Wnt signalling in pituitary development and tumorigenesis

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Abstract

Wnt signalling is activated in both pituitary organogenesis and its mature function. Wnt ligands and Wnt signalling pathways are critical for the regulation of the formation of the pituitary. In the mature pituitary, Wnt signalling pathways control cell activity and may stimulate cell proliferation in both physiological and pathological processes. This review compares Wnt signalling pathways active in the developing and mature pituitary and explores how this gives us further insight into the development of pituitary adenomas.

Key Words

- ▶ pituitary
- ▶ Wnt
- ▶ adenoma
- ▶ tumorigenesis
- ▶ development

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The pituitary

The anterior pituitary produces peptide hormones under the control of feedback from hormones secreted by the hypothalamus and the peripheral endocrine glands. This is enabled by five different populations of cells: corticotrophs which make ACTH; gonadotrophs which secrete FSH and LH; somatotrophs making GH; thyrotrophs making TSH; and lactotrophs secreting prolactin. Homeostasis is maintained by adjusting hormone expression and release from the cells of the pituitary and by varying the size of the populations of cells producing each hormone.

The control of the size of the populations of cells within the pituitary is thus critical to maintain endocrine homeostasis, and when cell proliferation becomes uncontrolled, tumours develop. Pituitary adenomas are surprisingly common with an estimated prevalence of 17% (Ezzat *et al.* 2004). The proliferation of tumours differs from the physiological expansion in cell populations in that tumours are thought to be monoclonal. However, the distinction between these two processes is not always clear, and some pituitary microadenomas have been observed to be transient, spontaneously resolving,

indicating the maintenance of at least some control of the population size. Furthermore, malignant transformation in these tumours is extremely uncommon, showing that they do not behave like adenomas in other tissues (Levy & Lightman 2003).

In the fetus, the anterior pituitary develops from an invagination of the oral ectoderm which forms Rathke's pouch. Rathke's pouch later forms the anterior pituitary, making contact with the diencephalon which later becomes the posterior pituitary. During development, multipotent cells differentiate into the five hormone-producing cells of the anterior pituitary.

Wnt signalling is important both for the differentiation of pluripotent cells and in the proliferation of mature cells. This review covers the role that Wnt signalling plays in the development of the anterior pituitary and potential roles in tumorigenesis and plasticity in the adult gland.

Wnt signalling

Wnt proteins are cell-signalling molecules. Their main effects are to stimulate cell proliferation, differentiation and migration. *Wnt1*, initially called integration 1 (*Int-1*)

was identified in murine virally induced breast cancers. It was later found to be homologous with the wingless gene, which in *Drosophila* controls segment polarity. The combination of the terms wingless and integration gave rise to the name 'Wnt'. Nineteen *Wnt* genes are coded by most mammalian (including the human) genomes. Wnt proteins interact with frizzled (Fz) and other co-receptors, for example lipid-related peptide 5/6 (LRP5/6), to form heterodimeric receptors for Wnt proteins. There are thus multiple permutations of Wnts, Fzs and other co-receptors, which can exert multiple effects upon cell activity (Kestler & Kühl 2008, Clevers & Nusse 2012).

The intracellular signalling pathways of Wnts are becoming better understood. Three principal mechanisms are currently known: the 'canonical' (β -catenin), the 'calcium' and the 'planar cell polarity' pathways (Clevers & Nusse 2012, Nusse 2012). The specificity of the pathway triggered appears to be dependent upon the Wnt and Fz co-receptor combination, the cell type and the environment of the cell. Furthermore, these pathways may be simultaneously interacting with one another in a 'Wnt signalling network' (Kestler & Kühl 2008).

β -Catenin signalling manipulates cell activity by interacting with the T-cell factor (TCF)/lymphocyte-enhancing factor (LEF) group of transcription factors. TCFs contain a single high motility group (HMG) domain sufficient for DNA binding and an N-terminal β -catenin-binding domain. The isoforms TCF1, TCF3 and TCF4 and LEF1 all demonstrate the conservation of an HMG, and in the pituitary demonstrate specific temporal and spatial expression. The binding of β -catenin to TCF/LEFs results in the activation or de-repression of Wnt target genes (Yi et al. 2011, Archbold et al. 2012, Wu et al. 2012). In an unactivated state, cytoplasmic β -catenin is bound to adenomatous polyposis coli (APC), axin and glycogen synthase kinase 3 β (GSK3 β), resulting in the phosphorylation of β -catenin and its ubiquitination and breakdown. Canonical Wnt signalling is activated when a Wnt ligand binds to an Fz receptor. This causes the binding of APC and axin to this membrane complex and prevents the breakdown of β -catenin, thus allowing it to accumulate and translocate to the nucleus where it binds TCFs (Clevers & Nusse 2012, Nusse 2012).

The binding of Wnts to Fz receptors can also trigger the intracellular release of calcium, activating calcium sensitive enzymes such as protein kinase C (PKC), calcium-calmodulin-dependent kinase II (Kremenevskaja et al. 2005), and calcium-sensitive phosphatase calcineurin which subsequently activates the transcription factor NF-AT (Kestler & Kühl 2008).

The Wnt/planar cell polarity pathway involves the activation of GTPases that activate downstream targets including JNK or rho kinase. This signalling pathway is associated with transmembrane proteins and reconfiguration of the cytoskeleton, thus allowing epithelial cells to set up planar cell polarity (Kestler & Kühl 2008).

The role of Wnt in pituitary development

Gene expression for 12 of the *Wnt* proteins has been identified in the developing pituitary: *Wnts 2b, 3, 4, 5a, 5b, 6, 7a, 7b, 10a, 11, 15 and 16* (Treier et al. 1998, Olson et al. 2006, Potok et al. 2008). Of these, the investigation so far has focused on *Wnt4* and *Wnt5a* (Treier et al. 1998, Potok et al. 2008). Wnt signalling pathways control the expression of transcription factors that control cell differentiation. We will explore the factors controlled by Wnt signalling before looking at Wnt expression.

Homeodomain transcription factors The homeodomain-containing group of transcription factors are characterised by the presence of a helix–turn–helix DNA-binding domain that enables the factors to drive the transcription of target genes. The differentiation of mature cells from the progenitors of Rathke's pouch relies upon the sequential switching on and off of homeodomain transcription factors.

The generation of the somatotroph, lactotroph and thyrotroph cell lines is dependent on the expression of the transcription factor *Pit1* (also known as *Pou1f1*; Li et al. 1990). *Pit1* expression is thought to be stimulated by the paired-like homeodomain transcription factor 'prophet of pit1' (*Prop1*) and suppressed by *Hesx1* that recruits co-repressors Groucho/TLE and N-CoR (Dasen et al. 2001, Olson et al. 2006). *Pitx1* and *Pitx2* are expressed in Rathke's pouch and throughout the proliferation and differentiation of all the five populations of hormone-producing cells of the anterior pituitary. *Pitx2* is essential for the proliferation of the cells of Rathke's pouch, with its inactivation resulting in severe pituitary hypoplasia (Gage et al. 1999). *Pitx1*^{-/-} mice have demonstrated normal formation of Rathke's pouch but defects in hind limb and cleft formation. An overlapping and compensatory role has been suggested between *Pitx1* and *Pitx2* (Suh et al. 2002). The carefully timed expression of different factors in the cells of Rathke's pouch allows the maintenance of pluripotency and the subsequent differentiation of mature endocrine cells.

Pit1 *Pit1* expression is dependent upon an interaction between PROP1 and β -catenin. This complex including Prop1 and β -catenin also acts in an inhibitory manner to *Hesx1* (which itself would suppress *Pit1*). Transgenic mice expressing a mutant β -catenin in cells expressing *Pitx1* (pituitary precursors) fail to express *Pit1* or develop cells expressing GH, TSH β or PRL (Olson *et al.* 2006). This process is independent of TCF and LEF, factors traditionally thought to be the prime target for β -catenin activity. In *Lef1*-deficient mice, *Pit1* expression along with the number of GH- and TSH β -expressing cells was not reduced. *Lef1* was found to be present on the evolutionary conserved early enhancer of *Pit1*. However, two-step chromatin precipitation, pulling down firstly acetylated H3K9, and then *Prop1* or *Lef1* from the *Pit1* enhancer, revealed Prop1 but not *Lef1* to be present on the activated early enhancer promoter region of *Pit1* at embryonic day 14.5 (e14.5) (Olson *et al.* 2006). Furthermore, *in vitro*, in the GHFT-1 pituitary cell line, LEF1 reduced the activity of a *Pit1*-driven luciferase reporter under the stimulation of β -catenin and Prop1, suggesting that its effect might be predominantly repressive of *Pit1* expression (Olson *et al.* 2006). *Tcf4*-deficient mice develop larger anterior pituitaries that are none the less fully differentiated, indicating that *Pit1* must be functioning in its absence (Brinkmeier *et al.* 2003). *Tcf3* is required in the developing murine forebrain and dorsal aspect of Rathke's pouch, where it prevents the expression of β -catenin targets. As there is an interaction between *Tcf3* and *Hesx1* and as the inhibition of canonical signalling rescues *Hesx1* mutants, it is tempting to postulate that *Tcf3* could repress *Pit1* expression. However, *Tcf3* expression is temporally and spatially different from that of *Pit1* (Olson *et al.* 2006, Brinkmeier *et al.* 2007, Andoniadou *et al.* 2011), and therefore the possible effects of TCF3–*Pit1* interaction remain unproven at present.

Tcf4 *Tcf4* regulates the recruitment of cells from the ectoderm to Rathke's pouch and also the rate of cell proliferation within the newly formed pituitary. This process is dependent upon an up-regulation of fibroblast growth factor 10 (*Fgf10*) and bone morphogenic protein (*Bmp*) expression within the developing pituitary by *Tcf4*. Despite *Tcf4* stimulating proliferation and migration, in experiments in mice, the role for β -catenin in activating this process was not demonstrated, thus the mechanisms responsible for *Tcf4* expression have yet to be elucidated (Brinkmeier *et al.* 2007).

Pitx2 *Pitx2* expression is responsible for the proliferation of distinct areas of the embryo, for example the cardiac outflow tract and the anterior pituitary. In e9.75 mice, injection of LiCl, which stimulates canonical Wnt signalling by inhibiting GSK3 β , caused the displacement of histone deacetylase 1 (HDAC1) from the *Lef1* promoter of *Pitx2*, increasing its expression. A similar response was seen in the aT3-1 pituitary cell line in which a constitutively active form of β -catenin was expressed. The absence of *Pitx2* causes the arrest of pituitary growth by e10.5. This seems to be as a result of growth arrest as the BrdU labelling of e10.5 *Pitx2*^{-/-} pituitaries was reduced by around 65% compared with controls. The overexpression of *Pitx2* under the control of the *Pit1* promoter results in hyperplastic pituitaries with a twofold increase in the number of somatotrophs. This suggests that canonical signalling plays a role in the proliferation of either pituitary progenitors or mature pituitary endocrine cells (Kioussi *et al.* 2002). The effects of *Pitx2* are cell and/or environment specific, as they were, for example, not observed *in vitro* in the Rat1 cell line which would not normally express *Pitx2*. In the aT3-1 pituitary cell line, which does express *Pitx2*, silencing its effects with either siRNA or IgG reduced proliferation. LiCl treatment of the aT3-1 cell line resulted in the recruitment of *Pitx2* and the displacement of Hdac1 from the promoter of cyclin D2 (Kioussi *et al.* 2002). Despite the fact that there is clearly a role for canonical signalling in inducing *Pitx2*, the Wnt ligand (if indeed it is a Wnt) responsible has yet to be identified: in non-functioning pituitary adenomas excised from humans, increased *PITX2* has been demonstrated. No Wnt proteins were identified in these tumours, but a ninefold increase in secreted Fz-related peptide (sFRP) expression was observed. sFRP is usually associated with the inhibition of canonical signalling; however, it has been shown to have stimulatory effects in certain contexts (Moreno *et al.* 2005).

Hesx1 In mice in which degradation-resistant β -catenin was expressed in committed *Pit1* lineage cells, development of the differentiated populations was normal (Olson *et al.* 2006, Gaston-Massuet *et al.* 2011). However, where degradation-resistant β -catenin was expressed in *Hesx1*-expressing cells, there was a reduction in GH production (Gaston-Massuet *et al.* 2011). The normal development of the pituitary is thus dependent upon canonical signalling being activated at specific time points. The expression of these factors in the context of the developing murine pituitary is shown diagrammatically in Fig. 1.

Figure 1

Summary of the interactions between homeodomain transcription factors and the elements of Wnt signalling important for murine pituitary genesis. The blue boxes represent the expression of transcription factors within Rathke's pouch and the developing pituitary. Manipulation of transcription factor expression by canonical signalling is demonstrated by the orange

ovals. Direct involvement of the Wnt4 ligand is indicated by the purple oval. Up-regulation of transcription is indicated by the green plus signs; repression is indicated by the red minus signs. β -Catenin when acting as a co-factor is indicated by an oval positioned between two transcription factors. Time points, in embryonic mice, are indicated along the timeline.

Wnt4 *Wnt4* is expressed in Rathke's pouch. *Wnt4*-deficient mice have smaller pituitaries and, in particular, reduced populations of cells expressing GH, TSH β and the α -glycoprotein hormone subunit (CgA or POMC), but normal populations of cells expressing *Pomc* (Treier *et al.* 1998, Potok *et al.* 2008). *Wnt4* mutant mice also have fewer *Pit1*-expressing cells, suggesting a reduction in hormone-producing cell precursors and not simply the failure of the cells to proliferate. In these cells, β -catenin expression, immunoreactivity for Tcf4 and levels of *Lef1* mRNA were undisturbed, suggesting a non-canonical mechanism to be responsible (Potok *et al.* 2008).

Wnt5a *Wnt5a* expression is abundant in the diencephalon. In mice, *Wnt5a* deficiency results in altered morphology of the pituitary, although all the principal hormone-producing cell types still develop. This altered morphology does not appear to be reliant on changes to proliferation rates or to apoptosis and maybe related to cell-cell adhesion (Cha *et al.* 2004). The expression domain of *Wnt5a* in the developing murine pituitary correlates neatly with that of BMP, giving rise to the question as to whether the two signalling systems are linked (Treier *et al.* 1998, Potok *et al.* 2008). *Wnt5a* mutant embryos have demonstrated reduced spatial control of *Fgf10* expression and the activation of *BMP*, indicating

that it may have a regulatory function. There was no change in β -catenin expression or evidence of the activation of canonical signalling in these mutants, perhaps suggesting a non-canonical mode of action (Potok *et al.* 2008). In Rathke's pouch explant studies from e9.5 to 10.5 murine embryos, co-expression of both *Wnt5a* and *Bmp4* consistently induced the genesis of hormone-producing cells. However, not all mature cell types were consistently cultured in this model, for example cells expressing GH were much more abundant than cells expressing TSH β (Treier *et al.* 1998).

Wnt6 *Wnt6* is expressed around the developing pituitary, but appears not to be essential for cell differentiation or proliferation. *Wnt6* mutant mice exhibit no pituitary morphological changes or altered differentiation. *Wnt6* was not identified in Rathke's pouch by *in situ* hybridisation by Potok *et al.*, but mRNA was identified by qPCR of micro-dissected Rathke's pouch at e12.5 by Olson *et al.* (2006).

Sox2 *Sox2* is a member of the Sox family of transcription factors that have critical roles in embryonic development. In the human embryonic kidney 293 (HEK293) cell line, the co-transfection model *Sox2* has been shown to inhibit canonical signalling, reducing the readout from a β -catenin expression plasmid, TOPflash, that consists of

four TCF-binding sites driving a luciferase reporter. Mutation of *Sox2* has been associated with bilateral anophthalmia and hypopituitarism in humans. It has thus been proposed that reduced *Sox2* expression and thus increased canonical signalling might be responsible for this failure in pituitary development (Kelberman *et al.* 2008). *Sox2* haploinsufficiency has been associated in two cases of non-progressive pituitary tumours of early onset, implicating a possible role for canonical signalling in their formation (Alatzoglou *et al.* 2011).

Wnts in mature pituitary cells

In this section, we will explore the role of Wnt signalling in the proliferation and activity of mature pituitary cells. It should be noted that most studies have been conducted on immortalised cell lines that may have altered signalling pathways and thus different characteristics to primary cells or *in vivo* models.

Lactotrophs

Oestrogen stimulates the proliferation of lactotrophs. An array study comparing genes expressed in the pituitaries of Fischer 344 rats treated with oestradiol (E_2) or placebo indicated an increase in the expression of *Wnt4*, *Wnt10a* and Wnt inhibitory factor 1 (*Wif1*). In the GH3 somatolactotroph cell line, E_2 was also shown to induce *Wnt4* expression. Despite increasing the expression of *Wnt4*, E_2 treatment did not stimulate canonical signalling in these cells; there was no change in the *Tcf4* readout from the TOPflash luciferase reporter or the nuclear accumulation of β -catenin following stimulation by LiCl. GH3 cells are known to demonstrate spontaneous oscillations in intracellular calcium concentrations. Interestingly, *Wnt4* was shown to inhibit these calcium oscillations in 63% of the cells. This effect was lost when GH3 cells were treated with antibodies to immunoneutralise the effect of *Wnt4*. In addition, 63% of the cells were shown to be in the G1 phase of the growth cycle, suggesting a possible role for *Wnt4* in controlling the cell cycle in these cells. This indicates that Wnts may be acting in a non-canonical manner in these mature pituitary cells (Giles *et al.* 2011). Furthermore, in mutant mice in which degradation-resistant β -catenin is expressed in lactotrophs, there is no increase in prolactinomas or tumour formation, indicating that canonical signalling does not stimulate growth in these cells (Gaston-Massuet *et al.* 2011).

E_2 increases the expression of *Wnt4* in the mature pituitary, which is most convincing in rat primary

pituitary cultures where somatotrophs show the most striking response. This increase in *Wnt4* expression was not consistently shown in lactotrophs, cells which normally show increased proliferation in response to E_2 (Miyakoshi *et al.* 2009, Giles *et al.* 2011). In the MtT/S somatotroph cell line, E_2 induced *Wnt4* expression. As these cells do not express oestrogen receptor β (*ER β /Esr2*), the mechanism must either be via oestrogen receptor α (*ER α /Esr1*) or via a non-classical oestrogen signalling pathway (Miyakoshi *et al.* 2009). In neuronal primary cultures, E_2 treatment activated canonical signalling via an *ER α* - and *ER β* -dependent but non-classical mechanism that resulted in the inactivation of GSK3 β . Here, oestrogen induced different Wnt target genes than *Wnt3a* (Varea *et al.* 2009). Other examples of the endocrine activation of canonical Wnt signalling are discussed below.

Gonadotrophs

β -Catenin signalling has been investigated in the gonadotroph cell line L β T2. Here, it was shown that GnRH stimulated canonical signalling by inducing the nuclear accumulation of β -catenin and activating the TCF/LEF-dependent transcriptional activity. In HEK293 cells transfected with the GnRH receptor type I, GnRH acted by inducing the phosphorylation of GSK3 β at serine 9, as would be expected in canonical signalling. Interestingly, in the L β T2 cell line, the phosphorylation of GSK3 β was not shown, indicating that GnRH had an effect upon canonical signalling in an alternative manner. This demonstrates that canonical signalling can be stimulated by non-Wnt proteins, and also by the cell specificity of the signalling pathway (Gardner *et al.* 2007). GnRH induces *Lh β* expression in the L β 2T cell line. This too appears to depend upon canonical signalling as β -catenin binds to *SF-1* (or *Nr5a1*, a transcription factor for *Lh β*), leading to increased expression. This shows another mechanism by which β -catenin affects transcription by interacting with factors other than LEF and TCF and in response to a non-Wnt ligand (Salisbury *et al.* 2007).

Somatotrophs

Hesx1-Cre/degradation-resistant β -catenin mutant mice (*Hesx1*^{Cre/+}; *Ctnnb1*^{lox(ex3)/+}) exhibit a reduction in the number of cells expressing GH (Gaston-Massuet *et al.* 2011). *Pitx1*-expressing cells (*Pit1* precursors) in which β -catenin is conditionally knocked out have also demonstrated reduced numbers of mature somatotrophs (Olson *et al.* 2006). However, where degradation-resistant

β -catenin was expressed in mature cells expressing GH in *Cre-lox* mice, there was no sign of any increase in the prevalence of pituitary adenomas or proliferation, indicating a loss of the response to β -catenin following maturation (Gaston-Massuet *et al.* 2011). Somatolactotroph cells lines and primary cultures from rat pituitaries express *Wnt4* (Miyakoshi *et al.* 2009, Giles *et al.* 2011), although there is no literature thus far with respect to a role for Wnt4 signalling in these mature GH-producing cells.

Corticotrophs

Corticotrophin-releasing hormone has been shown to stimulate an increase in β -catenin expression in the AtT-20 corticotroph cell line. This appears to be via a mechanism involving the cAMP/PKA pathway, as this effect was lost when inhibitors were used alongside stimuli. Somatotropin release-inhibiting factor (*Srif/Sst*), an inhibitor of *Acth* (or *Pomc*) expression, had the opposite effect and appeared to increase the phosphorylation of β -catenin. A dominant negative *Tcf4* vector reduced cell proliferation in this cell line. Thus, it appears that canonical signalling may be active in AtT-20 cells and may be in part responsible for cell cycle regulation. The study did not investigate whether there was any effect by canonical signalling upon *Acth* expression. Interestingly, these effects were not reproducible in GH3 cells (Khattak *et al.* 2010). In studies of pituitary development, the canonical pathway has negligible effects upon corticotrophs (Treier *et al.* 1998, Potok *et al.* 2008).

Thyrotrophs

Normal thyrotrophs, corticotrophs, lactotrophs and gonadotrophs have all been shown to express *Wnt4* (Giles *et al.* 2011). Triiodothyronine (T_3) treatment of TtT-97 thyrotroph tumour cells (which negatively feeds back on TSH production and inhibits growth) resulted in the reduced expression of *Wnt10a* and *Bmp4* along with reduced intranuclear β -catenin and cyclin A expression. T_3 was also shown to impede the readout from the super-TOPflash transcription vector, which has eight TCF-binding elements upstream of a luciferase reporter, suggesting the reduced activation of canonical signalling in these cells. T_3 did not alter *Pitx2* or cyclin D1 expression but did reduce cyclin A2 and cyclin B1, indicating a possible mechanism for canonical signalling in controlling cell cycle progression in this model. Thus, there may be a role for canonical Wnt signalling in the proliferation of mature thyrotrophs (Kerr *et al.* 2005).

Wnt signalling in pituitary tumours

The study of patients with syndromes resulting in hypopituitarism suggests a potential role for β -catenin and Wnt signalling.

Multiple endocrine neoplasia type 1

In a mouse model of multiple endocrine neoplasia type 1 (MEN1), in which patients develop tumours of the anterior pituitary, pancreas and parathyroids, mice developed tumours of the ovary and testes. In these tumours, there was a down-regulation of *Wnt4*, *Wnt9a*, *Fz6/Fzd6* and *Lrp2*, although in this study, pituitary tumours were not specifically examined (Mould *et al.* 2009).

Carney complex

Carney complex is a MEN syndrome in which there is an increase in the development of tumours, in particular skin tumours, myxomas, schwannomas and endocrine neoplasms. Carney complex occurs due to a mutation resulting in the decreased activity of the gene *Prkar1a* that codes a regulatory subunit of PKA. There is thus an increase in PKA and cAMP signalling. In the mouse *Prkar1a*^{+/-} model, tumour formation was less frequent than seen in humans. By crossing these mice with mice in which the tumour-suppressor gene *p53* (or *Tp53*) was knocked down, the phenotype of mice better mirrored that which was expected and developed both thyroid and pituitary tumours of the pars intermedia. The analysis of these mice showed that there was an increased expression of *Wnt3*, *Wnt3a*, β -catenin and *Lrp5* mRNA. Furthermore, in an adrenal cell line bearing an inactivating mutation of *Prkar1a*, cell growth was arrested at the G0/1 phase by inhibiting *Wnt3* expression. Owing to the effect that *PRKAR1a* has upon PKA, it is possible that Wnt may inhibit growth by interacting with cAMP signalling (Almeida *et al.* 2010).

ANE syndrome

In the alopecia, neurological defects and endocrinopathy (ANE) syndrome, patients developed combined anterior hormone deficiency including gonadotrophs and corticotrophs (Spiegel *et al.* 2010). Skin biopsies taken from one patient with ANE demonstrated a loss of mature follicles and the presence of dermal cysts, mirroring the findings in the skin of mice with β -catenin deficiency. A reduction in β -catenin expression in this skin biopsy was also observed,

indicating that canonical signalling may play a role in the pituitary failure of these patients (Nousbeck *et al.* 2008, Spiegel *et al.* 2010).

Wnt1^{-/-} mice

In the *Wnt1* transgenic mouse model for oestrogen- and progesterone-responsive breast cancer, a loss of the tumour-suppressor gene *p27* (or *Cdkn1b*) led to an increase in pituitary, gastric and duodenal tumours (Glover *et al.* 2009). However, *p27* is known to affect pituitary cell proliferation, and thus this finding might be independent of Wnt signalling (Nakayama *et al.* 1996), especially as *Wnt1* was not shown to be expressed in the developing pituitary (Olson *et al.* 2006).

Human pituitary adenomas

In pituitary adenomas, Miyakoshi *et al.* have demonstrated an increased expression of WNT4 and FZ6 in tumours expressing GH, PRL and TSHB, but not in those expressing ACTH when compared with normal human pituitaries. In these tumours, there was no change in β -catenin distribution; thus, it appears that when Wnt acts, it exerts its effects in a non-canonical manner. The phosphorylation of CaMK-II, an indicator of calcium signalling, was no different in these tumours when compared with normal tissue, although the levels were raised in two ACTHomas and one gonadotrophinoma. C-jun, a target of the planar cell polarity pathway, was inconsistently observed in some of the adenomas but not in the normal pituitary tissue. Thus, there is evidence that WNT4 may play a role in the development of some adenomas, especially those of cells from the Pit1 lineage; however, there is little evidence to suggest what mechanism might be employed (Miyakoshi *et al.* 2008). There is also inconsistency between studies of pituitary adenomas. In one study investigating β -catenin expression, nuclear accumulation of β -catenin was found in 57% of tumours; however, it did not compare this finding with healthy pituitary tissue. In this study, the expression was especially high in the tumours of cells from the Pit1 lineage (Semba *et al.* 2001). In a study of 47 pituitary adenomas, Formosa *et al.* found no changes in β -catenin expression, but did find increased immunostaining for the Wnt target genes cyclin D1 and *c-Myc* (or *MYC*). Cyclin D1 was associated with a later age of presentation, whereas *MYC* was seen in younger patients. These markers demonstrated little correlation with tumour size, although patients with hypopituitarism demonstrated a reduction

in *MYC* expression. Interestingly, cyclin D1 was observed to be more abundant in male than female patients, indicating a possible endocrine stimulation for its expression. These results perhaps suggest a β -catenin-independent mechanism to be activated in these tumours (Formosa *et al.* 2012). In the α T3-1 gonadotroph tumour cell line, transfection with a dominant-negative *PITX2* and *PITX2* siRNA resulted in a gross reduction in cell viability by increasing apoptosis. This effect was replicated in primary cultures from five out of seven human gonadotroph adenomas. Given that *PITX2* is under the control of β -catenin, canonical signalling may be implicated in the pathogenesis of these tumours (Acunzo *et al.* 2011). Clearly, further work is warranted to clarify the activation of Wnt signalling in pituitary tumours and identify the pathway(s) involved.

sFRP acts as an inhibitor of the Fz co-receptor complex. The sFRP 'WIF1' inhibits Wnt signalling and has been shown to be down-regulated in a series of both functioning and non-functioning pituitary adenomas when compared with healthy pituitary tissue. This finding on a microarray was verified by qPCR and was associated with the methylation of the CpG island on the promoter of *WIF1*, demonstrating the potential epigenetic regulation of Wnt signalling. This finding was also demonstrated *in vitro* in the GH3 cell line, which demonstrated little *WIF1* expression but in which the transfection of *WIF1* resulted in reduced proliferation. No changes in β -catenin localisation were observed in this study of 42 pituitary adenomas (Elston *et al.* 2008).

Wilms tumours

WNT4 is regulated by Wilms tumour 1 (*WT1*) in paediatric nephroblastomas. It was postulated that *WT1* might also play a role in pituitary adenoma formation. In a series of 90 pituitary tumours, no evidence was found to support the role of *WT1* in tumorigenesis (Schittenhelm *et al.* 2009).

In summary, there is clear evidence that the expression of Wnt signalling molecules is altered in pituitary adenomas. The precise mechanisms underlying these changes and their causality still require further interrogation in order to form a clear overall view of the likely significance of these signalling pathways in pituitary tumorigenesis.

Wnt and craniopharyngiomas

A craniopharyngioma is a benign epithelial tumour arising from around the sella turcica and represents 3% of all

intracranial tumours. In the adamantinomatous subtype of craniopharyngiomas, there is good evidence that canonical signalling is important in tumorigenesis. Nuclear β -catenin has been demonstrated in several studies of these tumours in humans, including seven of nine tumours (Hassanein *et al.* 2003) and ten of ten tumours (Sekine *et al.* 2002). In the latter study, missense mutations were observed in the site at which GSK3 β phosphorylates β -catenin, indicating that activated canonical signalling may play a role in tumorigenesis and/or proliferation. Furthermore, in a series of 16 patients treated surgically for craniopharyngioma, eight of them exhibited mutations of β -catenin, six of which were in children. Of note, in these tumours, there were no mutations found in *HESX1*, *PROP1* or *PIT1*, although the study did not explore quantitative changes in the expression of these transcription factors which, under the control of canonical signalling, regulate differentiation (Campanini *et al.* 2010). In another study of 14 adamantinomatous craniopharyngiomas, heterozygous mutations in β -catenin were found in nine patients in regions that would affect the phosphorylation of β -catenin by GSK3 β . All 14 patients demonstrated the accumulation of nuclear β -catenin (Cani *et al.* 2011).

Hesx1^{Cre/+};Ctnnb1^{lox(ex3)/+} mutant mice develop large cystic pituitary tumours that were both histologically and immunohistologically similar to human adamantinomatous craniopharyngiomas. Interestingly, canonical signalling appeared to be activated in islands of cells in these pituitaries that did not express hormones, nor did

they stain with Ki67 or incorporate BrdU, suggesting that canonical signalling may be activated in cells that were not themselves replicating. Thus, it may be that these are tumour precursor cells or that they stimulate the growth of other cells in the pituitary. These cells may support evidence that populations of stem cells are present within the pituitary (Gaston-Massuet *et al.* 2011). Triple heterozygous *Hesx1^{Cre/+}; Ctnnb1^{lox(ex3)/+}*; BAT-gal mice express β -galactosidase in cells with nucleocytoplasmic (as opposed to membranous) β -catenin (β -catenin^{nc}) allowed these cells to be separated by flow cytometry. β -Catenin^{nc} cells expressed reduced levels of terminal differentiation markers such as Pit1, GH and POMC. Although more of these cells were capable of forming colonies than cells with membrane-bound β -catenin, only 5% of them were actually capable of forming colonies and thus stemness in these cells is likely to be related to another factor, for example *Sox2* expression. The separated β -catenin^{nc} cells expressed increased expression of the members of the BMP and FGF families of signalling molecules, which, as we have seen, are essential for normal pituitary development, perhaps indicating a paracrine role for these cells in the activity of pituitary stem-like cells (Andoniadou *et al.* 2012). In the (*Hesx1^{Cre/+};Ctnnb1^{lox(ex3)/+}*) pituitaries, the number of cells producing GH was significantly reduced, resulting in phenotypic dwarf mice. There was no change in the number of ACTH-producing cells, indicating that this effect was constrained to Pit1-derived cells (Gaston-Massuet *et al.* 2011).

Table 1 A summary of evidence regarding the role played by Wnt dysregulation in human pituitary tumours.

Tumour	Species	Wnt dysregulation	Reference
Multiple endocrine neoplasia type 1	Mouse	Down-regulation of <i>Wnt4</i> , <i>Wnt9a</i> , <i>Fz6</i> and <i>Lrp2</i> , but pituitaries not specifically examined	Mould <i>et al.</i> (2009)
Carney complex	Mouse	<i>Prkar1a^{+/-}p53^{+/-}</i> mice demonstrate increased <i>Wnt3</i> , <i>Wnt3a</i> , β -catenin and <i>Lrp5</i> expression	Almeida <i>et al.</i> (2010)
Alopecia, neurological deficits and endocrinopathy	Human	Reduction in dermal β -catenin	Nousbeck <i>et al.</i> (2008) and Spiegel <i>et al.</i> (2010)
GH-, PRL- and TSHB-expressing human pituitary adenomas	Human	Increased <i>WNT4</i> and <i>FZ6</i> expression, but no change in β -catenin	Miyakoshi <i>et al.</i> (2008)
Pituitary adenomas	Human	β -Catenin nuclear accumulation in 57% (no control)	Semba <i>et al.</i> (2001)
Pituitary adenomas		Increased cyclin D1 and <i>c-Myc</i> (Wnt target genes) in later presenting tumours	Formosa <i>et al.</i> (2012)
Pituitary adenomas		Methylation of the <i>WIF1</i> promoter increased, resulting in reduced <i>WIF1</i> expression	Elston <i>et al.</i> (2008)
Adamantinomatous craniopharyngioma	Mouse	<i>Hesx1^{Cre/+};Ctnnb1^{lox(ex3)/+}</i> mice develop cystic pituitary tumours which mimic adamantinomatous craniopharyngioma	Andoniadou <i>et al.</i> (2011) and Gaston-Massuet <i>et al.</i> (2011)
Pituitary adenomas	Human	Nuclear β -catenin accumulation	Sekine <i>et al.</i> (2002), Hassanein <i>et al.</i> (2003), Campanini <i>et al.</i> (2010) and Cani <i>et al.</i> (2011)

A summary of the effects of Wnt signalling dysregulation in human and murine examples of pituitary tumours can be found in Table 1.

Concluding remarks

There is accumulating evidence that Wnt signalling plays a role in pituitary organogenesis. Most compelling is the role that β -catenin plays in the regulation of transcription factor expression by interacting to form complexes that both stimulate and inhibit gene expression. It is also clear that some of the Wnt proteins themselves play a role in the process as the pituitaries of *Wnt4*-deficient mice are lacking in the cells of the Pit1 lineage. What remains to be elucidated is a connection between the Wnt ligand and the intracellular effect. Further work is likely to identify the protein complexes responsible for switching on and off canonical signalling during the development of the pituitary. So far, experimental work has focused on tractable mouse models; however, it will be interesting to see how findings are transferable between species. This is particularly important in the translation to understanding tumours in humans, where pituitary lineage development may have significant differences from that in rodents (Pope et al. 2006).

In adult pituitaries, Wnt signalling plays a role in the activity and proliferation of mature hormone-producing cells. Here, there is building evidence for alternative Wnt signalling pathways being activated, for example *Wnt4* affecting calcium oscillations in GH3 cells (Giles et al. 2011). It is also interesting to note that trophic hormones have been found to stimulate canonical signalling in these mature cells (Kerr et al. 2005, Gardner et al. 2007, Khattak et al. 2010). Wnt signalling has been mainly demonstrated in cell lines, which, by their very nature, may have altered proliferative properties, thus it is essential for further *in vivo* studies to confirm the effects seen *in vitro*. mRNA expression patterns seem to indicate that Wnt signalling does play a role in *in vivo* models.

Canonical signalling clearly plays a different role in embryonic and mature cells. For example, activating β -catenin in differentiated cells had little effect compared with those still to mature (Gaston-Massuet et al. 2011). This altered response may help us improve ideas about pituitary plasticity; as most *ex vivo* studies of pituitary tumours fail to identify activated canonical signalling, it might indicate a mature as opposed to stem cell origin for these tumours. Further questions lie in changes to the mechanism of Wnt signalling with time; for example, how does a Wnt ligand stimulate canonical signalling in immature cells and then calcium signalling in adults?

The exploration of Wnt signalling in pituitary cells has demonstrated the complexity of the mechanisms involved in maintaining homeostasis. Wnt signalling is clearly important but appears to play only part of a network of signals, balancing cell activity, proliferation and apoptosis. The composition of the pituitary, with its multiple cell types distributed among each other, makes it a complex model in which to study a signalling network; nevertheless, it is this complexity that makes it ideal for us to better understand the interactions responsible for the actions of Wnt molecules.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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