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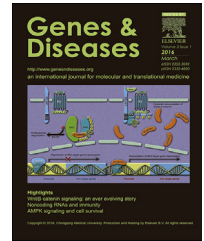


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REVIEW ARTICLE

Current understanding and dispute on the function of the Wnt signaling pathway effector TCF7L2 in hepatic gluconeogenesis



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Abstract Approximately 10 years ago, the Wnt signaling pathway effector TCF7L2 (=TCF-4) was recognized as a type 2 diabetes (T2D) risk gene through a genome wide association study (GWAS). As the correlation between *TCF7L2* polymorphisms and T2D susceptibility has been reproducibly observed by numerous follow-up investigations among different ethnic groups, great efforts have been made to explore the function of TCF7L2 in metabolic organs including the pancreas, liver and adipose tissues. Although these explorations have enriched our general knowledge on the Wnt signaling cascade in metabolic homeostasis, studies conducted to date have also generated controversial suggestions. Here I will provide a brief review on the Wnt signaling pathway as well as the milestone GWAS discovery and the follow-up studies. I will then discuss the two different opinions on the correlation between *TCF7L2* variants and T2D risk, a gain-of-function event versus a loss-of-function event. This will be followed by summarizing the relevant investigations on the metabolic function of hepatic TCF7L2 and presenting our view on the discrepancy and perspectives.

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The recognition of *TCF7L2* as a diabetic risk gene

Although several early investigations have indicated the role of Wnt signaling pathway in the production and function of certain metabolic hormones,^{1–4} the intensive global

attention to the function of this pathway on glucose homeostasis started in 2006, after a genome wide association study (GWAS) performed by Grant and colleagues revealed the linkage between the polymorphisms of the Wnt signaling pathway effector TCF7L2 and the risk of type 2 diabetes (T2D).⁵

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Back to 1999 and 2003, investigators had revealed that a region on chromosome 10q is linked to T2D susceptibility.^{6,7} Briefly, using a variance-components technique for conducting multi-point linkage analyses in a Mexican American population, Duggirala et al obtained evidence that there is a T2D susceptibility locus on chromosome 10q.⁶ In 2003, a genome wide linkage study performed by Reynisdottir et al in an Icelandic population yielded the linkages of T2D susceptibility to regions on chromosome 5q34-q35.2, 12q, as well as 10q.⁷ Three years later, Grant et al defined the genetic linkage on chromosomal 10q.⁵

Grant et al genotyped 228 microsatellite markers in a cohort of Icelandic subjects with T2D and healthy controls across a 10.5-Mb interval on the chromosome 10q. The microsatellite, DG10S478, located within the intron 3 region of the *TCF7L2* gene (previously known as *TCF-4*) was found to be associated with the T2D susceptibility. This correlation was then replicated in a U.S. cohort as well as a Danish cohort. Furthermore, two single nucleotide polymorphisms (SNPs) known as rs12255372 and rs7903146 were found to be in strong linkage disequilibrium with DG10S478 and also showed similar robust associations with T2D susceptibility. By comparing with the non-carriers, they calculated that heterozygous and homozygous carriers of the at-risk alleles have relative risks of T2D of 1.45 and 2.41, respectively.⁵ Importantly, this association has been replicated by numerous investigations among different ethnic populations.^{8–14} Studies have also revealed the linkage between these T2D susceptibility SNPs with cardiovascular and other complications of T2D.^{15,16}

Wnt signaling pathway and its key effector β -catenin/TCF

The Wnt signaling pathway was initially identified in cancer research and embryologic developmental studies^{17,18}, while the physiological role of Wnt signaling in metabolic homeostasis and its implications in metabolic disorders have received broad attention since last decade,^{1,19,20} especially after *TCF7L2* is recognized as an important T2D risk gene.⁵

The key effector of Wnt pathway is β -catenin (β -cat)/TCF (cat/TCF), formed by free β -cat and a member of the TCF transcription factor family, including *TCF7L2*.²¹ TCFs possess a high mobility group box (HMG) DNA binding domain while β -cat provides the transcriptional activation domain. In resting cells, free β -cat levels are tightly controlled by the proteasome-mediated degradation process (Fig. 1A, left panel). This involves the actions of the degradation complex on β -cat. The key components of this degradation complex are two tumor suppressors, adenomatous polyposis coli (APC) and axin/conduction; as well as two protein kinases, the serine/threonine kinase glycogen synthase kinase-3 (GSK-3) and casein kinases 1 α (CK-1 α). Following binding of a canonical Wnt ligand to the Frizzled receptor and LRP5/6 co-receptor, the degradation complex is dissociated, with the participation of the protein namely dishevelled (Dvl). This will prevent the degradation of free β -cat, which will accumulate and enter the nucleus, resulting in the formation of cat/TCF and the activation of cat/TCF (or Wnt signaling pathway)

downstream target gene expression (Fig. 1A, right panel). Importantly, in the absence of β -cat, however, TCFs may repress Wnt target gene expression via recruiting nuclear co-repressors, such as histone deacetylases (HDACs), C-terminal binding protein 1 (CtBP1) and transducin-like enhancer of split (TLE), the mammalian homologue of *Drosophila* Groucho.

Fig. 1A also shows that in addition to serving as the effector of Wnt ligands, cat/TCF can mediate the function of certain hormonal factors, such as the two important metabolic hormones, insulin and glucagon-like peptide-1 (GLP-1).^{22,23} This can be achieved by regulating *TCF7L2* expression and by stimulating the phosphorylation on β -cat C-terminal serine residues (S675 and S552) (Fig. 1B).^{22,24–29} Our experimental results demonstrated that feeding increased hepatic *Tcf7l2* mRNA and protein levels in mice, while *in vitro* insulin treatment in primary hepatocytes increased both *Tcf7l2* expression and β -cat S675 phosphorylation.²² In pancreatic β -cells, Liu and Habener, as well as my team demonstrated the effect of GLP-1 on β -cat S675 phosphorylation.^{30,31}

Mouse brain was shown to express dominant negative *Tcf7l2* molecules during the early embryonic developmental stage.³² Whether such native dominant negative molecules are expressed during adulthood in any peripheral organ is unknown. Scientists, however, can generate dominant negative *TCF7L2* (*TCF7L2DN*) for attenuating Wnt signaling in various systems or cell lineages.^{2,24,31,33–38}

TCF7L2 structure and the positions of T2D risk SNPs

Fig. 2 shows the intron-exon structure of *TCF7L2* and the SNPs that were found to be associated with T2D susceptibility. In addition to rs12255372 and rs7903146 which are known to be strongly associated with T2D risk in Caucasian populations, two other SNPs, known as rs290487 and rs11196218 were also indicated. They were recognized as the risk SNPs for an Asian population study and a study in a Hong Kong Chinese population.^{12,13} The *TCF7L2* gene consists of 17 exons. Among them, exon 4, 13, 14, 15 and 16 can be alternatively spliced, leading to the generation of 13 different transcripts. Such overall organization is conserved between humans and rodents. However, the sizes of protein products detected by Western blot for most tissues are 78 kDa and 58 kDa, respectively. A recent mouse embryonic study revealed the existence of brain specific promoters Ex1b-e, located within intron 5.³² Transcripts from these promoters will lead to the generation of *TCF7L2* that lack the N-terminal β -cat interaction domain, anticipating function as native dominant negative molecules.³² We cannot detect such transcripts in adult mouse liver or pancreas, although it is expressed in the mouse brain (data not shown). Whether such transcripts exist in any of the human tissues remains unknown.

TCF7L2 variants in T2D risk, gain-of-function or loss-of-function?

TCF7L2 T2D risk SNPs are located within the intron regions (Fig. 2). These SNPs may affect *TCF7L2* expression, although it is difficult to determine the underlying

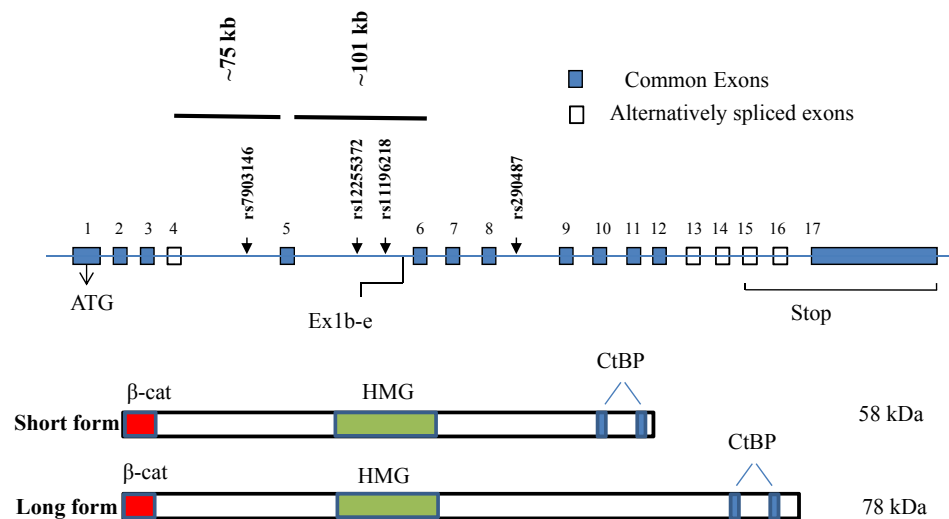


Fig. 2 TCF7L2 genetic structure and the positions of the T2D risk SNPs. TCF7L2, located on chromosome 10q25.3, consists of 17 exons. Among them, five can be alternatively spliced, leading to the generation of 13 different sized transcripts. Two T2D risk SNPs in Caucasian population, namely rs7903146 and rs1225372, are located within intron 4 and 5 respectively. Two other risk SNPs, rs1196218 and rs290487 were identified in Asian and Chinese populations. Ex1b-e are promoters identified in the brain during embryonic developmental stage. Their transcription leads to the generation of native dominant negative molecules. Western blot can detect two different sized TCF7L2 proteins, with estimated molecular weights of 58 kDa (short) and 78 kDa (long), respectively. For both forms, relative positions for the β -cat interaction domain, the HMG DNA binding domain and the CtBP interaction domains are indicated.

reported that liver-specific *Tcf7l2* overexpression increased hepatic glucose production.³⁹

Observations that support the view of loss-of-function

The above observations on the potential deleterious effect of TCF7L2/Tcf7l2, however, were not supported by a number of other investigations. Shu and others in Maedler's team made a series of observations that supported the beneficial effect of TCF7L2 in pancreatic islet.^{40–44} They found that in human or mouse pancreatic islets, siRNA-mediated TCF7L2/Tcf7l2 knockdown resulted in a significant elevation in β -cell apoptosis and a decrease in β -cell proliferation, associated with the attenuation of glucose-stimulated insulin secretion. In contrast, TCF7L2 overexpression protected islets from glucose- and cytokine-mediated apoptosis.⁴⁴ Furthermore, they found that Tcf7l2 knockdown reduced the expression of the two incretin hormone receptors.⁴³ A number of other investigations also supported the view that TCF7L2 exerts beneficial effects in pancreatic β -cells.^{37,45–51} Finally, several investigations suggested the repressive effect of TCF7L2 on hepatic gluconeogenesis,^{22,33,52–54} which are also in contrast with the observations made by Boj and colleagues.³⁹ (see below Session 4 for details).

Controversial observations made on the exploration of metabolic function of hepatic TCF7L2

The function of TCF7L2 on hepatic gluconeogenesis was initially investigated by Lyssenko and colleagues in 2007.

They observed that the carrier of the TCF7L2 T2D risk SNP rs7903146 had enhanced the rates of hepatic glucose production.¹⁴ A subsequent follow-up study by Pilgaard et al in human subjects also demonstrated that this T2D risk TCF7L2 allele was associated with increased hepatic glucose production, when a hyperinsulinemic clamp was applied.⁵⁵

Norton et al found that *in vitro* TCF7L2 silencing resulted in a marked increase in basal hepatic glucose production, accompanied by the increases in the expression of a battery of gluconeogenic genes, including those genes encoded the rate limiting enzymes fructose-1,6-bisphosphatase (Fbp1), phosphoenolpyruvate carboxykinase 1 (Pck1) and glucose 6-phosphatase (G6pc).⁵² Overexpression of TCF7L2, however, reversed the above phenotypes. TCF7L2 silencing did not affect the half-maximal inhibitory concentration of insulin or metformin on glucose production. They suggested that this was due to the fact that basal hepatic glucose production level remains elevated in TCF7L2-silenced cells.⁵² This team has recently reported their RNAseq and ChIP-Seq studies in defining the TCF7L2 transcriptional network in hepatocytes.⁵⁶

The Wnt signaling pathway is essential for the development and zonation of the embryonic liver. By taking the advantage of the existence of the TOPGAL transgenic mouse model,⁵⁷ Ip et al in my team examined Wnt signaling activity in adult mice. They detected the strong Wnt activity in the liver, especially in pericentral hepatocytes.²² They then observed that feeding increased hepatic Tcf7l2 mRNA and protein expression levels, while *in vitro* insulin treatment in mouse primary hepatocytes increased both Tcf7l2 mRNA and protein levels, as well as β -cat S675 phosphorylation. Wnt-3a treatment was shown to reduce gluconeogenic gene expression and glucose production in

hepatic cells *in vitro*. siRNA-mediated Tcf7l2 knock-down, however, increased glucose production and gluconeogenic gene expression in cultured hepatocytes.²² Our observations collectively support the view that Wnt signaling pathway negatively regulates hepatic gluconeogenesis; and that the Wnt pathway effector cat/TCF also mediates the function of postprandial insulin elevation in repressing glucose production.²²

Very recently, Ip et al presented us *in vivo* transgenic mouse work, further supporting the notion that Wnt signaling activity negatively regulates hepatic glucose production.³³ Briefly, we have generated a novel transgenic mouse model namely *LTCFDN*. In this mouse line, TCF7L2DN expression was driven by the liver-specific albumin promoter. *LTCFDN* mice showed a progressive impairment in response to pyruvate challenge, in the absence of insulin intolerance. *LTCFDN* hepatocytes displayed elevated gluconeogenic gene expression and glucose production. These hepatocytes also showed the loss of Wnt-3a-mediated repression of gluconeogenesis. The above observations were then reproduced in primary mouse hepatocytes with adenovirus mediated TCF7L2DN expression.³³

Several other lines of research also supported the suggestion that Wnt signaling activation and TCF7L2 negatively regulates hepatic gluconeogenesis.

A comprehensive *in vitro* and *in vivo* investigation on the role of TCF7L2 in hepatic gluconeogenesis was presented by Oh et al in 2012.⁵³ They found that Tcf7l2 expression was reduced in mice with insulin resistance, either due to the generic defect (leptin receptor deficient *db/db* mice) or after HFD feeding. They had then utilized the tail vein injection approach to knockdown hepatic Tcf7l2 with the adenovirus that expresses Tcf7l2 shRNA. Hepatic Tcf7l2 knockdown resulted in increased blood glucose levels and glucose intolerance, associated with elevated gluconeogenic gene expression. Furthermore, overexpression of a nuclear isoform of Tcf7l2 in HFD-fed mice improved glucose tolerance. The authors had also assessed the binding of Tcf7l2 to promoters of gluconeogenic genes (*Pck-1* and *G6pc*), as well as the effect of its binding on inhibiting the promoter occupancies of gluconeogenic transactivators, including *cAMP response element-binding* protein (CREB), CREB regulated transcription coactivator 2 (CRTC2), and FoxO.⁵³

Neve et al had made a quantitative comparison of *TCF7L2* transcript expression in the liver in carries of rs7903416 with diabetes and with normoglycemia. Five C-terminal *TCF7L2* transcripts were showed to be increased in the risk allele carriers with diabetes, as well as in diabetes patients regardless of their genotype.⁵⁸ Similar to what we had observed, in the human HepG2 cell line, TCF7L2 expression levels were also shown to be increased upon high glucose or insulin incubation. More importantly, insulin stimulated TCF7L2 expression is correlated with reduced HNF4 α levels, while selected TCF7L2 transcripts interact with HNF4 α , a transactivator of *G6pc* and *Pck-1*.⁵⁸

Together, these investigations collectively support the notion that hepatic TCF7L2 level can be postprandially regulated. This is likely due to the elevation of the key gluconeogenic repressive hormone insulin. Thus, in response to the elevation of insulin secretion, Wnt signaling negatively regulates hepatic gluconeogenesis and hence

reduces the plasma glucose level. However, a study on liver-specific Tcf7l2 knockout mice suggested the opposite.

Tcf7l2^{-/-} mice were generated in 1998 by Korinek and colleagues, eight years before the recognition of its human counterpart *TCF7L2* as an important T2D risk gene.⁵⁹ *Tcf7l2*^{-/-} mice died in the immediate postnatal period, accompanied with the lack of stem cells in their intestinal crypts.⁵⁹

Since Boj et al observed no metabolic defects in pancreatic β -cell specific *Tcf7l2* KO mice 39; they had reassessed the new born *Tcf7l2*^{-/-} mice. They found that immediately after birth, plasma glucose levels were indistinguishable between *Tcf7l2*^{+/+}, *Tcf7l2*^{+/-}, and *Tcf7l2*^{-/-} mice. Three hours after birth, however, *Tcf7l2*^{-/-} newborns showed hypoglycemia, which was not due to excessive insulin secretion. They were able to extend the life span of these mice to beyond 5 h with glucose injection, and then demonstrated that new born *Tcf7l2*^{-/-} mice show reduced gluconeogenic gene expression. This team then generated liver-specific *Tcf7l2* KO mice during adulthood. For this purpose, they mated the *Tcf7l2loxP* mice with the tamoxifen-inducible liver-specific Cre recombinase strain SACre-ERT2. They found that these liver-specific adult *Tcf7l2* knockout mice had reduced hepatic glucose production during fasting period and exhibited improved glucose homeostasis after HFD challenge. Finally, they also tested the metabolic effect of transient hepatic TCF7L2 overexpression with the adenovirus tail vein injection approach. In contrast to what was reported by Oh et al,⁵³ Boj et al found that liver TCF7L2 over-expression increased serum glucose level under fasting conditions. Again, this increase was not due to a reduction in circulating insulin levels.³⁹ Together, this team presented an opposite view with a number of other investigators, that TCF7L2 positively regulates hepatic gluconeogenesis and they suggested that the inhibition of Wnt signaling may bring the beneficial effect in metabolic diseases.³⁹

A current popular view on the discrepancy

As presented above, although enormous efforts have been made to explore the metabolic function of TCF7L2, as facilitated by the reproducible GWAS discovery, opposite views have been generated regarding the role of this Wnt signaling pathway effector on hepatic glucose production. Here I will first of all present the view shared by my colleagues and I, arguing that Wnt signaling pathway is indeed a negative regulator of hepatic gluconeogenesis. Physiologically, it serves as the effector of the most important repressive hormone insulin postprandially. I will then discuss the potential technical and other problems that may contribute to the generation of the discrepancy.

We suggest that the activation of Wnt signaling pathway negatively regulates hepatic gluconeogenesis. Firstly, both TCF7L2 expression and β -cat activation (S675 phosphorylation) were shown to be stimulated by insulin, which is the most important repressor of hepatic gluconeogenesis. It is unlikely that insulin represses gluconeogenesis but at the same time activates the Wnt signaling pathway effector to stimulate its secretion. As shown in Fig. 3, elevated hepatic gluconeogenesis occurs during fasting, as glucagon

stimulates the transactivators of gluconeogenic genes Pck-1 and G6Pc (Pck-1 is utilized as an example), including CREB, peroxisome proliferator-activated receptor-gamma coactivator (PGC-1 α), CRTC2 and FoxOs. It needs to be pointed out that FoxOs also utilize β -cat as its co-factor in stimulating gluconeogenic gene expression.⁶⁰ Following food intake, the rise of plasma insulin levels leads to the inactivation of FoxOs. In addition, insulin stimulates both TCF7L2 expression and β -cat stability. Thus, cat/TCF functions as the novel effector of the insulin signaling cascade, which contributes to the repression of gluconeogenesis. Wnt ligand (such as Wnt-3a) treatment did not change TCF7L2 levels, although it is known to stabilize β -cat (preventing its N-terminal serine/threonine residue phosphorylation as shown in Fig. 1). It remains unknown whether Wnt ligand has an intrinsic repressive effect on hepatic gluconeogenic gene expression.²² Secondly, it has been generally accepted that the competition between the developmental Wnt signaling and the stress FoxO signaling controls the homeostatic nature of the life, including catabolic and metabolic homeostasis. It is unlikely that both pathways positively regulate hepatic gluconeogenesis. It is reasonable to suggest that FoxO pathway mediates the function of the catabolic hormone glucagon in response to starvation (a category of stress) while Wnt pathway mediates the function of the metabolic hormone insulin after food intake.

We suggest that the controversial observations made by different laboratories may not be simply due to the differences in mouse strains utilized or other experimental details. We need to be extremely careful in applying our basic knowledge of the Wnt signaling pathway as well as TCF7L2 into this important research field.

Human TCF7L2 gene transcription leads to the generation of 13 different sized transcripts, while Western blotting can detect at least two different sized protein products, the 78 kDa one and the 58 kDa one. Some smaller sized proteins could also be detected in certain tissues.^{53,61} In addition, as discussed above, a native dominant negative TCF7L2 was shown to be expressed in mouse brain during embryonic development stage. Whether such dominant negative molecule is also expressed in the liver is unknown. The hepatic function of these different sized products could be both redundant and different. Furthermore, the expression of these transcripts was shown to be influenced by not only the risk SNPs, but also the hormonal factors during health and diseases. Thus, it may be too early to claim, based on assessing limited liver and pancreatic islet samples from carriers of TCF7L2 T2D risk SNPs, that this represents the gain-of-function event.

Hepatic gluconeogenesis is precisely controlled by complicated hormonal factors, including glucagon and insulin, in response to fasting and food intake. On the other hand, a given TCF protein, including TCF7L2 is only a "half"

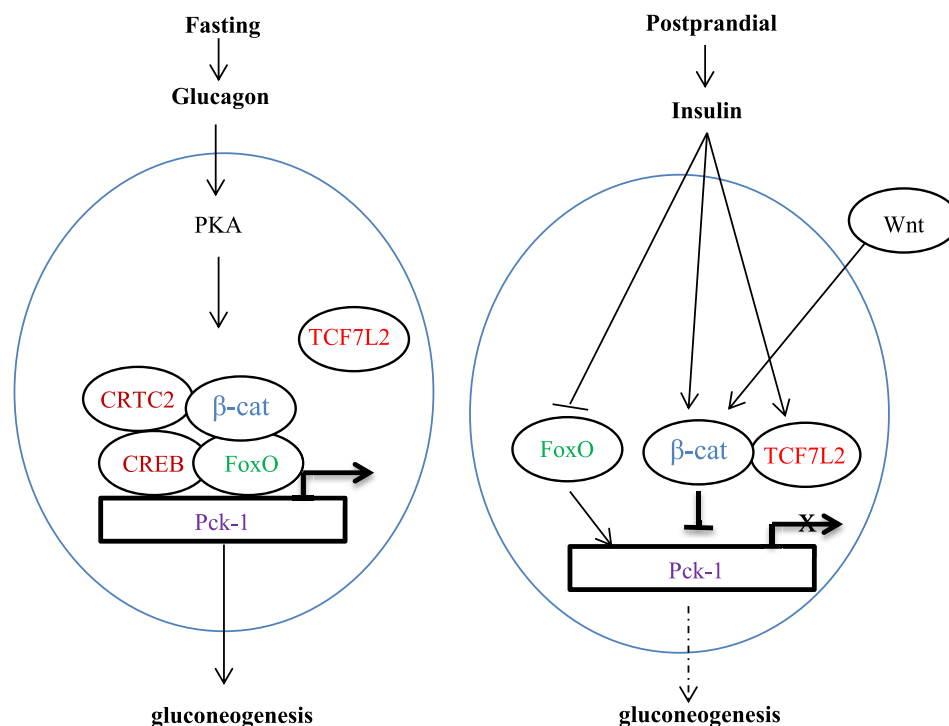


Fig. 3 Potential mechanisms contributing to the repression of hepatic gluconeogenesis by Wnt signaling activation. The gluconeogenic gene Pck-1 is utilized here for the illustration purpose. The elevation of plasma glucagon level during fasting leads to PKA activation and the stimulation of gluconeogenesis, with the participation of Pck-1 transactivators CREB, FoxO, CRTC2 and β -cat. Postprandial elevation of plasma insulin level leads to repressed hepatic gluconeogenesis, which is achieved by the inactivation of FoxO. In addition, insulin can simultaneously stimulate TCF7L2 expression and β -cat S675 phosphorylation. Thus, more β -cat molecules will team up with TCFs instead of with FoxOs, which finally contributes to the repression of Pck-1 and other gluconeogenic genes.

effector of the Wnt signaling. It needs to be teamed up with β -cat to exert its function as Wnt pathway effector. Furthermore, as shown in Fig. 1, the function of TCF is bi-directional. When β -cat is not available, TCF can repress, instead of stimulate, the expression of Wnt pathway downstream target gene. In addition, although we have learned that β -cat is involved in both Wnt and FoxO signaling, mechanism that controls the switch is still elusive.⁶² Thus, simply knockout or over-expressing TCF7L2 may not be a sufficient approach to determine its complicated function, especially in metabolic homeostasis. In addition, hepatocytes also express two other TCF members, TCF7 and TCF7L1.²²

Summary and perspectives

Despite the existence of dispute, our mechanistic understanding on the role of Wnt signaling pathway and TCF7L2 in metabolic homeostasis has been advanced significantly. We anticipate that in the near future, we will see the application of inducible expression of TCF7L2DN in combining with the manipulation of β -cat levels and phosphorylation status in the liver, as well as the utilization of other Wnt pathway gain-of-function and loss-of-function tools, for the further clarification of the role of hepatic Wnt signaling in gluconeogenesis and other features of metabolic homeostasis. Advanced techniques, such as RNAseq and ChIP-seq should be further utilized to define the hepatic TCF7L2 transcriptional network for health and diseases. Finally, expanded functional analyses need to be conducted on different sized TCF7L2 transcripts and protein products, in combination with expanded quantitative assessment on their expression in the risk SNP carriers.

Conflicts of interest

None declared.

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