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REVIEW

The WNT signalling pathway and diabetes mellitus

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Abstract The WNT signalling pathway is involved in many physiological and pathophysiological activities. WNT ligands bind to Frizzled receptors and co-receptors (LDL receptor-related protein 5/6), triggering a cascade of signalling events. The major effector of the canonical WNT signalling pathway is the bipartite transcription factor β-catenin/T cell transcription factor (β-cat/TCF), formed by free β-cat and one of the four TCFs. The WNT pathway is involved in lipid metabolism and glucose homeostasis, and mutations in *LRP5* may lead to the development of diabetes and obesity. β-Cat/TCF is also involved in the production of the incretin hormone glucagon-like peptide-1 in the intestinal endocrine L cells. More recently, genome-wide association studies have identified *TCF7L2* as a diabetes susceptibility gene, and individuals carrying certain *TCF7L2* single nucleotide polymorphisms could be more susceptible to the development of type 2 diabetes. Furthermore, β-cat is able to interact with forkhead box transcription factor subgroup O (FOXO) proteins. Since FOXO and TCF proteins compete for a limited pool of β-cat, enhanced FOXO activity during ageing and oxidative stress may attenuate WNT-mediated activities. These observations shed new light on the pathogenesis of type 2 diabetes as an age-dependent disease.

Keywords β-cat/TCF · FOXO · GLP-1 · Stress · TCF7L2 · WNT

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Abbreviations

β-cat	β-catenin
CK-1α	casein kinase-1α
Dvl	Dishevelled protein
FOXO	forkhead box transcription factor subgroup O
GLP-1	glucagon-like peptide-1
GSK-3	glycogen synthase kinase-3
GWA	genome-wide association
LEF-1	lymphoid enhancer-binding factor 1
LRP	LDL receptor-related protein
LUC	luciferase
Pdx-1	pancreas/duodenum homeobox protein 1
PI3K	phosphatidylinositol 3-kinase
PKB	protein kinase B
ROS	reactive oxygen species
SNP	single nucleotide polymorphism
TCF	T cell factor

Glossary

- *β-cat/TCF* This bipartite transcription factor functions as the major effect of the canonical WNT signalling pathway. It is formed by free β-catenin (β-cat) and a T cell factor (TCF)
- *FOXO* Forkhead box transcription factors subgroup O are mediators of stress. FOXO proteins compete with TCF proteins for the limited pool of free β-cat
- *Frizzled* Frizzled proteins (11 different kinds in mammals) receptors with seven transmembrane domains. WNTs can bind the cysteine-rich domain of Frizzled, an extracellular part of the receptor
- *LRP5/6* Low-density lipoprotein receptor-related proteins. They are co-receptors of the WNT ligands
- *S33Yβ-cat* A constitutive active mutant of β-cat that is resistant to proteasome-mediated degradation
- *TCF* There are four T cell factors (TCFs) in mammals, namely, TCF7 (also known as TCF-1), LEF-1, TCF7L1 (also known as TCF-3) and TCF7L2 (also known as TCF-4). In the absence of β-cat, a TCF protein binds to the consensus sequence (CTTTG[A/T][A/T]) in the WNT target gene promoters and represses their expression. β-Cat is able to convert a TCF protein into a transcriptional activator
- *TCF7L2* Also known as TCF-4. Recent genome-wide association studies revealed that polymorphisms in *TCF7L2* have by far the biggest effect on the risk of type 2 diabetes
- *WNT* WNT glycoproteins (encoded by 19 different genes in mammals) function as ligands for the WNT signalling pathway. Although certain WNT ligands can stimulate c-Jun NH_2-terminal kinase (JNK), calcium and protein kinase C signalling pathways, this review only discusses the role of the canonical WNT pathway, which uses the bipartite transcription factor β-cat/TCF as the major effector

Introduction to the WNT signalling pathway

The WNT signalling pathway (referred to as the WNT pathway hereafter) was initially recognised in colon cancer research and in embryonic development studies of the fruit fly (*Drosophila melanogaster*), frog (*Xenopus laevis*) and other organisms [1, 2]. Aberrant activation of the WNT pathway may lead to the development of colorectal and other types of tumours [3]. The major effector of the canonical WNT pathway is the bipartite transcription factor β-catenin/T cell factor (β-cat/TCF). This is formed by the heterodimerisation of free β-cat with one of the four members of the TCF family (TCF7 [also known as TCF-1], lymphoid enhancer-binding factor 1 [LEF-1], TCF7L1 [also known as TCF-3] and TCF7L2 [also known as TCF-4]), TCF7L2 being the major partner of β-cat in the intestinal epithelia [3, 4]. As shown in Fig. 1a, in the absence of WNT, the cellular concentration of free β-cat is tightly controlled by a 'destructive complex', consisting of the tumour suppressor adenomatous polyposis coli (APC), axin, the serine threonine kinase glycogen synthase kinase-3 (GSK-3), casein kinase-1α (CK-1α) and phosphorylated ERK (pERK) [5, 6]. This complex interacts with β-cat and phosphorylates it. The phosphorylated β-cat is then destroyed via the proteasome-mediated degradation process. The WNT ligands exert their effect via the Frizzled receptors and the LDL receptor-related protein 5/6 (LRP5/6) co-receptors (Fig. 1b). Following receptor binding, WNT signals are transmitted by the association of WNT receptors with Dishevelled protein (Dvl). This event triggers the disruption of the destructive complex, preventing the phosphorylation-dependent degradation of β-cat [4], which then enters the nucleus and interacts with a member of the TCF family to form a complex that stimulates the β-cat/TCF (or WNT) downstream target genes (Fig. 1b).

Many in vitro and in vivo examinations have shown that several components of the WNT pathway are involved in pancreatic beta cell proliferation [7–9], normal cholesterol metabolism and glucose-induced insulin secretion [10] and the production of the incretin hormone glucagon-like peptide-1 (GLP-1) [4, 11–13]. More recently, extensive genome-wide association (GWA) studies have identified *TCF7L2* as a type 2 diabetes susceptibility gene. Of all the polymorphisms studied to date, *TCF7L2* polymorphisms have been demonstrated to have by far the biggest effect on the risk of developing type 2 diabetes [14–20]. In addition, the human *LRP5* gene was mapped to within the *IDDM4* region, which is linked to type 1 diabetes on chromosome 11q13 [21–23]. Polymorphisms in *LRP5* have been shown to be associated with obesity phenotypes, and missense mutations in *LRP6* have been shown to be associated with the risk of bone loss, early coronary disease and the metabolic syndrome [24, 25]. Moreover, polymorphisms in *Wnt5B* have been shown to be associated with the risk of type 2 diabetes [26].

The WNT pathway co-receptors (LRP5/6), a WNT ligand (WNT5B), and the major component of the WNT pathway effector (TCF7L2), are evidently involved in preventing the development of type 2 diabetes and other metabolic diseases

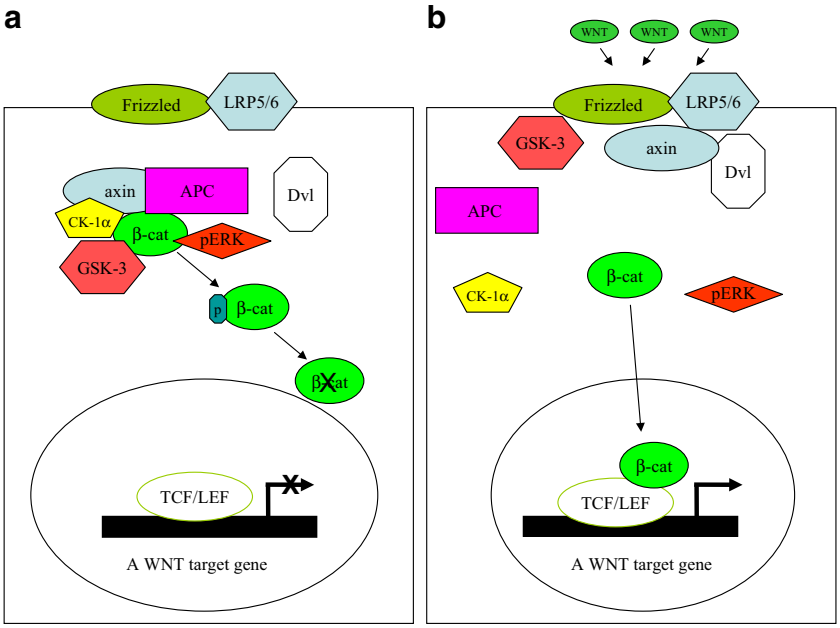

Fig. 1 Summary of the canonical WNT pathway. **a** In the absence of WNT stimulation, β-cat is located within the 'destructive complex', phosphorylated by GSK-3, CK-1α and pERK, and subsequently destroyed by proteasome-mediated protein degradation. **b** Following WNT stimulation, the phosphorylation/destructive complex disassembles. This results in an accumulation of free β-cat, which enters the nucleus and forms the bipartite transcription factor β-cat/TCF, leading to enhanced expression of the WNT target genes. APC, adenomatous polyposis coli; pERK, phosphorylated ERK

In this review, I will first discuss the laboratory experimental studies on the role of the WNT pathway in the development/genesis of mouse pancreatic islets, pancreatic beta cell growth and the production of the incretin hormone glucagon-like peptide-1 (GLP-1). This will be followed by a brief summary of GWA studies of *TCF7L2* and the risk of type 2 diabetes. Finally, I will discuss recent findings indicating that forkhead box transcription factor subgroup O (FOXO) and TCF proteins are able to compete for the limited pool of β-cat, and ageing will lead to increased FOXO-mediated gene transcription and reduced TCF-mediated gene transcription. These findings give us new insights into type 2 diabetes as an age-dependent disease.

WNT signalling is involved in the genesis of pancreatic islets and the proliferation of pancreatic beta cells

Investigations into how β-cat, the major effector of WNT signalling, influences pancreatic islet development using transgenic and knockout mice has produced inconsistent results. Although several WNT ligands and the Frizzled receptors were shown to be produced in the embryonic and postnatal pancreas [27–29], an early study showed that the loss of β-cat did not significantly perturb pancreatic islet endocrine cell mass or function, although β-cat is essential for pancreatic exocrine acinar cell development [30]. Utilising the pancreatic and duodenal homeobox protein 1 (Pdx1)–Cre system to specifically delete the gene encoding β-cat (*Ctnnb1*) in the epithelium of the pancreas and duodenum revealed that *Ctnnb1*-deleted cells had a competitive disadvantage during pancreas development [31]. Although there was a reduction in endocrine islet numbers during early embryonic development and the mice developed pancreatitis perinatally because of the disruption of acinar epithelial structure, the mice later recovered from the pancreatitis and regenerated normal pancreas and duodenal villi from the wild-type cells that escaped the *Ctnnb1* deletion [31]. However, a more recent study found that inducing the production of a stabilised form of β-cat (a mutant one that is more resistant to the proteasome-mediated degradation process) at different stages of development has different effects. During the early stage of organogenesis, robust production of stabilised β-cat drives changes in Hedgehog and fibroblast growth factor signalling and blocks the expression of the gene encoding Pdx-1, an important homeodomain protein transcription factor involved in the genesis of pancreatic beta cells. Induction of the stabilised form of β-cat at a later time point in pancreas development enhances proliferation and increases the size of this organ [32].

Seemingly contradictory results can be resolved if we assume that β-cat and β-cat/TCF exert different functions in a very precise dose-dependent manner at different developmental stages. It appears that mouse embryo is able to overcome a substantial reduction in β-cat via complicated compensatory mechanisms, which allow the generation of a normal pancreas

Rulifson et al. recently examined the effect of WNT signalling in regulating beta cell genesis and proliferation using both in vitro and in vivo approaches [7]. Purified WNT3a (which is known to activate the canonical WNT pathway) stimulated proliferation of both the mouse beta cell line MIN6 and primary mouse pancreatic beta cells, possibly through the cell cycle regulators cyclin D1, cyclin D2 and cyclin-dependent kinase 4, as well as the homeodomain transcription factor Pitx2. Immunohistological examinations of 3-month-old bi-transgenic rat insulin I promoter (RIP)-Cre and β-cat^{active} mice revealed a threefold increase in the production of Ki67 by pancreatic beta cells, which occurred in parallel with a 2.5-fold increase in beta cell mass [7]. Furthermore, axin production led to impaired *Pitx2* gene expression, along with impaired beta cell expansion [7]. Taken together, these observations suggest that WNT signalling is necessary and sufficient for pancreatic beta cell proliferation.

The incretin hormone GLP-1 has been shown to stimulate WNT activity in adult mouse pancreatic islets, and both TCF7L2 and β-cat are required for GLP-1-stimulated proliferation of the rat pancreatic beta cell line INS-1 [9]. Furthermore, adipocyte-derived WNT molecules have been reported to induce beta cell proliferation and insulin secretion in vitro [33].

In vivo mouse and in vitro cell line studies have revealed the essential function of WNT signalling in pancreatic beta cells during embryonic developmental stages and in adulthood

The WNT co-receptor LRP5 is essential for normal lipid metabolism and glucose-induced insulin secretion

Besides the effectors of WNT signalling, the co-receptors of the WNT ligands, LRP-5/6 [34, 35], are also important for normal lipid and glucose metabolism. An early study demonstrated that loss of function mutations of *LRP5* were associated with the development of the autosomal recessive disorder osteoporosis-pseudoglioma syndrome [36]. The co-receptor was shown to be important in transducing WNT signalling and to play critical roles in modulating bone accrual and eye development [37]. The human *LRP5* gene maps within the IDDM4 region on chromosome 11q13, which is linked to type 1 diabetes [21–23]. A recent GWA study has also shown that polymorphisms in *LRP5* are associated with obesity phenotypes [38]. Pancreatic production of LRP5 has been reported [39, 40].

It has been demonstrated that LRP5 can interact with axin, one of the inhibitors of the WNT pathway (Fig. 1). When *Lrp5* was expressed in fibroblast cells, the LRP5 protein alone exerted no effect on the WNT pathway, but acted synergistically with the WNT ligands [41]. Furthermore, LRP5 molecules without the extracellular domain were constitutively active. They induced TCF/LEF-mediated transcription and stabilised β-cat [41]. The addition of WNT ligands to the medium triggered the translocation of axin to the cell membrane and enhanced the interaction between axin and LRP5. Finally, the LRP5 domain involved in the interaction with axin is also required for TCF/LEF-mediated transcriptional activation. These observations collectively suggest that binding of axin by LRP5 and its translocation to the cell membrane is an important part of WNT signal activation [41] (Fig. 1b).

After being fed a high-fat diet, *Lrp5*$^{-/-}$ mice showed increased plasma cholesterol levels and after being fed a normal diet they showed markedly impaired glucose tolerance [10]. Furthermore, in response to the administration of high concentrations of glucose, the mice showed significant reductions in intracellular ATP and calcium levels and decreased glucose-induced insulin secretion [10]. The WNT ligands WNT3a and WNT5a stimulated insulin secretion in the wild-type mice but not in the *LRP5*$^{-/-}$ mice [10], suggesting that WNT ligands require a functional LRP5 to regulate insulin secretion.

In mice, functional LRP5 is important for normal lipid and glucose metabolism. In humans, mutations in *LRP5* may result in obesity, type 1 diabetes and other metabolic diseases

Both WNT and insulin pathways are involved in the production of the incretin hormone GLP-1

GLP-1 is an important incretin hormone that is encoded by the *Gcg* gene, which is expressed in the intestinal endocrine L cells [42–44]. In these cells, expression of *Gcg* mRNA and production of GLP-1 can be activated by lithium, which mimics the function of the WNT ligands [45], or the overproduction of the constitutively active S33Y β-cat mutant [12], indicating that *Gcg* is a downstream target of the WNT signalling pathway. Activation was subsequently attributed to a TCF binding site within the G2 enhancer element of the *Gcg* promoter and the production of TCF7L2 in the intestinal endocrine L cells [4]. It is well known that *Gcg* expression and GLP-1 production can be activated by elevations in cAMP levels [46–52]. Since the G2 enhancer element has been shown to mediate the stimulatory effect of both cAMP and calcium on *Gcg* promoter activity [53], it is

possible that cAMP pathway cross-talks with the WNT pathway to regulate *Gcg* expression [12].

Insulin inhibits *Gcg* expression in pancreatic alpha cells [54, 55]. This inhibition is physiologically important because *Gcg* mRNA expression in pancreatic islets leads to the production of glucagon, the primary counter-regulatory hormone of insulin [54, 55]. A recent study showed a significant stimulatory effect of insulin on *Gcg* mRNA expression and GLP-1 production in intestinal L cells. Interestingly, insulin activated a *Gcg*–luciferase (LUC) reporter gene construct containing the wild-type TCF binding site within the G2 enhancer element of the *Gcg* promoter, but not the one carrying a mutation at this site. Either 'knocking-down' β-cat production or the function of TCF7L2 completely blocked insulin-stimulated intestinal *Gcg* expression. Thus, insulin, at least partially uses effectors of the WNT pathway to exert this stimulatory effect [13]. Interestingly, the stimulatory effect of insulin on *Gcg* promoter activity was blocked by phosphatidylinositol 3-kinase (PI3K) inhibition, but not by protein kinase B (PKB) inhibition [13]. This, along with the observations of the existence of PKB-independent PI3K activity in other cell lineages [56–58], suggest that an unknown signalling component mediates PI3K-mediated cross-talk between insulin and WNT signalling pathways [13]. In hyperinsulinaemic and insulin-resistant MKR mice [59, 60], *Gcg* mRNA expression and GLP-1 production in the distal ileum were significantly higher than in sex- and age-matched controls [13], indicating that hyperinsulinaemia and/or insulin resistance may affect the homeostasis of GLP-1 production. Figure 2 shows that both WNT and cross-talk between insulin and WNT are involved in GLP-1 production, although detailed mechanisms and the pathophysiological significance of the cross-talk need to be further explored. Figure 2 also shows that lithium and cAMP may stimulate *Gcg* expression by affecting the production of free β-cat.

It appears that β-cat/TCF functions as the effector of other signalling pathways to regulate *Gcg* expression and GLP-1 production. It is likely that cross-talk between insulin and WNT pathways is involved in the homeostasis of GLP-1 production

TCF7L2 polymorphisms are associated with the risk of type 2 diabetes

In 2006, a study reported that inheritance of specific single nucleotide polymorphisms (SNPs) within the *TCF7L2* gene was related to an increased risk of type 2 diabetes [20]. The investigators of this study have genotyped 228 microsatellite

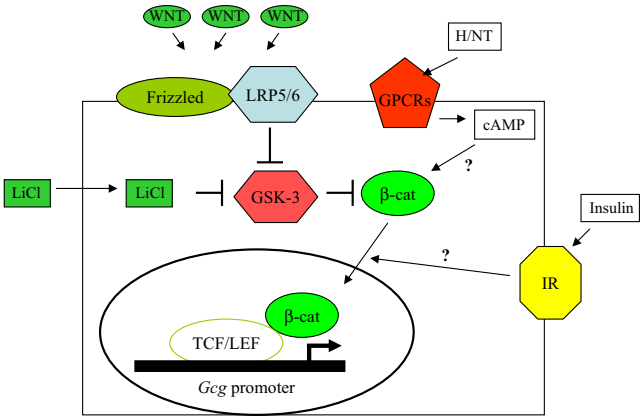

Fig. 2 Multiple signalling cascades utilise β-cat/TCF as the effector for the regulation of *Gcg* expression. Both WNT and LiCl increase levels of free β-cat through inhibition of GSK-3. Hormone and neurotransmitters (H/NT) may enhance β-cat/TCF activity by interacting with their G-protein-coupled receptors (GPCRs). Insulin may enhance the translocation of β-cat into the nucleus. The question marks indicate that detailed mechanisms for these events are still under investigation. IR, insulin receptor

markers in Icelandic individuals with type 2 diabetes and healthy controls across a 10.5 Mb interval on chromosome 10q. A microsatellite, DG10S478, located within the intron 3 region of *TCF7L2*, was found to be associated with type 2 diabetes [20]. This observation was subsequently replicated in both a Danish and US cohort [20]. The investigators found that two of five SNPs investigated within introns 4 and 5 of *TCF7L2*, namely rs12255372 and rs7903146, were in strong linkage disequilibrium with DG10S478 and showed similarly robust associations with type 2 diabetes [20]. This discovery has drawn attention globally [15–19, 61–71], and studies in many other ethnic groups have confirmed that rs12255372 and rs7903146 are the two SNPs most strongly associated with type 2 diabetes [72, 73], with the SNP rs7903146 reported to have the greatest effect in white individuals [73, 74]. The two SNPs occur at relatively low frequencies in Asian populations, although an association with type 2 diabetes was identified in two large Japanese cohorts [75, 76]. Recent studies have revealed two novel SNPs associated with the risk of type 2 diabetes. The SNP rs290487 was identified in a study of a Han Chinese population in Taiwan [77], and the SNP rs11196218 was identified in a study of Hong Kong Chinese individuals [78]. It has been reported that non-diabetic carriers of the risk-associated *TCF7L2* SNPs do not have defects in GLP-1 secretion [64].

All the SNPs identified to date in *TCF7L2* are located within the intron regions of the gene. How these SNPs affect the expression of *TCF7L2*, and therefore the risk of type 2 diabetes, remains largely unknown

Since TCF7L2 is known as an intestinal cell specific transcription factor [79] and is an important regulator of intestinal *Gcg* expression and GLP-1 production [11], it was suggested that the *TCF7L2* SNPs may modify disease susceptibility by affecting intestinal *Gcg* expression and plasma levels of GLP-1 [20]. More recently, genotyping 1,100 non-diabetic German individuals for the five known *TCF7L2* SNPs indicated that *TCF7L2* variants are associated with reduced insulin secretion [64]. In contrast, plasma GLP-1 levels during an OGTT were not significantly influenced by the *TCF7L2* variants [64]. The CT/TT genotypes of the SNP rs7903146 were shown to strongly predict future type 2 diabetes in two independent Scandinavian cohorts [63]. The risk T allele was associated with impaired insulin secretion, incretin effects, and an enhanced rate of hepatic glucose production [63]. Furthermore, investigators found that islet *TCF7L2* expression was increased fivefold in individuals with the TT genotype. Although *TCF7L2* expression was positively correlated with the expression of *INS*, which encodes insulin, it was inversely correlated with glucose-stimulated insulin release [63]. Furthermore, an ex vivo examination demonstrated that *TCF7L2* knockdown (with small interfering RNA) increased human pancreatic beta cell apoptosis and reduced beta cell proliferation and glucose-stimulated insulin secretion [8]. Overexpression of *TCF7L2*, on the other hand, protected islets from glucose- and cytokine-induced apoptosis and from impaired functions [8]. As discussed above, both TCF7L2 and β-cat are required as effectors for GLP-1-stimulated beta cell proliferation [9].

Although these recent studies indicate that *TCF7L2* SNPs may directly affect *INS* expression and/or insulin secretion, we still do not have a clear picture of how these SNPs affect the function of pancreatic beta cells. As discussed by Schafer and colleagues, the involvement of changes in GLP-1 production and secretion influenced by *TCF7L2* variants in the increased risk of type 2 diabetes cannot be eliminated [64]. It should also be pointed out that the participants in their study were non-diabetic individuals. It is possible that a certain compensatory response(s) attenuated the defect in GLP-1 secretion in these *TCF7L2* SNP carriers in the pre-diabetic stages.

FOXOs compete with TCFs for the limited pool of β-cat

Over the last few years, investigations have led to the discovery of the insulin–FOXO protein signalling cascade [80–83]. This regulatory system controls metabolic homeostasis and other important physiological and pathophysiological events. In the absence of insulin or growth factors, FOXOs are mainly located within the nuclei and upregulate the expression of a set of target genes, thereby promoting cell cycle arrest, stress resistance and apoptosis (Fig. 3a). In the presence of insulin or growth factors, FOXOs are phosphorylated by PKB and serum- and glucocorticoid-regulated protein kinase and stay in the cell cytosol (Fig. 3b) [81]. In this way, FOXOs function to control the growth, development, metabolism, and possibly, longevity of the cell and the organism in response to insulin, insulin-like growth factor-1 and many other growth factors [81]. In contrast to the effect on insulin signalling, oxidative stress induces the activation of FOXO signalling [84]. It appears that this is due to the activation of the small GTPase Ral, which leads to the c-Jun NH_2-terminal kinase (JNK)-dependent phosphorylation of FOXOs, followed by their translocation to the nucleus and increased FOXO-mediated transcriptional activities [84].

An evolutionarily conserved interaction between the WNT pathway effector β-cat and FOXOs was discovered in 2005 [85]. In mammalian cells, a yeast two-hybrid screen detected an interaction between β-cat and FOXO1 and FOXO3. The gene *bar-1* (also known as *C54D1.6*) encodes the *Caenorhabditis elegans* (nematode worm) homologue of β-cat [86], while the FOXO gene homologue in this organism is *daf16* [87]. An interaction between BAR-1 and DAF16 was also detected [85]. In mammalian cells, binding of β-cat to FOXO enhanced the transcriptional activity of FOXO. In *C. elegans*, the loss of BAR-1 reduced the activity of DAF16 in dauer formation and life span [85]. More importantly, the association between β-cat and FOXO was shown to be enhanced in cells exposed to oxidative stress [85].

The interaction between FOXO and β-cat prompted scientists to explore the pathophysiological role of this interaction in age-dependent diseases, including osteoporosis. It was reported that male and female mice with sufficient sex hormones could still lose bone mass and strength progressively during the ages of 4–31 months, and that this was associated with enhanced osteoblast and osteocyte apoptosis, reduced osteoblast number and bone formation rate, and elevated levels of reactive oxygen species (ROS) [88]. Furthermore, in the C57BL/6 mice, ageing was shown to be associated with the reduced expression of several WNT target gene mRNAs, including *Axin2* and *Opg* (also known as *Tnfrsf11b*); and increased expression of the FOXO target genes, such as *Gadd45* (also known as *Gadd45a*) [89]. However, hydrogen peroxide treatment in an uncommitted mesenchymal cell line, C2C12, increased FOXO-mediated transcription and attenuated both basal and WNT3A-stimulated levels of *Axin2* and other WNT target genes. Opposite effects of hydrogen peroxide on FOXO- and TCF-mediated transcription were confirmed by measuring the reporter gene activity of FOXO-LUC and TCF-LUC in response to different dosages of hydrogen peroxide. More recently, the concept of that the interaction

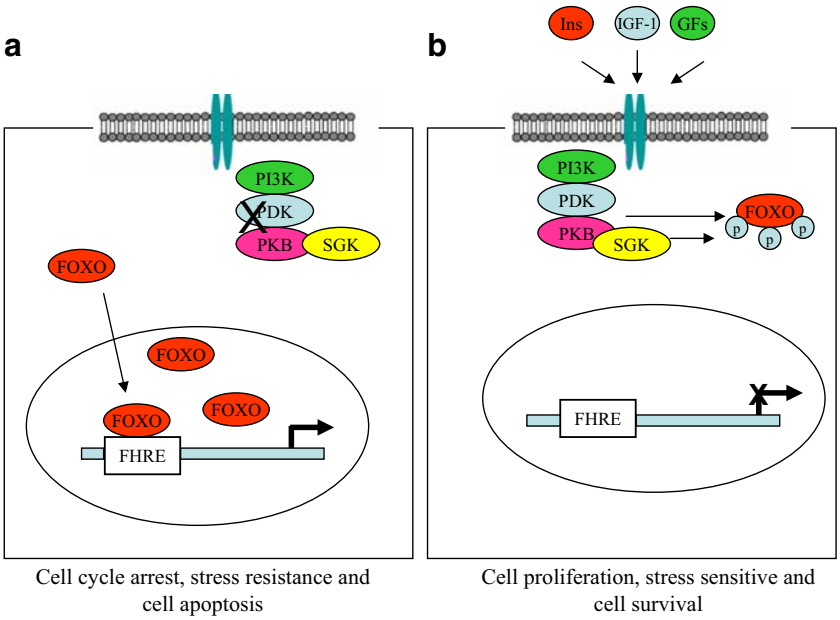

Fig. 3 Schematic representation of FOXO signalling. **a** Without insulin or a growth factor, FOXO enters the nucleus, binds to the forkhead response element (FHRE) of its downstream target genes. The activation of these genes (such as *p27* [also known as *Cdkn1b*], *Bim* [also known as *Bcl2l11*], *Gadd45* and *Fasl*) will lead to cell cycle arrest, stress resistance and cell apoptosis. **b** Insulin (Ins), insulin-like growth factor-1 (IGF-1) and other growth factors (GFs) are able to activate the PI3K–PDK-1–PKB signalling pathway. Both PKB and serum- and glucocorticoid-inducible kinase (SGK) phosphorylate FOXOs, trapping them in the cytosol. PDK-1, 3′-phosphoinositide-dependent kinase-1

of FOXO with β-cat inhibits β-cat/TCF activity was further confirmed [90]. It has been demonstrated that small interfering RNA (siRNA)-mediated knockdown of FOXO reverted the loss of β-cat binding to TCF after cellular oxidative stress [90]. The production and function of FOXO proteins in the pancreatic beta cells have been extensively investigated during the past few years [91]. It will be interesting to examine whether insulin and growth

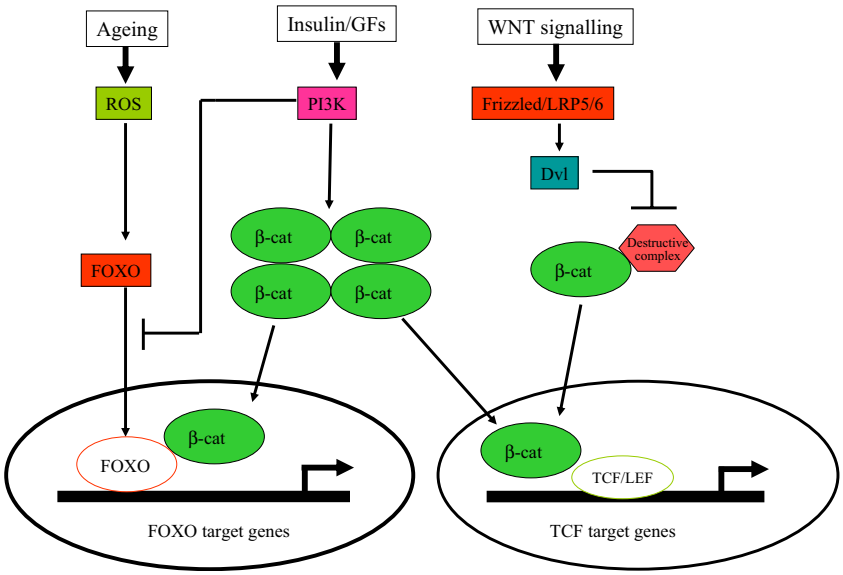

Fig. 4 Insulin/growth factors control the balance between FOXO- and TCF-mediated gene expression. FOXOs and TCFs compete for the limited pool of β-cat. During ageing and oxidative stress, the production of ROS leads to increased FOXO-mediated gene transcription and reduced TCF-mediated gene transcription. This will lead to reduced WNT activity, which is important for lipid and glucose metabolism, pancreatic beta cell proliferation and function and the production of the incretin hormone GLP-1. Insulin/growth factors (GFs) help to restore the balance by two means. First, they stimulate the nuclear exclusion of FOXOs via phosphorylation mediated by PKB/serum- and glucocorticoid-inducible kinase (SGK) [81, 82, 91]. Second, they enhance the nuclear content of β-cat and the binding of β-cat/TCF to the WNT target gene promoters via a yet to be identified PKB-independent mechanism [13]

factors control the balance between FOXO-mediated and WNT-mediated gene transcription (see below).

FOXOs and TCF proteins compete for the common co-factor β-cat, while insulin/growth factors inhibit FOXO-mediated gene expression by trapping FOXOs in the cell cytosol

Summary and perspective

Comprehensive in vitro and in vivo studies by multiple laboratories have shown that WNT signalling is important in normal pancreatic islet development, as well as in pancreatic beta cell function and genesis. It appears that the WNT/β-cat pathway plays direct, precise, and even opposing roles during different stages of pancreatic islet development [32]. Obviously, to exert such precise and opposing effects, WNT/β-cat signalling needs to interact with other signalling pathways and regulate the downstream gene expression profiles, both temporally and spatially.

WNT signalling is also important in activating intestinal *Gcg* transcription and, therefore, the production of the incretin hormone GLP-1 [11–13], which has been shown to utilise WNT signalling effectors, i.e. β-cat/TCF7L2, to exert its effect on beta cell proliferation [9]. Further examinations are required to verify whether TCF7L2, certain WNT ligands and Frizzled receptors are also involved in the genesis of pancreatic islets and intestinal endocrine L cells.

GWA have revealed relationships between SNPs in *LRP5*, which encodes a co-receptor of the WNT ligands, and the risk of type 1 diabetes [22, 23], as well as obesity [29]. *LRP6* mutations are possibly related to the development of bone loss, coronary disease and the metabolic syndrome [24, 25]. More importantly, extensive recent studies have identified associations between SNPs in *TCF7L2* and the risk of type 2 diabetes. These observations suggest that WNT signalling is not only involved in pancreatic islet development during embryogenesis, but also in the function of pancreatic and intestinal endocrine cells during adulthood. Since all the known risk-associated SNPs of *TCF7L2* are located within the intronic regions, the effect of these SNPs on *TCF7L2* expression should be examined. To ultimately understand why these SNPs affect the risk of type 2 diabetes, we need to explore mechanisms underlying TCF7L2 production in pancreatic and intestinal endocrine cells under both physiological and pathological conditions.

Type 2 diabetes is a chronic and age-dependent disease. As shown in Fig. 4, nuclear FOXOs increase during ageing because of the accumulation of ROS and JNK signalling pathway activation [92]. FOXOs compete with TCF proteins, including TCF7L2, for the limited pool of β-cat. This leads to reduced WNT activity, which is important for lipid and glucose metabolism, pancreatic beta cell proliferation and function, and the production of the incretin hormone GLP-1. Insulin and growth factors, on the other hand, may restore the balance between FOXO- and TCF-mediated gene transcription by trapping FOXOs within the cell cytosol. The establishment of this concept offers a new perspective on the pathogenesis of type 2 diabetes and other age-dependent diseases. Further examination of the cross-talk between insulin/growth factors and WNT signalling pathways may lead to the development of novel therapeutic approaches for the treatment of type 2 diabetes and other age-dependent diseases.

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

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