

# The Wnt/beta-catenin pathway in adrenocortical development and cancer

Abeer El Wakil, Enzo Lalli

### ▶ To cite this version:

Abeer El Wakil, Enzo Lalli. The Wnt/beta-catenin pathway in adrenocortical development and cancer. Molecular and Cellular Endocrinology, 2010, 332 (1-2), pp.32. 10.1016/j.mce.2010.11.014 . hal-00654958

### HAL Id: hal-00654958 https://hal.science/hal-00654958

Submitted on 24 Dec 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

### Accepted Manuscript

Title: The Wnt/beta-catenin pathway in adrenocortical development and cancer

Authors: Abeer EL Wakil, Enzo Lalli

PII:	S0303-7207(10)00542-3
DOI:	doi:10.1016/j.mce.2010.11.014
Reference:	MCE 7684
To appear in:	Molecular and Cellular Endocrinology
Received date:	2-9-2010
Revised date:	22-10-2010
Accepted date:	12-11-2010



Please cite this article as: Wakil, A.E.L., Lalli, E., The Wnt/beta-catenin pathway in adrenocortical development and cancer, *Molecular and Cellular Endocrinology* (2010), doi:10.1016/j.mce.2010.11.014

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

#### The Wnt/beta-catenin pathway in adrenocortical development and cancer

Abeer EL WAKIL\*<sup>1, 2</sup>, Enzo LALLI<sup>1, 2</sup>

<sup>1</sup>Institut de Pharmacologie Moléculaire et Cellulaire CNRS UMR 6097 and <sup>2</sup>Université de Nice - Sophia Antipolis, Valbonne, France

\* Permanent address: Department of Biological Sciences, Faculty of Education, Alexandria University, Egypt.

**Corresponding author:** Enzo Lalli, Institut de Pharmacologie Moléculaire et Cellulaire, 660 route des Lucioles, 06560 Valbonne, France. Phone: +33 4 93 95 77 55; fax : +33 4 93 95 77 08; E-mail: ninino@ipmc.cnrs.fr

Keywords: Adrenal cortex, Wnt,  $\beta$ -catenin, development, cancer

Conflict of interest: None declared.

#### Abstract

Signaling by the Wnt family of secreted glycolipoproteins plays key roles in embryonic development of organisms ranging from nematodes to mammals and is also implicated in several types of human cancers. Canonical Wnt signaling functions by regulating the translocation of  $\beta$ -catenin to the nucleus, where it controls key gene expression programs through interaction with Tcf/Lef and other families of transcription factors. Wnts can also act through non-canonical pathways that do not involve  $\beta$ -catenin activation, but implicate small GTPases/JNK kinase and intracellular calcium.

Here we review recent studies that have revealed the expression of several components of  $Wnt/\beta$ -catenin signaling in the adrenal cortex and discovered a key role for this pathway in the regulation of proliferation/differentiation of progenitor cells and in tumorigenesis of that endocrine organ.

#### Introduction

The adrenal gland contains a neuroendocrine medulla within a steroidogenic cortex. The medulla produces catecholamines in response to sympathetic inputs, while the cortex produces essential steroids in response to hormonal inputs. Aldosterone secretion from the adrenal cortex regulates sodium retention and plasma volume (Takeda, 2004; Whaley-Connell et al., 2010), and has been classically considered to be regulated by the renin-angiotensin system. Glucocorticoid output, which is an essential component of the stress response (Sapolsky et al., 2000), is modulated by the hypothalamic-pituitary-adrenal (HPA) axis. Although adrenocortical function requires precise control of the differentiation of steroidogenic cell lineages, the developmental and postnatal mechanisms that regulate their production and maintenance have only recently started to be defined (Kim and Hammer, 2007).

Wnt signaling has been identified as one of the key signaling pathways in both normal development (regulating cell growth, motility and differentiation) and tumorigenesis. Deregulation of this pathway is in fact often present in human cancers (Kikuchi, 2003). Here, we will review what is known about the role of Wnt signaling in normal adrenal gland development and its alterations in adrenocortical tumors.

#### Wnt signaling in development and function of the adrenal cortex

### Mechanisms of Wnt signaling

Signaling by the Wnt family of secreted glycolipoproteins plays key roles in embryonic development of organisms ranging from nematodes to mammals. A critical and most studied Wnt pathway is the canonical Wnt signaling, which functions by regulating the amount of the transcriptional co-activator  $\beta$ -catenin that controls essential developmental gene expression programs (for an exhaustive overview of Wnt signaling see the web resource <u>http://www.stanford.edu/%7Ernusse/wntwindow.html</u>). In the absence of Wnt, casein kinase 1 $\beta$  (CK1 $\beta$ ) and glycogen synthase kinase-3 $\beta$  (GSK-3 $\beta$ ) sequentially phosphorylate the amino terminal region of  $\beta$ -catenin in the Axin complex, which is composed of the scaffolding protein Axin, the tumor suppressor *adenomatous* 

*polyposis coli* gene product (APC), CK1 $\beta$ , and GSK-3 $\beta$  Phosphorylated  $\beta$ -catenin is subsequently ubiquitinated, resulting in its proteasomal degradation. This continual elimination of  $\beta$ -catenin by the action of the Axin complex keeps its level low, preventing it from reaching the nucleus, and Wnt target genes are thereby repressed by the DNA-bound T cell factor/lymphoid enhancer factor (Tcf/Lef) family of proteins complexed with Groucho/TLE corepressors. The Wnt/β-catenin pathway is activated when a Wnt ligand binds its cell-surface receptor consisting of a seven-pass transmembrane Frizzled (Fz) receptor and its co-receptor, low-density lipoprotein receptor related protein 6 (LRP6) or its close relative LRP5. The formation of a likely Wnt-Fz-LRP6/5 complex together with the recruitment of the scaffolding protein Dishevelled (DvI), which antagonizes the action of GSK-3<sup>β</sup> through an unknown mechanism, results in the inhibition of  $\beta$ -catenin phosphorylation. At that stage  $\beta$ catenin dissociates from the Axin complex and is no longer degraded, accumulating in the cytoplasm and also being translocated to the nucleus, where it competes with Groucho/TLE corepressors for binding to Tcf/Lef (Daniels and Weis, 2005), thereby stimulating the expression of various Wnt target genes (Huang and He, 2008; MacDonald et al., 2009; Mulholland et al., 2005; van Amerongen and Nusse, 2009). This constitutes the most widely accepted current model of Wnt/β-catenin signaling (Figure 1). To increase the complexity of the system, Wnts can also act through "noncanonical" pathways that do not involve  $\beta$ -catenin activation, but implicate small GTPases/JNK kinase and intracellular calcium (Rao and Kuhl, 2010). Additionally, natural antagonists of Wnt signaling exist, which can bind either to 1) Wnt proteins or to 2) their receptors. Some of the most prominent members of the first class are secreted Frizzled-related proteins (sFrp), which display a high sequence homology with the extracellular Wnt binding domain of Frizzled in their N-terminal region and function as Wnt antagonists for both canonical and non-canonical signaling (Kawano and Krypta, 2003), while *Dickkopf* family members encode secreted proteins which antagonize Wnt signaling by inhibiting the Wnt coreceptors LRP5 and 6 (Niehrs, 2006).

#### Zonal patterns of expression of Wnt-related genes in the adrenal cortex

A role for Wnt signaling in the development of the adrenal cortex is suggested by the zonal expression of several of its component elements. Wnt4 is a signaling

factor with multiple roles in organogenesis (Brisken *et al.*, 2000; Jordan *et al.*, 2003; Vainio *et al.*, 1999). Its deficiency leads to abnormal development of the kidney, pituitary gland, female reproductive system, and mammary gland. *Wnt4* is expressed in the cortical region of the developing adrenal gland from embryonic d11.5 onward, especially in the outermost part, the zona glomerulosa. Its expression correlates temporally with that of the transcription factor *Sf1* (see below). *Wnt4*-deficient mice have a smaller number of glomerulosa cells, which leads to a decrease in the production of aldosterone. Such a defect in aldosterone biosynthesis is associated with an abnormal distribution of aldosterone synthase, Cyp11b2, in the adrenal (Heikkila *et al.*, 2002). Cyp11b2 is the terminal enzyme in the pathway of aldosterone biosynthesis and is normally confined to the zona glomerulosa.

The nuclear localization of  $\beta$ -catenin, a downstream effector of Wnt signaling, can serve as a marker for Wnt pathway activation. Nuclear  $\beta$ -catenin expressing cells are localized in a subcapsular position in the mouse adrenal cortex (Figure 2). Eberhart and Argani have published a survey of  $\beta$ -catenin nuclear localization in normal human fetal and adult tissues in which they have shown its localization in the glomerulosa of the adrenal cortex (Eberhart and Argani, 2001). Knockout (KO) mice that are globally deficient in β-catenin undergo embryonic lethality during gastrulation and lack mesoderm, precluding analysis of potential defects in adrenal development (Haegel *et al.*, 1995). To get further insight into the role of  $\beta$ -catenin in the adrenal cortex, recent studies shutting down  $\beta$ -catenin expression through the use of a Sf1 promoter-Cre transgene in adrenocortical cells produced mice with either complete adrenal aplasia or defects in maintenance of the adult cortex resulting in depletion of adrenocortical cells (Kim *et al.*, 2008). These results show the essential role of  $\beta$ catenin in mouse adrenocortical development. At least part of this phenotype may be attributed to the lack of  $\beta$ -catenin function in cell-cell adhesion and be independent of What signaling, since this defect is considerably more severe than that seen in Whatdeficient mice (Heikkila et al., 2002). However, Wnt4 may have additional roles in noncanonical Wnt signaling (Brisken et al., 2000; Vainio et al., 1999), the canonical pathway may be activated by additional Wnt ligands in the adrenal gland, or βcatenin activation may be the target of other, Wnt-independent signaling pathways (Hino et al., 2005; Taurin et al., 2006).

Gene expression profiling studies on human and mouse adrenal glands have shown that among the Dickkopf factors only *Dkk3* was expressed at a detectable level. *In situ* hybridization on adult human adrenal gland sections showed that *Dkk3* is differentially expressed with a higher expression in the zona glomerulosa than in the zona fasciculata or the zona reticularis (Suwa *et al.*, 2003). Studies screening for expression of other gene families involved in the Wnt pathway, the *frizzled* (*Fz*) and *dishevelled* (*Dvl*) families, in the adrenal cortex have reported that *Fz1*, *Fz2* and *Dvl3* transcripts are found in human, whereas only *Fz2* and *Dvl3* are present in the mouse (Suwa *et al.*, 2003). Recently, Bernichtein *et al.* have also reported the presence of *Sfrp1* mRNA expression in mouse adrenal gland and assessed its expression by immunohistochemistry, showing an intense staining for the sFrp1 protein throughout the adrenal cortex (Bernichtein *et al.*, 2008).

To summarize, *Wnt4* and  $\beta$ -catenin expression in mice and *DKK3* expression in human are confined to the zona glomerulosa, whereas *Sfrp1* is distributed throughout the whole cortical region of the mouse adrenal. The colocalization of Wnt4 and  $\beta$ -catenin in the glomerulosa implies an action of the canonical Wnt signaling pathway on glomerulosa-specific function, which is probably locally limited by a potential inhibitor like DKK3. Altogether, these data show that a variety of genes of the Wnt signaling pathways are expressed in the adrenal cortex and suggest a role for Wnt signaling in the development and/or maintenance of functional zonation in the adrenal cortex.

#### Effect of $\beta$ -catenin on SF-1-mediated transcription

Steroidogenic Factor-1 (SF-1) is a critical regulator of adrenogonadal development and function. Its overexpression also has an important role in the pathogenesis of adrenocortical tumors (reviewed in Schimmer and White, 2010; Lalli, 2010).  $\beta$ -catenin has been shown to physically associate with SF-1 and increase its transactivation of several gene promoters (Hossain and Saunders, 2003; Jordan *et al.*, 2003; Mizusaki *et al.*, 2003). One report also showed that in the testis Wnt4 represses steroidogenesis by antagonizing SF-1/ $\beta$ -catenin synergy (Jordan *et al.*, 2003). These studies raised the possibility that  $\beta$ -catenin also plays important roles in adrenocortical function through the modulation of SF-1 action.

#### Action of Wnt signaling components on steroidogenesis

Dkk3 and Wnt4 have multiple actions on steroidogenesis in normal human adrenocortical cells, including effects on overall steroidogenesis (aldosterone and cortisol biosynthesis) and distinct effects on steroidogenic enzyme mRNA levels. Dkk3 inhibits aldosterone and cortisol biosynthesis in primary human adrenocortical cells, both in basal conditions or in the presence of cyclic AMP (cAMP) (Chen and Hornsby, 2006). Conversely, Wnt4 increases steroidogenesis, as evidenced by the increase of both Cyp17 and Cyp21 when added alone, but decreases it in the presence of cAMP (Chen and Hornsby, 2006). Dkk3 also decreases cAMP-stimulated Cyp17 expression. Both Dkk3 and Wnt4 increase the level of Cyp11b2. Overall, these data indicate that the overlap of Dkk3 expression with Wnt4 and  $\beta$ -catenin in the zona glomerulosa and the stimulation of Cyp11b2 by Dkk3 and Wnt4 might imply an action on glomerulosa-specific function.

#### Wnt signaling in adrenocortical tumors

Adrenocortical tumors (ACTs) in the literature refer to both adrenocortical adenoma (ACA) and adrenocortical carcinoma (ACC), even though they are different entities. ACA are frequent in the general population and nowadays they are most often found incidentally, while ACC is a rare tumor with 2 peaks occuring mostly in the first decade of life as well as in the fourth and fifth decades, with an incidence of 4-12 per million per year in adults (Bertherat and Bertagna, 2009). They are more common in women. ACTs have been classified as either functional (75%) or nonfunctional (25%) tumors, depending on hormone hypersecretion syndromes. In contrast to adult tumors, over 95% of pediatric tumors are functional and are frequently associated with congenital abnormalities and secondary tumors. Functional tumors produce symptoms depending on their production of an excess of glucocorticoid (Cushing's syndrome), androgen (virilization) or mineralocorticoid (Conn's syndrome) hormones. It is difficult to distinguish between benign and malignant ACTs. However, a number of criteria can be applied, including size: benign tumors of the adrenal cortex are generally smaller than malignant tumors. The presence of distant metastases is an absolute indication of malignancy.

Although our understanding of ACT biology has increased after the results obtained by analysis of their gene expression profiles (de Reyniès *et al.*, 2009; Giordano *et al.*, 2003; Giordano *et al.*, 2009; West *et al.*, 2007) the precise sequence of events involved in their pathogenesis remains to be elucidated. Adrenocortical tumorigenesis in rodents and humans involves both genetic and epigenetic alterations, with accumulation of chromosomal alterations being a key mechanism during tumor progression.

#### Mutations of APC and adrenocortical tumors

Genetic alterations of the Wnt signaling pathway were initially identified in patients with familial adenomatous polyposis coli (FAP) and have since then been extended to a variety of cancers. Patients with FAP have constitutive activation of the Wnt/ $\beta$ -catenin signaling pathway due to germline loss-of-function mutations of the *APC* gene. They are predisposed to both colonic and extra-colonic tumors, among which adrenocortical tumors. Interestingly, most of these ACT in patients with FAP are benign and reveal no mutations in the  $\beta$ -catenin gene (Schinner *et al.*, 2009; Smith *et al.*, 2000).

#### $\beta$ -catenin and adrenocortical tumors

It has been reported that activating mutations of exon 3 of the  $\beta$ -catenin gene (CTNNB1), encoding a specific serine/threonine rich domain which is phosphorylated by GSK-3 $\beta$  and is essential for the targeted degradation of  $\beta$ -catenin, are found in a wide variety of human cancers including adrenocortical tumors (Gaujoux *et al.*, 2008; Polakis, 2000; Tadjine *et al.*, 2008a; Tadjine *et al.*, 2008b; Tissier *et al.*, 2005). These mutations were reported with similar frequencies in ACAs and ACCs. However, abnormal cytoplasmic or nuclear immunolocalization of  $\beta$ -catenin was observed in 85% of adrenocortical carcinomas compared to 38% of ACAs (Tissier *et al.*, 2005). Similarly, a somatic mutation of  $\beta$ -catenin has been observed in a heterogeneous ACT with an apparently benign part adjacent to a malignant one. The  $\beta$ -catenin mutation was localized only in the malignant part of this tumour, suggesting that its occurrence would be associated with a more aggressive ACT (Gaujoux *et al.*, 2008).  $\beta$ -catenin nuclear accumulation produces consequent activation of Tcf/Lef transcription factors, which induce expression of *c-myc* and/or *cyclin D* genes, as

reported in colon cancer cell lines (He et al., 1998; Tetsu and McCormick, 1999). Moreover, knock-down of  $\beta$ -catenin blocks colon cancer cells in G1, providing proof of principle that interference with aberrant Wnt signaling could be of therapeutical value (van de Wetering *et al.*, 2003). In addition, β-catenin immunoreactivity has also been observed in the nuclei of neoplastic cells that accumulate in the adrenal cortex of gonadectomized inbred mice, suggesting that the Wnt signaling pathway may participate in gonadectomy-induced adrenocortical neoplasia (Bielinska et al., 2005). Berthon et al. have recently developed transgenic mice expressing a constitutively active  $\beta$ -catenin in their adrenal cortex. These mice show adrenal hyperplasia and dysplasia and develop malignant tumors over a 17-month time course. Moreover, profound changes in cellular and zonal adrenocortical identity are present. They manifest as ectopic differentiation of Cyp11b2-expressing cells at the expense of fasciculata cells, leading therefore to primary hyperaldosteronism. These data demonstrate that constitutively active  $\beta$ -catenin is an adrenal oncogene which can trigger aberrant differentiation of aldosterone-secreting cells and promotes malignancy (Berthon et al., 2010). Of note, the H295R adrencortical cancer cell line harbors a  $\beta$ -catenin-activating mutation. This leads to constitutive  $\beta$ -catenindependent transcription. Treatment of this cell line with the Tcf/β-catenin antagonist PKF115-584 inhibits the transcriptional effect of  $\beta$ -catenin and decreases cell proliferation (Doghman et al., 2008). Altogether these findings establish the Wnt signaling pathway as a potential therapeutic target in patients with adrenocortical tumors displaying abnormal  $\beta$ -catenin accumulation.

#### Secreted Frizzled-related proteins (sFrps) and adrenocortical tumorigenesis

Alterations in the levels of sFrps expression caused by hypermethylation of their promoter region are among the most frequently occurring epigenetic alterations in different pathological conditions including tumor formation and make these molecules relevant to investigations in diverse fields of biology and biomedical sciences. Such epigenetic alterations are associated with transcriptional gene silencing (Baylin and Herman, 2000; Feinberg and Tycko, 2004) and put the spotlight on the study of the correlations between sFrps and human cancer development.

*Sfrp1* and/or *Sfrp2* hypermethylation seems to occur in a wide variety of tumor types (Dahl *et al.*, 2007; Huang *et al.*, 2007; Lodygin *et al.*, 2005; Suzuki *et al.*, 2004;

Suzuki et al., 2008). It has been therefore proposed, at least for bladder carcinomas, that the status of Sfrp1 promoter methylation could serve as an epigenetic biomarker for cancer detection and progression (Lee et al., 2004; Veeck et al., 2006). Although Sfrp1 re-expression in breast, hepatocellular and prostate tumors, where Sfrp1 had been inactivated, induces apoptosis and suppresses cell proliferation, the incidence of spontaneous tumor formation is not increased in Sfrp1 null mice (Trevant et al., 2008). These data suggest that Sfrp1, and possibly the related Sfrp2 gene, act as tumor suppressor genes. Their inactivation may predispose to neoplastic progression rather than to tumorigenesis and other additional genetic alterations would in this case be necessary for tumor onset. It is remarkable that, in the gonadectomized mice adrenocortical tumor model, linkage studies have evidenced Sfrp1 as a candidate gene positively influencing tumorigenesis (Bernichtein et al., 2008). In these mice, Sfrp1 levels are lower in the tumor tissue compared to the adjacent normal adrenal cortex. Given the oncogenic potential of constitutive Wnt signaling, it has been generally accepted that antagonism of Wnt/β-catenin signaling constitutes the mechanism by which Sfrp1 inhibits tumor cell growth and prevents metatastatic invasion (Esteve and Bovolenta, 2010). In contrast to these anti-tumorigenic effects of Sfrp1, other groups suggested that it can also act as an oncogene that is responsible for the metastatic properties of renal carcinoma and promotes prostate carcinogenesis (Joesting et al., 2005). This dual function suggests that the cellular context is an important determinant of Sfrp1 activity. Also, studies in colorectal tumors showed that another Sfrp gene, Sfrp4, may not act as a tumor suppressor gene and have quite different biological properties compared to other Sfrp family members (Feng Han et al., 2006).

In summary, the fact that several oncogenes are Wnt responsive genes argues for the involvement of constitutive Wnt signaling in the development of cancer after the shutting down of *Sfrp1* expression. It is thus evident that studies of the interactions between the different sFrp molecules and Wnt-related proteins will be of key importance to clarify the role of this family of soluble factors in tumor pathogenesis. These studies will also open the possibility for development of novel therapeutic approaches that target this pathway.

#### **Conclusions and perspectives**

The Wnt/ $\beta$ -catenin pathway plays an important role in the regulation of the stem/progenitor cell pool and of the differentiation process in several tissues (Katoh, 2007). Remarkably, in the gonads, which share a common embryological origin with the adrenal gland, activation of the Wnt/ $\beta$ -catenin pathway has an essential role in regulating ovarian differentiation (Chassot *et al.*, 2008; Liu *et al.*, 2009; Maatouk *et al.*, 2008; Tomizuka *et al.*, 2008). Studies in the mouse have shown that  $\beta$ -catenin is involved in the regulation of proliferation and differentiation of progenitor cells in the adrenal cortex and that its constitutive activation may lead to tumor formation (Berthon *et al.*, 2010; Kim *et al.*, 2008). We believe that now among the most relevant questions to be addressed are the following:

- What is the nature of the Wnt factors responsible for  $\beta$ -catenin activation in the subcapsular area of the adrenal cortex? Are other, Wnt-independent mechanisms involved?

- How does  $\beta$ -catenin crosstalk with other signaling pathways (growth factors, hedgehog, Notch) to regulate the size of the progenitor cell population and their differentiation?

- What is the precise relationship of Wnt signaling with adrenal cell differentiation into aldosterone-producing cells?

- An interesting study reported regulation of downstream components of Wnt signaling at the post-transcriptional level by miRNAs in PPNAD, a benign neoplasm of the adrenal cortex (Iliopoulos *et al.*, 2009). Can miRNAs regulate Wnt signaling also during normal development and in other pathological settings?

It is easy to predict that in the years to come we shall witness a burgeoning of studies concerning the Wnt/ $\beta$ -catenin pathway in the adrenal cortex that will considerably increase our understanding of its role in adrenocortical development and cancer.

#### Acknowledgements

We thank F. Aguila for artwork. Research in E.L. laboratory is supported by INCa, ANR GENOPAT and CNRS (LIA).

### References

- Baylin, S.B., Herman, J.G., 2000. DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet. 16, 168-174.
- Bernichtein, S., Petretto, E., Jamieson, S., Goel, A., Aitman, T.J., Mangion, J.M., Huhtaniemi, I.T., 2008. Adrenal gland tumorigenesis after gonadectomy in mice is a complex genetic trait driven by epistatic loci. Endocrinology 149, 651-661.
- Bertherat, J., Bertagna, X., 2009. Pathogenesis of adrenocortical cancer. Best Pract. Res. Clin. Endocrinol. Metab. 23, 261-271.
- Berthon, A., Sahut-Barnola, I., Lambert-Langlais, S., de Joussineau, C., Damon-Soubeyrand, C., Louiset, E., Taketo, M.M., Tissier, F., Bertherat, J., Lefrançois-Martinez, A.M., Martinez, A., Val, P., 2010. Constitutive betacatenin activation induces adrenal hyperplasia and promotes adrenal cancer development. Hum. Mol. Genet. 19, 1561-1576.
- Bielinska, M., Genova, E., Boime, I., Parviainen, H., Kiiveri, S., Leppäluoto, J., Rahman, N., Heikinheimo, M., Wilson, D.B., 2005. Gonadotropin-induced adrenocortical neoplasia in NU/J nude mice. Endocrinology 146, 3975-3984.
- Brisken, C., Heineman, A., Chavarria, T., Elenbaas, B., Tan, J., Dey, S.K., McMahon, J.A., McMahon, A.P., Weinberg, R.A., 2000. Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling. Genes Dev. 14, 650-654.
- Chassot, A.A., Ranc, F., Gregoire, E.P., Roepers-Gajadien, H.L., Taketo, M.M., Camerino, G., de Rooij, D.G., Schedl, A., Chaboissier, M.C., 2008. Activation of beta-catenin signaling by Rspo1 controls differentiation of the mammalian ovary. Hum. Mol. Genet. 17, 1264-1277.
- Chen, M., Hornsby, P.J., 2006. Adenovirus-delivered DKK3/WNT4 and steroidogenesis in primary cultures of adrenocortical cells. Horm. Metab. Res. 38, 549-555.
- Dahl, E., Wiesmann, F., Woenckhaus, M., Stoehr, R., Wild, P.J., Veeck, J., Knüchel, R., Klopocki, E., Sauter, G., Simon, R., Wieland, W.F., Walter, B., Denzinger, S., Hartmann, A., Hammerschmied, C.G., 2007. Frequent loss of SFRP1 expression in multiple human solid tumours: association with aberrant promoter methylation in renal cell carcinoma. Oncogene 26, 5680-5691.
- Daniels, D.L., Weis, W.I., 2005. Beta-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt- mediated transcription activation. Nat. Struct. Mol. Biol. 12, 364-371.
- de Reyniès, A., Assié, G., Rickman, D.S., Tissier, F., Groussin, L., René-Corail, F., Dousset, B., Bertagna, X., Clauser, E., Bertherat, J., 2009. Gene expression profiling reveals a new classification of adrenocortical tumors and identifies molecular predictors of malignancy and survival. J. Clin. Oncol. 27, 1108-1115.
- Doghman, M., Cazareth, J., Lalli, E., 2008. The T cell factor/beta-catenin antagonist PKF115-584 inhibits proliferation of adrenocortical carcinoma cells. J. Clin. Endocrinol. Metab. 93, 3222-3225.
- Eberhart, C.G., Argani, P., 2001. Wnt signaling in human development: beta-catenin nuclear translocation in fetal lung, kidney, placenta, capillaries, adrenal, and cartilage. Pediatr. Dev. Pathol. 4, 351-357.

- Esteve, P., Bovolenta, P., 2010. The advantages and disadvantages of *sfrp1* and *sfrp2* expression in pathological events. Tohoku J. Exp. Med. 221, 11-17.
- Feinberg, A.P., Tycko, B., 2004. The history of cancer epigenetics. Nat. Rev. Cancer 4, 143-153.
- Feng Han, Q., Zhao, W., Bentel, J., Shearwood, A.M., Zeps, N., Joseph, D., Iacopetta, B., Dharmarajan, A., 2006. Expression of sFRP-4 and beta-catenin in human colorectal carcinoma. Cancer Lett. 231, 129-137.
- Gaujoux, S., Tissier, F., Groussin, L., Libé, R., Ragazzon, B., Launay, P., Audebourg, A., Dousset, B., Bertagna, X., Bertherat, J., 2008. Wnt/beta-catenin and 3',5'-cyclic adenosine 5'-monophosphate/protein kinase A signaling pathways alterations and somatic beta-catenin gene mutations in the progression of adrenocortical tumors. J. Clin. Endocrinol. Metab. 93, 4135-4140.
- Giordano, T.J., Thomas, D.G., Kuick, R., Lizyness, M., Misek, D.E., Smith, A.L., Sanders, D., Aljundi, R.T., Gauger, P.G., Thompson, N.W., Taylor, J.M.G., Hanash, S.M., 2003. Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis. Am. J. Pathol. 162, 521-531.
- Giordano, T.J., Kuick, R., Else, T., Gauger, P.G., Vinco, M., Bauersfeld, J., Sanders, D., Thomas, D.G., Doherty, G., Hammer, G., 2009. Molecular classification and prognostication of adrenocortical tumors by transcriptome profiling. Clin. Cancer Res. 15, 668-676.
- Haegel, H., Larue, L., Ohsugi, M., Fedorov, L., Herrenknecht, K., Kemler, R., 1995. Lack of beta-catenin affects mouse development at gastrulation. Development 121, 3529-3537.
- He, T.C., Sparks, A.B., Rago, C., Hermeking, H., Zawel, L., da Costa, L.T., Morin, P.J., Vogelstein, B., Kinzler, K.W., 1998. Identification of c-MYC as a target of the APC pathway. Science 281, 1509-1512.
- Heikkila, M., Peltoketo, H., Leppaluoto, J., Ilves, M., Vuolteenaho, O., Vainio, S., 2002. Wnt-4 deficiency alters mouse adrenal cortex function, reducing aldosterone production. Endocrinology 143, 4358-4365.
- Hino, S., Tanji, C., Nakayama, K.I., Kikuchi, A., 2005. Phosphorylation of betacatenin by cyclic AMP-dependent protein kinase stabilizes beta-catenin through inhibition of its ubiquitination. Mol. Cell. Biol. 25, 9063-9072.
- Hossain, A., Saunders, G.F., 2003. Synergistic cooperation between the beta-catenin signaling pathway and steroidogenic factor 1 in the activation of the Mullerian inhibiting substance type II receptor. J. Biol. Chem. 278, 26511-26516.
- Huang, H., He, X., 2008. Wnt/beta-catenin signaling: new (and old) players and new insights. Curr. Opin. Cell. Biol. 20, 119-125.
- Huang, J., Zhang, Y.L., Teng, X.M., Lin, Y., Zheng, D.L., Yang, P.Y., Han, Z.G., 2007. Down-regulation of SFRP1 as a putative tumor suppressor gene can contribute to human hepatocellular carcinoma. BMC Cancer 7, 126.
- Iliopoulos, D., Bimpaki, E.I., Nesterova, M., Stratakis, C.A., 2009. MicroRNA signature of primary pigmented nodular adrenocortical disease: clinical correlations and regulation of Wnt signaling. Cancer Res. 69, 3278-3282.
- Joesting, M.S., Perrin, S., Elenbaas, B., Fawell, S.E., Rubin, J.S., Franco, O.E., Hayward, S.W., Cunha, G.R., Marker, P.C., 2005. Identification of SFRP1 as a candidate mediator of stromal-to-epithelial signaling in prostate cancer. Cancer Res. 65, 10423-10430.
- Jordan, B.K., Shen, J.H., Olaso, R., Ingraham, H.A., Vilain, E., 2003. Wnt4 overexpression disrupts normal testicular vasculature and inhibits testosterone

synthesis by repressing steroidogenic factor 1/beta-catenin synergy. Proc. Natl. Acad. Sci. U S A 100, 10866-10871.

- Katoh, M., 2007. WNT signaling pathway and stem cell signaling network. Clin. Cancer Res. 13, 4042-4045.
- Kawano, Y., Krypta, R., 2003. Secreted antagonists of the Wnt signaling pathway. J. Cell Sci. 116, 2627-2634.
- Kikuchi, A., 2003. Tumor formation by genetic mutations in the components of the Wnt signaling pathway. Cancer Sci. 94, 225-229.
- Kim, A.C., Hammer, G.D., 2007. Adrenocortical cells with stem/progenitor cell properties: recent advances. Mol. Cell. Endocrinol. 265-266, 10-16.
- Kim, A.C., Reuter, A.L., Zubair, M., Else, T., Serecky, K., Bingham, N.C., Lavery, G.G., Parker, K.L., Hammer, G.D., 2008. Targeted disruption of beta-catenin in Sf1-expressing cells impairs development and maintenance of the adrenal cortex. Development 135, 2593-2602.
- Lalli, E., 2010. Adrenocortical development and cancer: focus on SF-1. J. Mol. Endocrinol. 44, 301-307.
- Lee, J.L., Lin, C.T., Chueh, L.L., Chang, C.J., 2004. Autocrine/paracrine secreted Frizzled-related protein 2 induces cellular resistance to apoptosis: a possible mechanism of mammary tumorigenesis. J. Biol. Chem. 279, 14602-14609.
- Liu, C.F., Bingham, N., Parker, K., Yao, H.H., 2009. Sex-specific roles of betacatenin in mouse gonadal development. Hum. Mol. Genet. 18, 405-417.
- Lodygin, D., Epanchintsev, A., Menssen, A., Diebold, J., Hermeking, H., 2005. Functional epigenomics identifies genes frequently silenced in prostate cancer. Cancer Res. 65, 4218-4227.
- Maatouk, D.M, DiNapoli, L., Alvers, A., Parker, K.L., Taketo, M.M., Capel, B., 2008. Stabilization of beta-catenin in XY gonads causes male-to-female sexreversal. Hum. Mol. Genet. 17, 2949-2955.
- MacDonald, B.T., Tamai, K., He, X., 2009. Wnt/beta-catenin signaling: components, mechanisms, and diseases. Dev. Cell 17, 9-26.
- Mizusaki, H., Kawabe, K., Mukai, T., Ariyoshi, E., Kasahara, M., Yoshioka, H., Swain, A., Morohashi, K., 2003. Dax-1 (dosage-sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1) gene transcription is regulated by wnt4 in the female developing gonad. Mol. Endocrinol. 17, 507-519.
- Mulholland, D.J., Dedhar, S., Coetzee, G.A., Nelson, C.C., 2005. Interaction of nuclear receptors with the Wnt/beta-catenin/Tcf signaling axis: Wnt you like to know? Endocr. Rev. 26, 898-915.
- Niehrs, C., 2006. Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene 25, 7469-7481.
- Polakis, P., 2000. Wnt signaling and cancer. Genes Dev. 14, 1837-1851.
- Rao, T.P., Kuhl, M., 2010. An updated overview on Wnt signaling pathways: a prelude for more. Circ. Res. 106, 1798-1806.
- Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55-89.
- Schimmer, B.P., White, P.C., 2010. Minireview: Steroidogenic Factor 1: its roles in differentiation, development, and disease. Mol. Endocrinol. 24, 1322-1337.
- Schinner, S., Willenberg, H.S., Schott, M., Scherbaum, W.A., 2009. Pathophysiological aspects of Wnt-signaling in endocrine disease. Eur. J. Endocrinol. 160, 731-737.

- Smith, T.G., Clark, S.K., Katz, D.E., Reznek, R.H., Phillips, R.K., 2000. Adrenal masses are associated with familial adenomatous polyposis. Dis. Colon Rectum 43, 1739-1742.
- Suwa, T., Chen, M., Hawks, C.L., Hornsby, P.J., 2003. Zonal expression of dickkopf-3 and components of the Wnt signaling pathways in the human adrenal cortex. J. Endocrinol. 178, 149-158.
- Suzuki, H., Toyota, M., Carraway, H., Gabrielson, E., Ohmura, T., Fujikane, T., Nishikawa, N., Sogabe, Y., Nojima, M., Sonoda, T., Mori, M., Hirata, K., Imai, K., Shinomura, Y., Baylin, S.B., Tokino, T., 2008. Frequent epigenetic inactivation of Wnt antagonist genes in breast cancer. Br. J. Cancer 98, 1147-1156.
- Suzuki, H., Watkins, D.N., Jair, K.W., Schuebel, K.E., Markowitz, S.D., Chen, W.D., Pretlow, T.P., Yang, B., Akiyama, Y., Van Engeland, M., Toyota, M., Tokino, T., Hinoda, Y., Imai, K., Herman, J.G., Baylin, S.B., 2004. Epigenetic inactivation of SFRP genes allows constitutive WNT signaling in colorectal cancer. Nat. Genet. 36, 417-422.
- Tadjine, M., Lampron, A., Ouadi, L., Bourdeau, I., 2008a. Frequent mutations of betacatenin gene in sporadic secreting adrenocortical adenomas. Clin. Endocrinol. 68, 264-270.
- Tadjine, M., Lampron, A., Ouadi, L., Horvath, A., Stratakis, C.A., Bourdeau, I., 2008b. Detection of somatic beta-catenin mutations in primary pigmented nodular adrenocortical disease (PPNAD). Clin. Endocrinol. 69, 367-373.
- Takeda, Y., 2004. Vascular synthesis of aldosterone: role in hypertension. Mol. Cell. Endocrinol. 217, 75-79.
- Taurin, S., Sandbo, N., Qin, Y., Browning, D., Dulin, NO., 2006. Phosphorylation of beta-catenin by cyclic AMP-dependent protein kinase. J. Biol. Chem. 281, 9971-9976.
- Tetsu, O., McCormick, F., 1999. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. Nature 398, 422-426.
- Tissier, F., Cavard, C., Groussin, L., Perlemoine, K., Fumey, G., Hagneré, A.M., René-Corail, F., Jullian, E., Gicquel, C., Bertagna, X., Vacher-Lavenu, M.C., Perret, C., Bertherat, J., 2005. Mutations of beta-catenin in adrenocortical tumors: activation of the Wnt signaling pathway is a frequent event in both benign and malignant adrenocortical tumors. Cancer Res. 65, 7622-7627.
- Tomizuka, K., Horikoshi, K., Kitada, R., Sugawara, Y., Iba, Y., Kojima, A., Yoshitome, A., Yamawaki, K., Amagai, M., Inoue, A., Oshima, T., Kakitani, M., 2008. Rspondin1 plays an essential role in ovarian development through positively regulating Wnt-4 signaling. Hum. Mol. Genet. 17, 1278-1291.
- Trevant, B., Gaur, T., Hussain, S., Symons, J., Komm, B.S., Bodine, P.V., Stein, G.S., Lian, J.B., 2008. Expression of secreted frizzled related protein 1, a Wnt antagonist, in brain, kidney, and skeleton is dispensable for normal embryonic development. J. Cell. Physiol. 217, 113-126.
- Vainio, S., Heikkila, M., Kispert, A., Chin, N., McMahon, A.P., 1999. Female development in mammals is regulated by Wnt-4 signaling. Nature 397, 405-409.
- van Amerongen, R., Nusse, R., 2009. Towards an integrated view of Wnt signaling in development. Development 136, 3205-3214.
- van de Wetering, M., Oving, I., Muncan, V., Pon Fong, M.T., Brantjes, H., van Leenen, D., Holstege, F.C., Brummelkamp, T.R., Agami, R., Clevers, H.,

2003. Specific inhibition of gene expression using a stably integrated, inducible small-interfering-RNA vector. EMBO Rep. 4, 609-615.

- Veeck, J., Niederacher, D., An, H., Klopocki, E., Wiesmann, F., Betz, B., Galm, O., Camara, O., Dürst, M., Kristiansen, G., Huszka, C., Knüchel, R., Dahl, E., 2006. Aberrant methylation of the Wnt antagonist SFRP1 in breast cancer is associated with unfavourable prognosis. Oncogene 25, 3479-3488.
- West, A.N., Neale, G.A., Pounds, S., Figueiredo, B.C., Rodriguez-Galindo, C., Pianovski, M.A., Oliveira Filho, A.G., Malkin, D., Lalli, E., Ribeiro, R., Zambetti, G.P., 2007. Gene expression profiling of childhood adrenocortical tumors. Cancer Res., 67, 600-608.
- Whaley-Connell, A., Johnson, M.S., Sowers, J.R., 2010. Aldosterone: role in the cardiometabolic syndrome and resistant hypertension. Prog. Cardiovasc. Dis. 52, 401-409.

### Figure legends

**Figure 1**. The canonical Wnt signaling pathway. Binding of secreted Wnt factors to Frizzled receptors on the cell membrane transduces a signal to the Axin complex that inhibits phosphorylation, and then degradation, of  $\beta$ -catenin. It then accumulates in the cytoplasm and can translocate in the nucleus, where it interacts with Tcf/Lef and other families of transcription factors to regulate expression of target genes.

*Figure 2.* Immunostaining of  $\beta$ -catenin in the mouse adrenal cortex. Nucleocytoplasmic accumulation of  $\beta$ -catenin is found in the subcapsular zone and in the glomerulosa. 40x magnification.

A cooled Manus



Figure 2

