REVIEW

Pathophysiological aspects of Wnt-signaling in endocrine disease

Sven Schinner, Holger S Willenberg, Matthias Schott and Werner A Scherbaum

Department of Endocrinology, Diabetes and Rheumatology, University Hospital Düsseldorf, Moorenstrasse 5, 40225 Düsseldorf, Germany

(Correspondence should be addressed to S Schinner; Email: sven.schinner@uni-duesseldorf.de)

Abstract

Wnt-signaling has recently been identified as a regulator of a number of endocrine functions in health and disease in addition to its original attribution to developmental biology. Wnts are extracellular ligands on frizzled receptors and on lipoprotein receptor-related protein co-receptors. Ligand binding leads eventually to the activation of intracellular signaling cascades; based on the involvement of the transcriptional co-activator β -catenin it can be distinguished between canonical (i.e. β -catenin) and non-canonical Wnt-signaling. Recent studies revealed that canonical Wnt-signaling regulates the function of endocrine organs and contributes to a number of endocrine disorders. In this review, we would like to focus on a) recent mechanistic data on Wnts in pancreatic β -cell function; b) human genetic studies on Wnt signaling in type 2 diabetes mellitus; c) crosstalk between adipocytes and endocrine cells through Wnt-signaling molecules (with a focus on the role of Wnt-signaling in adrenocortical cells).

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Introduction: the Wnt-signaling pathway

Wnt signaling molecules can bind to cell-surface receptors called frizzled and to lipoprotein receptor-related protein (LRP) co-receptors (low density LRP). Frizzled receptors are G-protein-coupled seven-transmembrane receptors. Binding to both receptors activates the canonical Wnt-signaling pathway. By targeting a complex containing adenomatous polyposis coli (APC) and axin the activation of this pathway leads to an inhibition of glycogen synthase kinase-3 (GSK3B). This results eventually in the stabilization of β -catenin. Subsequently, β -catenin, a transcriptional co-activator, translocates to the nucleus to activate T-cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors on canonical Wnt target-genes (see Fig. 1) (1). Originally, the Wnt signaling pathway has been identified as a regulator of embryogenesis and has thereafter been associated with tumor development (2). In addition, the role of Wnt-signaling agonists and antagonists in adipocyte differentiation has been subjected to a number of studies since its original description in 2000 (3–6). This has been the evidence linking Wnt-signaling with metabolic regulation.

Wnts and β -cell function

Wnt-signaling molecules are expressed in the human endocrine pancreas (7). Recent studies demonstrate

that Wnt-signaling regulates insulin secretion and the proliferation of pancreatic β -cells. By using *in vitro* and *in vivo* approaches independent groups have recently reported that canonical Wnt signaling induces β -cell proliferation. Our group found adipocyte-derived Wnt-signaling molecules to induce cyclin D1 transcription and the proliferation of β -cell lines and primary murine β -cells (see below) (8). In line with this, Rulifson *et al.* demonstrated that recombinant Wnt3a protein induced cell cycle regulators like cyclin D2 and Pitx2 and the proliferation rate in cultured β -cells (see Fig. 2). In addition, overexpression of active β -catenin was sufficient to induce proliferation markers and to increase β -cell mass *in vivo* using a transgenic mouse model. Conversely, inhibition of Wnt-signaling by overexpression of axin led to a decrease in the expression of cell cycle regulators and reduced β -cell expansion *in vivo* (9).

Furthermore, Liu & Habener investigated the effects of glucagon-like peptide-1 (GLP-1) and the long acting GLP-1 receptor agonist exendin-4 (Exd4) on Wnt-signaling and proliferation of pancreatic β -cells. They used TCF/LEF-driven reporter-gene assays and found GLP-1 and Exd4 to induce canonical Wnt-signaling in insulin-producing Ins-1-cells and primary murine β -cells. Consistently, activation of β -catenin was induced by Exd4. In chromatin immuno-precipitation assays, they showed that Exd4 stimulates the interaction of β -catenin and TCF7L2 with the cyclin D1 promoter. TCF7L2 is a Wnt-regulated transcription

Figure 1 The intracellular Wnt-signaling cascade. (a) Wnt-ligands bind to frizzled cell-surface receptors and to LRP5 co-receptors. Ligand-binding activates dishevelled and recruits the axin-APC-GSK3B complex to the cell membrane, whereby the phosphorylation of β -catenin by GSK3B is inhibited. As a consequence of ligand binding β -catenin can eventually translocate to the nucleus to co-activate transcription factors on target genes. In adrenocortical cells β -catenin co-activates SF-1 driven transcription, for example at the StAR promoter resulting in an induction of steroidogenesis and eventually cortisol and aldosterone secretion. (b) In the absence of Wnt-ligand GSK3B phosphorylates β -catenin thereby inducing its degradation.

factor and polymorphisms in *TCF7L2* are associated with an increased risk for type 2 diabetes (see below). Most importantly, inhibiting *TCF7L2* by stably transfecting a dominant-negative mutant inhibited the basal proliferation rate of Ins-1 cells and abolished the stimulatory effect of Exd4 on β -cell proliferation. How can GLP-1 activate canonical Wnt-signaling? Liu & Habener carried out experiments using kinase inhibitors and overexpression of various dominant-negative kinase constructs in order to dissect the involved signaling pathways. They identified the protein kinase A (PKA), a known effector of GLP-1, to be required for the stimulation of Wnt-signaling by GLP-1. They found PKA to phosphorylate β -catenin at Ser-675 in response to GLP-1. In other studies, this phosphorylation has been shown to stabilize β -catenin and to enhance its transcriptional activity (10). Therefore, PKA appears to be a link between activation of the GLP-1 receptor and canonical Wnt-signaling in β -cells. In addition, protein kinase B (PKB) was found to

mediate basal and Exd4-stimulated TOPFLASH transcription, indicating that both PKA and PKB are involved in linking GLP-1 to canonical Wnt-signaling (11).

In extension to this, Shu *et al.* demonstrated that a knock-down of *TCF7L2* in isolated mouse and human islets by siRNA resulted in a blunted insulin-release in response to glucose in static incubation studies and in perfusion assays. Similarly, the effect of GLP-1 on glucose-stimulated insulin-secretion was decreased after depletion of *TCF7L2*. In addition, depletion of *TCF7L2* led to a decrease in proliferation and an increase in apoptosis of isolated human islets. Conversely, overexpression of *TCF7L2* protected islets from glucotoxicity and cytokine toxicity with respect to glucose-induced insulin-secretion, proliferation, and apoptosis (12).

In line with these findings, Fujino and colleagues reported that mice deficient in the Wnt co-receptor LRP5 had impaired glucose tolerance. These mice also showed blunted plasma insulin concentrations in response to glucose but no insulin resistance. Isolated islets from LRP5 deficient mice showed diminished glucose-induced insulin-secretion. However, the insulin response to α -ketoisocaproate and tolbutamide were normal, whereas the ATP/ADP ratio under high-glucose exposure was decreased as compared with wild type islets. Glucose is metabolized in the glycolytic pathway to generate ATP which in turn inhibits the ATP-dependent K^+ -channel (so called 'glucose-sensing'). Tolbutamide and α -ketoisocaproate inhibit the ATP-dependent K^+ -channel independently of the glycolytic pathway (the latter possibly indirectly by generating ATP from

Figure 2 Wnt-signaling stimulates insulin secretion and β -cell proliferation. Activation of canonical (β -catenin) Wnt-signaling induces the expression of the glucokinase gene, a key enzyme in glucose-sensing. In addition, canonical Wnt-signaling induces β -cell proliferation.

mitochondria). Eventually, the inhibition of ATP-dependent K^+ -channels leads to a calcium influx into β -cells, which then triggers insulin release.

Consistently, Fujino *et al.* found that treatment with Wnt-ligands increased glucose-induced insulin-secretion in wild-type islets but not in LRP5 deficient islets. These data suggested a deficit in glucose sensing in islets when canonical Wnt-signaling is impaired (13). Consistently, we demonstrated that β -catenin activates glucokinase gene transcription in pancreatic β -cells. Glucokinase is the crucial regulator of glucose-sensing in β -cells. Molecular studies suggest that β -catenin coactivates the transcriptional activity of PPAR γ at the glucokinase promoter (8) (see Fig. 2).

In contrast to these findings in postnatal mice, it has been shown that a depletion of β -catenin in the prenatal pancreas leads to acinar cell hypoplasia but did not change the mass and architecture of islets. Interestingly, these β -catenin deficient mice show normal glucose tolerance after birth (14). This discrepancy with Fujino's data (LRP5 $^{-/-}$ mice) might be explained by β -catenin-independent signal-transduction downstream of LRP5. In this scenario, depletion of LRP5 might affect various signaling molecules in addition to the canonical β -catenin pathway contributing to the impaired insulin secretion. Another important difference between these two mouse models (LRP5- versus β -catenin-deficiency) is the age of the mice when tested for glucose tolerance and insulin secretion. Murtaugh *et al.* found normal glucose tolerance of the β -catenin deficient mice at 3–4 months of age. Fujino *et al.* analyzed glucose tolerance and islet function when the LRP5 deficient mice were 6–8 months old. One might speculate that the β -cell decompensation and the insulin-deficient phenotype develop over time. However, at the present stage, these explanations remain speculative and further studies are needed to unravel the role of LRP5 and β -catenin in islet biology.

Human genetic studies on Wnt signaling in type 2 diabetes mellitus

The above-mentioned *in vitro* and animal studies suggested a link between Wnt signaling and glucose metabolism. However, only very recently, human genetic studies actually supported the contribution of Wnt signaling to type 2 diabetes mellitus in humans. In 2006, two pioneer studies demonstrated that polymorphisms in *TCF7L2*, a Wnt-regulated transcription factor downstream from β -catenin, are associated with an increased risk for type 2 diabetes mellitus (15, 16). In genome wide association studies, the association of polymorphisms within *TCF7L2* with diabetes mellitus type 2 has been confirmed. Presently, *TCF7L2* is the strongest susceptibility gene for diabetes mellitus type 2. The reported polymorphisms in *TCF7L2* increased the risk of developing type 2 diabetes mellitus by 30–50%

for each inherited allele which is almost twice the risk as for other diabetes candidate genes. Since then a number of large studies confirmed the association of *TCF7L2* with diabetes mellitus in various ethnical groups (17–22). Among the various single nucleotide polymorphisms in *TCF7L2*, the variants rs12255372 and rs7903146 have been identified to have the strongest association with diabetes mellitus type 2.

Notably, all the identified SNPs in the *TCF7L2* gene are located within intron regions. It is not yet clear how these intronic polymorphisms can cause altered expression or function of *TCF7L2*. Of note, *TCF7L2* expression in pancreatic islets was found to be higher in diabetics than in non-diabetic subjects. Furthermore, *TCF7L2* expression is upregulated in pancreatic islets in risk allele carriers. Presently, we do not understand how these expression patterns can contribute to the pathophysiological processes in the context of the *in vitro* findings described above (for further reading on the association studies of *TCF7L2* and diabetes mellitus type 2 a number of excellent reviews can be recommended (23–25).

How can these SNPs in *TCF7L2* contribute to the diabetic phenotype?

The polymorphisms in *TCF7L2* appears to affect insulin secretion rather than insulin sensitivity (26) and a link with the incretin system has been suggested. The proglucagon gene encodes for glucagon in pancreatic α -cells and for GLP-1 in intestinal L-cells. *In vitro* studies by Jin's group have pointed out the role of β -catenin and *TCF7L2* in the regulation of proglucagon gene transcription in enteroendocrine cells: they showed that lithium induced proglucagon mRNA expression and the transcription of the proglucagon gene promoter. As lithium acts as an inhibitor of GSK3 β , thereby preventing the degradation of β -catenin they tested the direct effect of β -catenin on the proglucagon gene promoter. In fact, over-expression of constitutively active β -catenin activated the proglucagon gene promoter. Furthermore, *TCF7L2* (also known as TCF4) binds to the proglucagon gene promoter together with β -catenin and constitutively active β -catenin coactivates transcription from a truncated proglucagon promoter carrying an intact TCF binding site. *TCF7L2* activates the transcription of target genes when bound to β -catenin but acts as a transcriptional repressor in the absence of β -catenin as it lacks an activation domain. In fact, a dominant-negative *TCF7L2* mutant lacking the β -catenin binding domain repressed proglucagon gene transcription and GLP-1 synthesis. These studies underline the importance of β -catenin/TCF signaling in the regulation of proglucagon gene expression and GLP-1 synthesis (27, 28). Therefore, it has initially been speculated that polymorphisms of *TCF7L2* might regulate GLP-1 expression and GLP-1 serum levels thereby indirectly

modulating insulin release from pancreatic β -cells (15). This 'entero-insular' hypothesis is based on the interaction between enteroendocrine cells and pancreatic β -cells. This crosstalk is illustrated by the so called 'incretin effect': physiologically, intestinally administered glucose provokes a greater insulin release from β -cells than the same amount of glucose given i.v. This is explained by the fact that enteroendocrine cells produce hormones like GLP-1 upon glucose stimulation which act directly on pancreatic β -cells to stimulate insulin secretion.

However, a study by Schäfer *et al.* found no effect of *TCF7L2* polymorphisms on GLP-1 secretion in non-diabetic subjects (29). Similarly, Lyssenko *et al.* reported a blunted insulin response to oral and i.v. glucose load in patients carrying *TCF7L2* polymorphisms, arguing against this interpretation of the entero-insular hypothesis (30). In the light of the work by Liu & Habener, however, a role for *TCF7L2* in modulating GLP-1 action within the β -cell seems more probable, given that *TCF7L2* has been demonstrated to mediate GLP-1 effects in β -cells *in vitro* (11).

Crosstalk between adipocytes and endocrine cells through Wnt-signaling molecules

In 2000 Ross *et al.* showed in murine cell lines that inhibition of Wnt-signaling is a prerequisite for the differentiation of adipocytes (5). In a number of succeeding studies, it has been shown that constitutive endogenous Wnt-signaling keeps pre-adipocytes in an undifferentiated state (3, 6, 31). Animal studies in mice confirmed the importance of Wnts for adipogenesis: transgenic overexpression of Wnt10b under the control of an adipocyte-specific promoter leads to reduced body fat content and impaired adipocyte maturation (32).

In addition to these auto- and paracrine effects on adipogenesis, we found Wnt signaling molecules secreted by adipocytes to be capable of acting on endocrine target cells. Adipocytes are endocrine active cells as they secrete signaling molecules to regulate metabolic functions. From a clinical point of view, it is well established that obesity is associated with metabolic co-morbidities: arterial hypertension – at least partly in consequence to hypersecretion of aldosterone and cortisol from adrenocortical cells – and pancreatic β -cell dysfunction are frequent in obesity.

At a cellular level, it has been shown that adipocytes can induce aldosterone and cortisol release from cultured adrenocortical cells (33). Given that Wnt3a and Wnt10b are secreted by mature human adipocytes, we performed *in vitro* assays to assess whether adipocytes are able to elicit Wnt-mediated responses on target cells. In fact, adipocytes were found to induce canonical Wnt-signaling in adrenocortical cells as well as in pancreatic β -cells (8, 34). Functionally, we could

show that adipocytes induce the transcription of the *StAR* gene through secreted Wnt-signaling molecules and this led to an increase in aldosterone and cortisol release from adrenocortical cells (34). These observations suggest that adipocytes target endocrine cells through secreted Wnt-signaling molecules.

How can adipocyte-derived Wnts reach endocrine cells?

Wnt-signaling molecules have been detected in the systemic circulation in humans (35, 36) allowing for endocrine actions. However, paracrine actions might contribute to adipo-endocrine interactions as well: the adrenal gland is surrounded by adipose tissue and adipocytes are frequently found within the adrenal cortex (33). Similarly, obesity has been associated with increased adipocyte content within the pancreas in humans (37). Consistently, a systemic analysis of an obese, insulin resistant and hyperinsulinemic mouse model New Zealand-obese mouse revealed a massive increase of intra-pancreatic adipocyte deposition compared with wild-type animals. In addition, histological studies showed proximity of adipocytes and islets in these animals, suggesting paracrine effects on adipocytes on β -cells (38).

Wnt signaling controls adrenal tumorigenesis and function

The first *in situ* hybridization studies revealed that *Xenopus* homologues for pontin 52 and reptin 52, both of which are interaction partners of β -catenin are expressed in a subpopulation of neural crest cells that give rise to the adrenal medulla (39). On the contrary, immunoreactive β -catenin protein was found in the human fetal adrenal cortex (40). Molecular investigations documented that a variety of members of the Wnt-signaling pathway are also present within the human and mice adrenocortical tissues, among them *Wnt4*, *dickkopf* (*DKK3*), *FZD1*, *FZD2*, *FZD3*, and *disheveled* (in human) (*DVL3*) (41).

Soon it became evident that the nuclear translocation of β -catenin may be associated with adrenal tumorigenesis. Early data on the expression of β -catenin in adult human endocrine tumors showed that the nuclear accumulation of β -catenin may be associated with the Ki-67 (MiB-1) labeling index that reflects the proliferative activity in these neoplasms (42). Surprisingly, the analysis of exon 3 of the β -catenin gene did not reveal any mutations in these tumors. The same group also looked into adrenocortical adenomas. However, since they did not find nuclear accumulation of β -catenin in adrenocortical tumors, they did not perform mutational analyses (42).

Figure 3 Wnts and adrenal function. This figure illustrates Wnt-actions in adrenocortical cell. On one hand, adipocyte-derived Wnt10b and Wnt3a can induce canonical Wnt-signaling in adrenocortical cells thereby stimulating steroidogenesis. On the other hand, in adrenocortical tumor cells different aberrations of Wnt-signaling may occur: (A) autocrine action of differentially secreted Wnt-molecules, e.g., Wnt4; (B) mutations in the APC gene leading to impaired degradation of β -catenin; (C) activating mutations in the β -catenin gene promotes cytoplasmic accumulation and nuclear translocation of the β -catenin protein; (D) high nuclear β -catenin immunoreactivity is observed in adrenocortical neoplasms; (E) constitutive activation of the cAMP protein kinase A pathway promotes β -catenin stabilization.

Later, it could be demonstrated that a considerable number of adrenocortical tumors showed abnormal immunohistological staining patterns for β -catenin and revealed indeed activating mutations in exon 3 of the β -catenin gene, frequently involving a serin residue (S45) (43–45). These mutations were found in sporadic benign and malignant adrenocortical neoplasms and also in the NCI-H295R cell line.

Interestingly, a study in patients with familial APC (FAP) who had extracolonic tumors, could not demonstrate mutations in the β -catenin gene but revealed somatic mutations in the APC gene, including a patient with an adrenocortical adenoma (46). Wnts signal via Dvl to disrupt the β -catenin destruction complex which includes axin, GSK3 β , and APC. The APC protein destabilizes a protein complex, comprised of GSK3 β , axin, and Dvl, promoting phosphorylation and degradation of β -catenin (see Fig. 1). Retrospective and prospective studies in patients with FAP support the significance of this finding because an increased rate of adrenal tumorigenesis was found in patients with FAP and mutations in the APC gene (47, 48) (see Fig. 3).

Of note, somatic β -catenin mutations were also detected in adenomas associated with primary

pigmented nodular adrenocortical disease (PPNAD), further implicating Wnt/ β -catenin signaling in adrenal tumorigenesis (44, 49).

In vitro studies suggest that the Wnt-signaling pathway is involved in the control of adrenal steroidogenesis. WNT4 has been shown to increase the steroidogenic enzymes 17 α -hydroxylase and aldosterone synthase in the absence of cyclic AMP (50). We found that fat-cell conditioned-medium that contained WNT3a and WNT10b molecules stimulated adrenocortical cells and synthesis of aldosterone *in vitro* (34). In agreement, WNT4-deficiency results in the dysfunction of the adrenal cortex and in reduced plasma levels of aldosterone (51). Of note, recent data show that Wnt4 may be differentially regulated by angiotensin II and abundant expression of Wnt4 was found in aldosterone-producing adenomas (52).

The upregulation of steroidogenic enzymes in response to Wnt-molecules is mediated by β -catenin-dependent activation of steroidogenic factor-1 and expression of the StAR (34, 53) (see Fig. 1).

Conclusion

The Wnt-signaling pathway has been shown to regulate a number of endocrine functions: molecular approaches performed *in vitro* and *in vivo* as well as human genetics studies have confirmed a crucial role for Wnt-signaling in adipogenesis, pancreatic β -cell function and diabetes mellitus type 2 as well as adrenal physiology. Notably, adipocytes have been found to secrete Wnt-signaling molecules to regulate the function of endocrine target cells, thereby suggesting that Wnts might act as adipokines.

Declaration of interest

The authors declare that there is no conflict of interest.

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