoto K, Kawabata Y, Hamagami K, Yamagata K, Miyagawa J, Baba A. Overexpression of pituitary adenylate cyclase-activating polypeptide in islets inhibits hyperinsulinemia and islet hyperplasia in agouti yellow mice. *J Pharmacol Exp Ther* 2004; **309**: 796-803 [PMID: 14742740 DOI: 10.1124/jpet.103.062919]
- 71 **Schäfer H**, Zheng J, Gundlach F, Günther R, Siegel EG, Fölsch UR, Schmidt WE. Pituitary adenylate-cyclase-activating polypeptide stimulates proto-oncogene expression and activates the AP-1 (c-Fos/c-Jun) transcription factor in AR4-2J pancreatic carcinoma cells. *Eur J Biochem* 1996; **242**: 467-476 [PMID: 9022670]
- 72 **Nakata M**, Shintani N, Hashimoto H, Baba A, Yada T. Intra-islet *PACAP* protects pancreatic  $\beta$ -cells against glucotoxicity and lipotoxicity. *J Mol Neurosci* 2010; **42**: 404-410 [PMID: 20461496 DOI: 10.1007/s12031-010-9383-4]
- 73 **Yamamoto K**, Hashimoto H, Tomimoto S, Shintani N, Miyazaki J, Tashiro F, Aihara H, Nanno T, Li M, Yamagata K, Miyagawa J, Matsuzawa Y, Kawabata Y, Fukuyama Y, Koga K, Mori W, Tanaka K, Matsuda T, Baba A. Overexpression of *PACAP* in transgenic mouse

- pancreatic beta-cells enhances insulin secretion and ameliorates streptozotocin-induced diabetes. *Diabetes* 2003; **52**: 1155-1162 [PMID: 12716746]
- 74 **Barz T**, Hoffmann A, Panhuysen M, Spengler D. Peroxisome proliferator-activated receptor gamma is a Zac target gene mediating Zac antiproliferation. *Cancer Res* 2006; **66**: 11975-11982 [PMID: 17178896 DOI: 10.1158/0008-5472.CAN-06-1529]
- 75 **Rosen ED**, Spiegelman BM. PPARgamma: a nuclear regulator of metabolism, differentiation, and cell growth. *J Biol Chem* 2001; **276**: 37731-37734 [PMID: 11459852]
- 76 **Fajas L**, Aubouef D, Raspé E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J. The organization, promoter analysis, and expression of the human PPARgamma gene. *J Biol Chem* 1997; **272**: 18779-18789 [PMID: 9228052]
- 77 **Ito E**, Ozawa S, Takahashi K, Tanaka T, Katsuta H, Yamaguchi S, Maruyama M, Takizawa M, Katahira H, Yoshimoto K, Nagamatsu S, Ishida H. PPAR-gamma overexpression selectively suppresses insulin secretory capacity in isolated pancreatic islets through induction of UCP-2 protein. *Biochem Biophys Res Commun* 2004; **324**: 810-814 [PMID: 15474499 DOI: 10.1016/j.bbrc.2004.08.238]
- 78 **Ravnskjaer K**, Boergesen M, Rubi B, Larsen JK, Nielsen T, Fridriksson J, Maechler P, Mandrup S. Peroxisome proliferator-activated receptor alpha (PPARalpha) potentiates, whereas PPARgamma attenuates, glucose-stimulated insulin secretion in pancreatic beta-cells. *Endocrinology* 2005; **146**: 3266-3276 [PMID: 15878969 DOI: 10.1210/en.2004-1430]
- 79 **Stiles BL**, Kuralwalla-Martinez C, Guo W, Gregorian C, Wang Y, Tian J, Magnuson MA, Wu H. Selective deletion of Pten in pancreatic beta cells leads to increased islet mass and resistance to STZ-induced diabetes. *Mol Cell Biol* 2006; **26**: 2772-2781 [PMID: 16537919 DOI: 10.1128/MCB.26.7.2772-2781.2006]
- 80 **Wang L**, Liu Y, Yan Lu S, Nguyen KT, Schroer SA, Suzuki A, Mak TW, Gaisano H, Woo M. Deletion of Pten in pancreatic B-cells protects against deficient B-cell mass and function in mouse models of type 2 diabetes. *Diabetes* 2010; **59**: 3117-3126 [PMID: 20852026 DOI: 10.2337/db09-1805]
- 81 **Yang KT**, Bayan JA, Zeng N, Aggarwal R, He L, Peng Z, Kassa A, Kim M, Luo Z, Shi Z, Medina V, Boddupally K, Stiles BL. Adult-onset deletion of Pten increases islet mass and beta cell proliferation in mice. *Diabetologia* 2014; **57**: 352-361 [PMID: 24162585 DOI: 10.1007/s00125-013-3085-8]
- 82 **Boettner B**, Van Aelst L. The RASputin effect. *Genes Dev* 2002; **16**: 2033-2038 [PMID: 12183358 DOI: 10.1101/gad.1020602]
- 83 **Font de Mora J**, Esteban LM, Burks DJ, Núñez A, Garcés C, García-Barrado MJ, Iglesias-Osma MC, Moratinos J, Ward JM, Santos E. Ras-GRF1 signaling is required for normal beta-cell development and glucose homeostasis. *EMBO J* 2003; **22**: 3039-3049 [PMID: 12805218 DOI: 10.1093/emboj/cdg280]
- 84 **Kowluru A**. Friendly, and not so friendly, roles of Rac1 in islet beta-cell function: lessons learnt from pharmacological and molecular biological approaches. *Biochem Pharmacol* 2011; **81**: 965-975 [PMID: 21300027 DOI: 10.1016/j.bcp.2011.01.013]
- 85 **Ljubicic S**, Bezzi P, Vitale N, Regazzi R. The GTPase RalA regulates different steps of the secretory process in pancreatic beta-cells. *PLoS One* 2009; **4**: e7770 [PMID: 19890390 DOI: 10.1371/journal.pone.0007770]
- 86 **Lopez JA**, Kwan EP, Xie L, He Y, James DE, Gaisano HY. The RalA GTPase is a central regulator of insulin exocytosis from pancreatic islet beta cells. *J Biol Chem* 2008; **283**: 17939-17945 [PMID: 18426794 DOI: 10.1074/jbc.M800321200]
- 87 **Valerio G**, Franzese A, Salerno M, Muzzi G, Cecere G, Temple KI, Shield JP. Beta-cell dysfunction in classic transient neonatal diabetes is characterized by impaired insulin response to glucose but normal response to glucagon. *Diabetes Care* 2004; **27**: 2405-2408 [PMID: 15451908]
- 88 **Schmidt-Edelkraut U**, Hoffmann A, Daniel G, Spengler D. Zac1 regulates astroglial differentiation of neural stem cells through Socs3. *Stem Cells* 2013; **31**: 1621-1632 [PMID: 23630160 DOI: 10.1002/stem.1405]
- 89 **Howard JK**, Flier JS. Attenuation of leptin and insulin signaling by SOCS proteins. *Trends Endocrinol Metab* 2006; **17**: 365-371 [PMID: 17010638 DOI: 10.1016/j.tem.2006.09.007]
- 90 **Krebs DL**, Hilton DJ. A new role for SOCS in insulin action. Suppressor of cytokine signaling. *Sci STKE* 2003; **2003**: PE6 [PMID: 12582200 DOI: 10.1126/stke.2003.169.pe6]
- 91 **Emanuelli B**, Glondu M, Filloux C, Peraldi P, Van Obberghen E. The potential role of SOCS-3 in the interleukin-1beta-induced desensitization of insulin signaling in pancreatic beta-cells. *Diabetes* 2004; **53** Suppl 3: S97-S103 [PMID: 15561930]
- 92 **Lindberg K**, Rönn SG, Tornehave D, Richter H, Hansen JA, Römer J, Jackerott M, Billestrup N. Regulation of pancreatic beta-cell mass and proliferation by SOCS-3. *J Mol Endocrinol* 2005; **35**: 231-243 [PMID: 16216905 DOI: 10.1677/jme.1.01840]
- 93 **Karlsen AE**, Rönn SG, Lindberg K, Johannesen J, Galsgaard ED, Pociot F, Nielsen JH, Mandrup-Poulsen T, Nerup J, Billestrup N. Suppressor of cytokine signaling 3 (SOCS-3) protects beta -cells against interleukin-1beta - and interferon-gamma -mediated toxicity. *Proc Natl Acad Sci USA* 2001; **98**: 12191-12196 [PMID: 11593036 DOI: 10.1073/pnas.211445998]
- 94 **Bruun C**, Heding PE, Rönn SG, Frobøse H, Rhodes CJ, Mandrup-Poulsen T, Billestrup N. Suppressor of cytokine signalling-3 inhibits Tumor necrosis factor-alpha induced apoptosis and signalling in beta cells. *Mol Cell Endocrinol* 2009; **311**: 32-38 [PMID: 19643162 DOI: 10.1016/j.mce.2009.07.019]
- 95 **Rezende LF**, Vieira AS, Negro A, Langone F, Boschero AC. Ciliary neurotrophic factor (CNTF) signals through STAT3-SOCS3 pathway and protects rat pancreatic islets from cytokine-induced apoptosis. *Cytokine* 2009; **46**: 65-71 [PMID: 19272793 DOI: 10.1016/j.cyto.2008.12.014]
- 96 **Rezende LF**, Santos GJ, Carneiro EM, Boschero AC. Ciliary neurotrophic factor protects mice against streptozotocin-induced type 1 diabetes through SOCS3: the role of STAT1/STAT3 ratio in beta-cell death. *J Biol Chem* 2012; **287**: 41628-41639 [PMID: 23038263 DOI: 10.1074/jbc.M112.358788]
- 97 **Zhang P**, Liégeois NJ, Wong C, Finegold M, Hou H, Thompson JC, Silverman A, Harper JW, DePinho RA, Elledge SJ. Altered cell differentiation and proliferation in mice lacking p57KIP2 indicates a role in Beckwith-Wiedemann syndrome. *Nature* 1997; **387**: 151-158 [PMID: 9144284 DOI: 10.1038/387151a0]
- 98 **Carey RG**, Li B, DiCicco-Bloom E. Pituitary adenylate cyclase activating polypeptide anti-mitogenic signaling in cerebral cortical progenitors is regulated by p57Kip2-dependent CDK2 activity. *J Neurosci* 2002; **22**: 1583-1591 [PMID: 11880488]
- 99 **Bertrand N**, Castro DS, Guillemot F. Proneural genes and the specification of neural cell types. *Nat Rev Neurosci* 2002; **3**: 517-530 [PMID: 12094208 DOI: 10.1038/nrn874]
- 100 **Bhattacharya A**, Baker NE. A network of broadly expressed HLH genes regulates tissue-specific cell fates. *Cell* 2011; **147**: 881-892 [PMID: 22078884 DOI: 10.1016/j.cell.2011.08.055]
- 101 **Schmidt-Edelkraut U**, Daniel G, Hoffmann A, Spengler D. Zac1 regulates cell cycle arrest in neuronal progenitors via Tcf4. *Mol Cell Biol* 2014; **34**: 1020-1030 [PMID: 24396065 DOI: 10.1128/MCB.01195-13]
- 102 **Rothschild G**, Zhao X, Iavarone A, Lasorella A. E Proteins and Id2 converge on p57Kip2 to regulate cell cycle in neural cells. *Mol Cell Biol* 2006; **26**: 4351-4361 [PMID: 16705184 DOI: 10.1128/MCB.01743-05]
- 103 **Avrahami D**, Li C, Yu M, Jiao Y, Zhang J, Naji A, Ziaie S, Glaser B, Kaestner KH. Targeting the cell cycle inhibitor p57Kip2 promotes adult human beta cell replication. *J Clin Invest* 2014; **124**: 670-674 [PMID: 24430183 DOI: 10.1172/JCI69519]
- 104 **Aynsley-Green A**. Nesidioblastosis of the pancreas in infancy. *Dev Med Child Neurol* 1981; **23**: 372-379 [PMID: 7018975]
- 105 **Nestorowicz A**, Inagaki N, Gono T, Schoor KP, Wilson BA, Glaser B, Landau H, Stanley CA, Thornton PS, Seino S, Permutt MA. A nonsense mutation in the inward rectifier potassium channel gene, Kir6.2, is associated with familial hyperinsulinism. *Diabetes* 1997;

- 46: 1743-1748 [PMID: 9356020]
- 106 **Thomas PM**, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, Aguilar-Bryan L, Gagel RF, Bryan J. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* 1995; **268**: 426-429 [PMID: 7716548]
- 107 **Ryan F**, Devaney D, Joyce C, Nestorowicz A, Permutt MA, Glaser B, Barton DE, Thornton PS. Hyperinsulinism: molecular aetiology of focal disease. *Arch Dis Child* 1998; **79**: 445-447 [PMID: 10193261]
- 108 **Kassem SA**, Ariel I, Thornton PS, Hussain K, Smith V, Lindley KJ, Aynsley-Green A, Glaser B. p57(KIP2) expression in normal islet cells and in hyperinsulinism of infancy. *Diabetes* 2001; **50**: 2763-2769 [PMID: 11723059]
- 109 **Yorifuji T**, Hashimoto Y, Kawakita R, Hosokawa Y, Fujimaru R, Hatake K, Tamagawa N, Nakajima H, Fujii M. Relapsing 6q24-related transient neonatal diabetes mellitus successfully treated with a dipeptidyl peptidase-4 inhibitor: a case report. *Pediatr Diabetes* 2014; **15**: 606-610 [PMID: 24552466 DOI: 10.1111/pedi.12123]
- 110 **Collins FS**, Varmus H. A new initiative on precision medicine. *N Engl J Med* 2015; **372**: 793-795 [PMID: 25635347 DOI: 10.1056/NEJMp1500523]

**P- Reviewer:** Hegardt FG, Lawen A **S- Editor:** Ji FF  
**L- Editor:** A **E- Editor:** Wang CH





Published by **Baishideng Publishing Group Inc**

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: [bpgoffice@wjgnet.com](mailto:bpgoffice@wjgnet.com)

Help Desk: <http://www.wjgnet.com/esps/helpdesk.aspx>

<http://www.wjgnet.com>

