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Biology of human craniopharyngioma: lessons from mouse models

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Abstract

Adamantinomatous craniopharyngiomas (ACP) are clinically relevant tumours that are associated with high morbidity, poor quality of life and occasional mortality. Human and mouse studies have provided important insights into the biology of these aggressive tumours, and we are starting to understand why, how and when these tumours develop in humans. Mutations in β-catenin that result in the over-activation of the WNT/β-catenin signalling pathway are critical drivers of most, perhaps of all, human ACPs. Mouse studies have shown that only pituitary embryonic precursors or adult stem cells are able to generate tumours when targeted with oncogenic β-catenin, which suggests that the cell context is critical in order for mutant β-catenin to exert its oncogenic effect. Interestingly, mutant stem cells do not generate the bulk of the tumour cells; instead, they induce tumours in a paracrine manner. Combining basic studies in mice and humans will provide further insights into the biology of these neoplasms and will reveal pathogenic pathways that could be targeted with specific inhibitors for the benefit of patients. These benign tumours may additionally represent a unique model for investigating the early steps that lead to oncogenesis.

Introduction

Craniopharyngiomas (CPs) are non-malignant epithelial tumours of the sellar region. Although the overall incidence is low (1.60–2.14 new cases/million per year), they are the most common tumour of this area in children under 15 years of age (1.53–2.92) (Nielsen *et al*. 2011, Muller 2014*a*). They are designated as grade I tumours according to the World Health Organization (WHO) classification (Louis *et al*. 2014). However, CPs often infiltrate into the hypothalamus, optic chiasm and local vascular structures, which leads to a high degree of morbidity (Holsken *et al*. 2010). Malignant CPs have

been described, but these are extremely rare (Aquilina *et al*. 2010, Sofela *et al*. 2014).

There are two different subtypes of CPs, adamantinomatous and papillary. Adamantinomatous CPs (ACPs) represent the most common non-neuroepithelial intracranial lesions in children, and they contribute to 1.5–11.6% of all paediatric CNS tumours (Bunin *et al*. 1998). There is a well-described bimodal age distribution in incidence, with peaks occurring between the ages of 5 and 14 (childhood-onset ACP) and between ages 65 and 74 (adult-onset ACP) (Bunin *et al*. 1998, Nielsen *et al*.

2011). ACPs are the archetypal suprasellar tumour for causing neuroendocrine disruption and dysfunction, not just from tumour pressure and invasive effects but also from treatments targeted to this area. ACP and its current therapies (surgery and radiotherapy) are associated with a high 5-year survival rate but also with tumour recurrence, considerable morbidity and poor health-related quality of life (Karavitaki *et al.* 2005, Muller 2010*a,b*, Cohen *et al.* 2013, Karavitaki 2014). The instillation of radionucleo-tides, chemotherapy and, more recently, the insertion of interferon alpha directly into the cysts are occasionally employed (Bartels *et al.* 2012, Liu *et al.* 2012, Wisoff 2012, Vanhauwaert *et al.* 2013, Karavitaki 2014, Zheng *et al.* 2014). Although these modalities can be effective in reducing the burden caused by the cysts, their use is controversial because of the lack of control studies and high morbidity associated with such therapies. In addition, they have little effect on the solid component of ACP. Tumour and treatment-related consequences include severe and morbid obesity and subsequent type 2 diabetes mellitus (in up to two-thirds of patients), learning difficulties (psychological, behavioural and educational problems), visual impairment, stroke, seizures and life-threatening panhypopituitarism. Additionally, diabetes insipidus is present in up to 95% of patients and is implicated in late mortality (Karavitaki *et al.* 2005, Cohen *et al.* 2013). Tumour recurrence is frequent, even

after radiotherapy, and repeated surgeries are often required (Merchant 2006, Kiehna & Merchant 2010, Iannalfi *et al.* 2013). Overall, these are clinically relevant tumours that are associated with poor quality of life; ACP survivors almost universally become chronic patients with health-related well-being problems for the rest of their lives.

The papillary variant (papillary craniopharyngioma, PCP) is very rare and appears almost exclusively in adults, where peak incidence rates were observed in the 40–44-year-old age group (Nielsen *et al.* 2011). Recently, over-activating mutations in *BRAF* p.Val600Glu have been described in association with PCP, which suggests that these mutations may drive tumorigenesis (Brastianos *et al.* 2014). This suggests the exciting possibility that PCP patients may benefit from the use of clinically proven BRAF inhibitors (Ribas & Flaherty 2011, Menzies & Long 2014, Rahman *et al.* 2014, Fedorenko *et al.* 2015).

In this Thematic Review, I focus on the aetiology and pathogenesis of ACP and discuss two particular areas: tumour heterogeneity and the notion that ACPs are mostly developmental disorders. For more specific clinical and molecular accounts of human CP or to complement this review, I recommend other published reviews (Cohen *et al.* 2011, Hussain *et al.* 2013, Larkin & Ansorge 2013, Karavitaki 2014, Muller 2014*b*, Martinez-Barbera 2015, Martinez-Barbera & Buslei 2015).
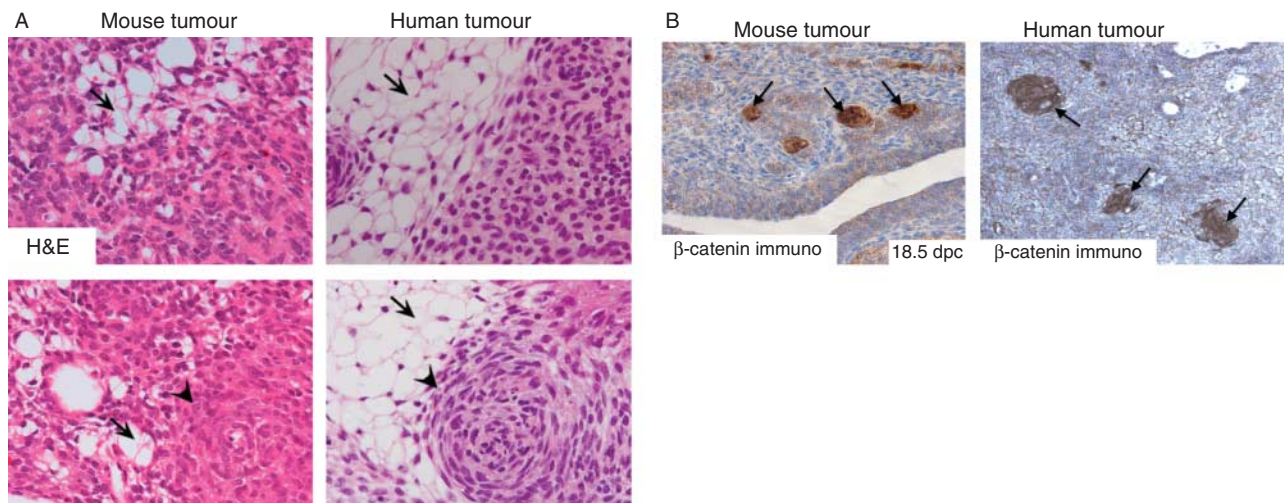

Figure 1
Histomorphological features of mouse and human ACP. (A) Haematoxylin and eosin staining of mouse and human tumours showing the presence of microcystic changes (stellate reticulum; arrows) and whorl-like nodular structures (cell clusters; arrowheads). (B) Immunohistochemistry with a specific anti-β-catenin antibody showing the presence of cell clusters with nucleo-cytoplasmic accumulation of β-catenin (arrows). Reproduced, with permission, from Gaston-Massuet C, Andoniadou CL, Signore M, Jayakody SA, Charolidi N, Kyeyune R, Vernay B, Jacques TS, Taketo MM, Le Tissier P, *et al.* (2011) Increased Wingless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors in mice and humans. *PNAS* **108** 11482–11487.

Mutations in *CTNNB1* cause mouse and human ACP

Initial molecular studies of human ACP revealed an association with mutations in *CTNNB1*, the gene that encodes β-catenin, a central regulator of the Wnt pathway (Sekine *et al.* 2002, Kato *et al.* 2004, Buslei *et al.* 2005, Oikonomou *et al.* 2005, Brastianos *et al.* 2014). These mutations were mostly located in exon 3, which encodes amino acids with important regulatory functions (Aberle *et al.* 1997). The prediction for these mutant proteins is that they would be resistant to degradation; hence, the half-life of mutant β-catenin would be significantly increased and would lead to over-activation of the Wnt pathway. This prediction was experimentally validated. For instance, specific immunohistochemistry of ACP histological sections revealed clusters of cells that exhibit nucleo-cytoplasmic accumulation of β-catenin, which is consistent with its stabilisation and increased half-life (Figs 1 and 2; Buslei *et al.* 2007, Cao *et al.* 2010, Gaston-Massuet *et al.* 2011). These β-catenin-accumulating clusters are unique to ACP and are not present in any other pituitary tumours, including the papillary form of CP (Hofmann *et al.* 2006). In addition, the Wnt/β-catenin pathway was shown to be active in the cell clusters, as was revealed by the expression of the transcriptional targets *AXIN2*, *LEF1* and bone morphogenetic protein 4 (*BMP4*; Sekine *et al.* 2004, Holsken *et al.* 2009, Gaston-Massuet

et al. 2011). These initial human subject studies provided enough evidence for a role of *CTNNB1* mutations in the aetiology of ACP. However, the presence of recurrent mutations in human tumours does not demonstrate *per se* that they are tumour drivers.

Further insights came from genetically engineered mouse models that allowed the activation of the Wnt pathway in the pituitary gland. In a first mouse model (*Hesx1*[Cre/+]/*Ctnnb1*[+/lox(ex3)] embryonic ACP model; Figs 1 and 2), a mutant form of β-catenin that lacked the amino acids encoded by exon 3 and therefore showed increased stabilisation and half-life was expressed in Rathke's pouch (RP) (Gaston-Massuet *et al.* 2011). RP contains the embryonic precursors of the pituitary gland that generate all of the hormone-producing cells of the anterior pituitary (Bilodeau *et al.* 2009, Davis *et al.* 2011, Jayakody *et al.* 2012). Based on patterns of cytokeratin expression, it has been suggested that ACP may derive from remnants of RP epithelium in humans. Therefore, in this mouse model, a form of β-catenin that was functionally equivalent to that identified in human ACP was expressed in the pituitary precursors from which human ACP is thought to derive. Indeed, the expression of oncogenic β-catenin in murine RP resulted in the formation of tumours that were similar to human ACP (Gaston-Massuet *et al.* 2011). For instance, histological analyses revealed that as in human ACP, mouse tumour cells did not express any pituitary hormones and were negative for synaptophysin, a marker
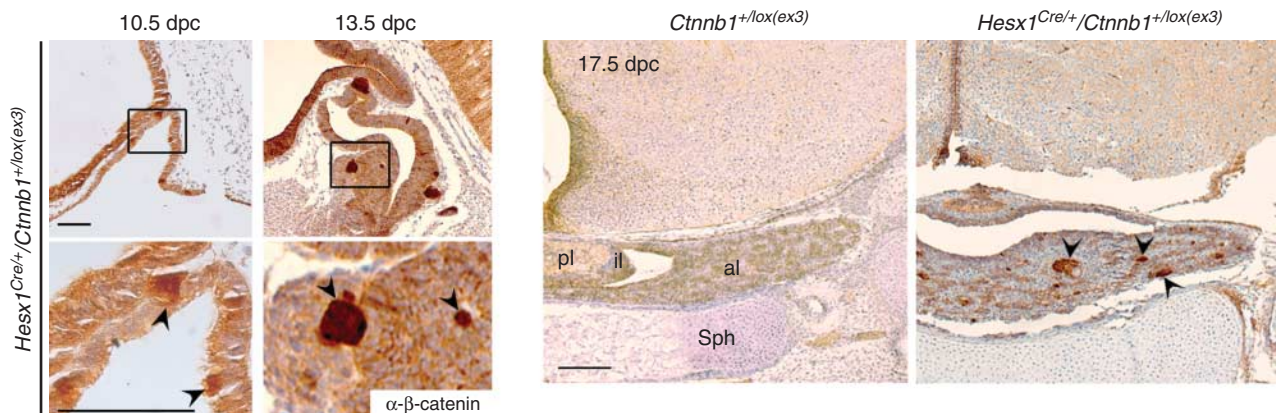

Figure 2

Cell clusters with nucleo-cytoplasmic accumulation of β-catenin are detectable in the embryonic ACP mouse model. Histological sections of the embryonic mouse ACP model (*Hesx1*[Cre/+]/*Ctnnb1*[+/lox(ex3)]), which developed pituitary glands from 10.5 to 17.5 days post coitum (dpc), were immunostained with a specific β-catenin antibody and counterstained with haematoxylin. Magnified views of the boxed regions are shown. Note that the accumulation of β-catenin begins in a few cells within Rathke's pouch epithelium, which contains the anterior pituitary progenitors. These clusters (arrowheads) are observed only in *Hesx1*[Cre/+]/*Ctnnb1*[+/lox(ex3)] but not in control *Ctnnb1*[+/lox(ex3)] pituitaries. al, anterior lobe; il, intermediate lobe; pl, posterior lobe; sph, sphenoid bone. Scale bars: 100 μm. Reproduced, with permission, from Gaston-Massuet C, Andoniadou CL, Signore M, Jayakody SA, Charolidi N, Kyeyune R, Vernay B, Jacques TS, Taketo MM, Le Tissier P, *et al.* (2011) Increased Wingless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors in mice and humans. *PNAS* **108** 11482–11487.

of neural and endocrine cells. Importantly, the mouse tumours contained groups of cells with nucleo-cyto-plasmic β-catenin that resembled the typical cell clusters observed in human ACP (Fig. 1). These clusters also showed activation of the Wnt pathway and expression of *Axin2*, *Lef1* and *Bmp4* (Gaston-Massuet *et al*. 2011, Andoniadou *et al*. 2012). All of the analysed mice developed tumours, but the time period required for tumour establishment varied broadly, with a median survival of 11 weeks. Some features of human ACP were not observed in the mouse tumours, for instance, calcification or wet keratin, which may require years rather than weeks to develop. Mouse tumours were localised within the sellar region, and no hypothalamic infiltrations were observed. It is possible that species-specific differences in pituitary morphogenesis between mice and humans may underlie the lack of hypothalamic infiltration and the location of the mouse tumours (discussed further later in this review). Overall, these initial murine studies demonstrated that the *CTNNB1* mutations that are present in human ACP were real drivers of ACP tumorigenesis, and they provided a unique genetically engineered mouse model for studying the pathogenesis of human ACP and testing novel treatments.

Undifferentiated pituitary precursors/stem cells play a critical role in ACP tumorigenesis

Mouse and human studies demonstrated that *CTNNB1* mutations drive tumorigenesis, but the cell type that is needed to sustain the mutation in order for the tumour to develop remained to be assessed. Interestingly, it was shown that the expression of oncogenic β-catenin in

differentiated cells of the mouse pituitary, including somatotrophs, lactotrophs, thyrotrophs, corticotrophs and gonadotrophs or even the Pit1 cell-lineage precursors, did not result in tumorigenesis (Fig. 3; Gaston-Massuet *et al*. 2011). These cells actively proliferate embryonically as well as during early postnatal life, which suggests that the absence of tumour development is not a consequence of the cells having exited cell cycle. In contrast, and as discussed in the previous section, tumours formed when mutant β-catenin was expressed in RP embryonic precursors. These precursors share typical features of stemness (i.e. self-renewal and the ability to generate all of the differentiated cell types in the pituitary). Therefore, these initial experiments revealed that undifferentiated precursor/stem cells might play a critical role in the genesis of CP.

It has been shown in the adult mouse pituitary that the $Sox2^+$ population contains pituitary stem cells that are capable of self-renewing and of generating all of the hormone-producing cells *in vitro* and *in vivo* (Fauquier *et al*. 2008, Andoniadou *et al*. 2013). Notably, $Sox2^+$ cells are also present in human pituitaries (Garcia-Lavandeira *et al*. 2009). To assess whether adult pituitary stem cells play a role in ACP formation, a second genetically engineered mouse model was generated ($Sox2^{CreERT2/+}$; $Ctnnb1^{+/lox(ex3)}$ adult ACP model). In this model, oncogenic β-catenin was expressed exclusively in $Sox2^+$ stem cells of the adult pituitary gland (Andoniadou *et al*. 2013). These mice developed pituitary tumours that were very similar to those observed in the embryonic ACP mouse model and in humans; for instance, tumours were synaptophysin-negative and contained the typical β-catenin-accumulating cell clusters that characterise ACP (Andoniadou *et al*. 2013).

Pit1-Cre/Ctnnb1$^{+/lox(ex3)}$ *Gh-Cre/Ctnnb1$^{+/lox(ex3)}$*   *Prl-Cre/Ctnnb1$^{+/lox(ex3)}$*

Figure 3

ACP-like tumours form only when pituitary progenitor/stem cells are targeted to express oncogenic β-catenin. Expression of mutant β-catenin in committed progenitors (*Pit1-Cre/Ctnnb1$^{+/lox(ex3)}$*) (left), terminally differentiated hormone-producing cells (*Gh-Cre/Ctnnb1$^{+/lox(ex3)}$*) (centre) and *Prl-Cre/Ctnnb1$^{+/lox(ex3)}$* (right) is not tumorigenic, and pituitaries from adult mice are normal. Reproduced, with permission, from Gaston-Massuet C, Andoniadou CL, Signore M, Jayakody SA, Charolidi N, Kyeyune R, Vernay B, Jacques TS, Taketo MM, Le Tissier P, *et al*. (2011) Increased Wingless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors in mice and humans. *PNAS* **108** 11482–11487.

Intriguingly, when Sox2$^+$ stem cells were simultaneously targeted to express β-catenin and a yellow fluorescent protein in order to trace the cellular progeny of mutant Sox2$^+$ stem cells, only cluster cells were found to derive from the targeted Sox2$^+$ cells, whereas the bulk of the tumour cells were not. This result was surprising, because according to the cancer stem cell paradigm, the tumour cells were expected to be descendants of mutant Sox2$^+$ cells, which express oncogenic β-catenin (Nguyen *et al.* 2012, Visvader & Lindeman 2012). Instead, these results suggest that cluster cells may act in a paracrine manner to cause Sox2$^-$ cells to transform, so that they become the tumour cell-of-origin. In agreement with this notion, cluster cells in human and mouse ACPs express numerous signalling molecules, such as Sonic Hedgehog, fibroblast growth factors, BMPs, epidermal growth factor as well as several chemokines and cytokines with pro-inflammatory action that are all heavily involved in human tumours and cancers (Buslei *et al.* 2007, Pettorini *et al.* 2010, Gaston-Massuet *et al.* 2011, Andoniadou *et al.* 2012, Gong *et al.* 2014, Gomes *et al.* 2015). Counteracting the effects of some of these signals may be beneficial for patients, for instance, by using small-molecule inhibitors against critical oncogenic pathways (Blanco Calvo *et al.* 2009, Rudin *et al.* 2009, Rothwell *et al.* 2010, Holsken *et al.* 2011, Wesche *et al.* 2011). This is very relevant because therapeutic agents that specifically target the Wnt/β-catenin pathway have only recently entered clinical trials, and none has yet been approved (Anastas & Moon 2013, Kahn 2014). Logically, the activities of the clusters must be required to support tumour viability; otherwise, one must call into question why the scarce non-dividing cells are not eliminated, even though normal pituitary cells are mostly killed by the dividing tumour cells. In fact, using a xenograft mouse model, the Buslei group has shown that clusters are critical players in controlling cell behaviour and the infiltration of other tumour cells (Stache *et al.* 2014). The paracrine model for the involvement of tissue-specific adult stem cells in tumorigenesis suggests that the cell that sustains the oncogenic mutation (mutant *CTNNB1*) and the cell-of-origin of the tumours are different (Fig. 4). Published literature supports a critical role for paracrine activities in tumours/cancers such as leukaemia (Kode *et al.* 2014), skin cancer (Nicolas *et al.* 2003, Demehri *et al.* 2009) and hepatocellular carcinoma (Lujambio *et al.* 2013), which suggests that these models may have broader implications in the oncology field.

Knowns and unknowns of genetic heterogeneity in human ACP

Tumours and cancers are composed of different cell types that are genetically heterogeneous. This heterogeneity is manifested by the presence of different DNA mutations in
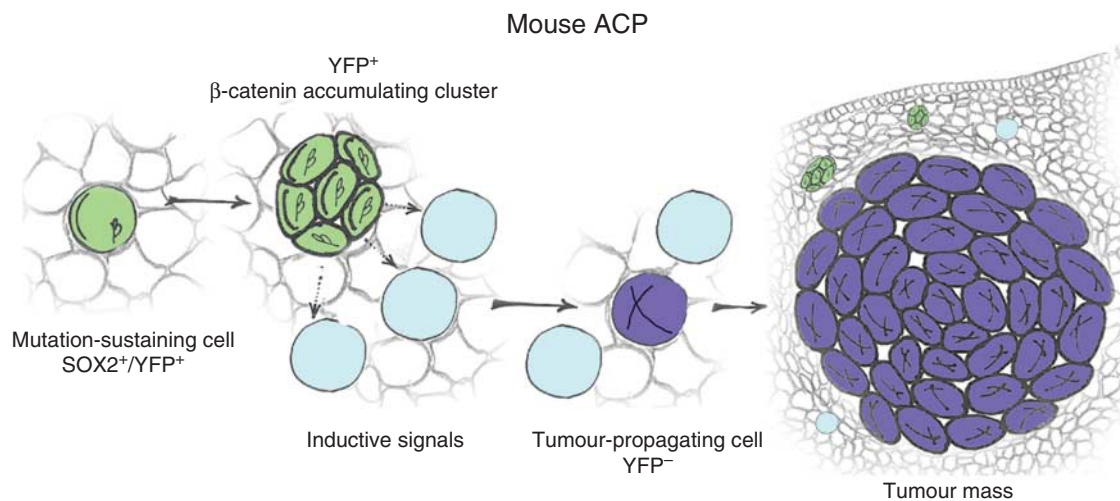

Mouse ACP

Figure 4
Paracrine model of involvement of pituitary stem cells in tumorigenesis. When targeted to express oncogenic β-catenin, SOX2$^+$ cells (green) accumulate nucleo-cytoplasmic β-catenin, proliferate transiently, stop dividing and form clusters. Clusters secrete signals to the surrounding cells to induce cell transformation and tumour growth from a cell that is not derived from the targeted SOX2$^+$ stem cell. Reprinted from *Cell Stem Cell*, **13**, Andoniadou CL, Matsushima D, Mousavy Gharavy SN, Signore M, Mackintosh AI, Schaeffer M, Gaston-Massuet C, Mollard P, Jacques TS, Le Tissier P, *et al.*, Sox2(C) stem/progenitor cells in the adult mouse pituitary support organ homeostasis and have tumor-inducing potential, pp 433–445, Copyright 2013, with permission from Elsevier.

specific cell clones and/or distinct gene expression profiles, even in homogeneous genomes, because of epigenetic variations. Tumour heterogeneity occurs between tumours (inter-tumour heterogeneity) and within tumours (intra-tumour heterogeneity). Generally speaking, benign tumours exhibit low intra-tumour heterogeneity, whereas malignant cancers are usually highly heterogeneous, with multiple cell clones competing within the tumour microenvironment to obtain sufficient resources to allow their expansion and survival. These genetically diverse populations contribute to a resistance to anti-cancer drugs; these therapies exert a selective pressure that kills only sensitive clones and gives resistant clones the opportunity to expand and the tumour the opportunity to recur, a phenomenon referred to as clonal evolution (Jamal-Hanjani *et al*. 2015, McGranahan & Swanton 2015, Walther *et al*. 2015). It is not surprising that tumours are heterogeneous; physiological homoeostasis of healthy tissues and organs may also result in heterogeneity, because stem cells and/or progenitors compete with each other during the lifetime of the human (Verovskaya *et al*. 2013, Babovic & Eaves 2014). We think of ourselves as homogeneous entities, but we are mosaic mixtures of differentiated cells that are derived from distinct clones with specific genetic and epigenetic variations.

There have not been many studies that have been aimed at assessing tumour heterogeneity in human ACP. Initial studies identified numerous karyotypic abnormalities in several chromosomes (Gorski *et al*. 1992, Karnes *et al*. 1992), whereas others (Griffin *et al*. 1992, Vagner-Capodano *et al*. 1992) reported no gross cytogenetic defects in human ACP. In these studies, karyotyping was performed on cultured cells isolated from human ACP samples, which are prone to artefacts. Cultured cells may theoretically accumulate aberrations, and culture conditions may select for the expansion of 'normal', non-tumour cells present in a tumour (i.e. host reactive tissue), which could explain the presence or absence of karyotypic defects in these studies respectively. Comparative genomic hybridisation was subsequently used, but similar inconsistencies were reported. Rienstein *et al*. (2003) analysed nine human ACP tumours and identified translocations, deletions and an increase in DNA copy numbers in six of the tumours. However, two independent groups failed to detect copy number variations in more than 30 ACP and nine PCP tumours; they concluded that chromosomal imbalances are a rare event (Rickert & Paulus 2003, Yoshimoto *et al*. 2004). So far, the most common genetic changes identified in human ACP are

mutations in *CTNNB1*, although mutation frequencies vary from 16 to 100% depending on the study (Sekine *et al*. 2002, Kato *et al*. 2004, Buslei *et al*. 2005, Oikonomou *et al*. 2005, Brastianos *et al*. 2014, Larkin *et al*. 2014). In a recent report, other mutations were identified in several loci by exome sequencing, but they were not recurrent and were possibly not involved in tumour survival or growth (Brastianos *et al*. 2014). In contrast, a human ACP sample was found to contain both mutations in *CTNNB1* and BRAFV600E, which suggests that tumour subtypes may exist (Larkin *et al*. 2014). Further analyses (e.g. whole-genome sequencing) and exome sequencing of more human ACP tumours is required to assess the degree of genetic heterogeneity in human ACP and to determine whether mutations in other genes also underlies the molecular aetiology of human ACP.

A prediction from the mouse model (Figs 1 and 2) is that human ACP are non-clonal and heterogeneous tumours, seeing as the model shows that cluster cells are derived from Sox2$^+$ stem cells, but the cell-of-origin of the tumours is of a different, yet-unidentified cell lineage. Few studies have addressed the clonality of human ACP. Sarubi *et al*. (2001) carried out a genetic analysis of eight human ACP tumours using the X-chromosome inactivation assay and found that only two were monoclonal and six were of polyclonal origin. The X-chromosome inactivation assay can determine clonality in female patients. This assay is based on the random inactivation by DNA methylation of a single X chromosome in each cell during early embryogenesis, and this inactivation is subsequently propagated to daughter cells. In a monoclonal cell population, all of the cells would be expected to have inactivated the same X chromosome, be it either maternal or paternal, whereas polyclonal tissues would carry a mixture of both maternal and paternal X chromosomes. The human androgen receptor gene is commonly used because of the presence of a hypervariable CAG short tandem repeat ($n=9$–36) in the first exon of the gene. Distinction between the paternally and maternally derived X chromosomes is only possible in females who are heterozygous for this repeat. However, because this CAG repeat is polymorphic in 90% of females, the assay is informative in most females. X-chromosome inactivation is correlated with hypermethylation; therefore, methylation-sensitive restriction enzymes digest only the active (i.e. unmethylated) alleles. In this way, it is possible to distinguish the active from the inactive alleles in a subsequent PCR (Boudewijns *et al*. 2007).

The results from the study by Sarubi *et al*. are difficult to interpret. The presumptive polyclonal origin of six of

the tumours could represent contaminating non-tumoural host cells (e.g. immune cells, glial cells, fibroblasts and endothelial cells), because human ACP causes a strong stromal reaction. Even if they are carefully dissected, these contaminating cells are very difficult to remove from fresh tumour samples. The finding that two tumours are monoclonal is also questionable, seeing as the resolution of the X-chromosome inactivation assay would not allow for the identification of small-cell populations of different origins. For instance, if cluster and non-cluster tumour cells were of different cell lineages, low numbers of clusters in some human tumours would be missed in this assay.

If all of the tumour cells carried *CTNNB1* mutations, the accumulation of nucleo-cytoplasmic β-catenin and the activation of the Wnt pathway might be expected to occur in all of the cells. However, this is not the case. Nucleo-cytoplasmic β-catenin and the activation of the Wnt pathway are confined to a specific population of tumour cells that either form clusters or are sparsely found throughout the tumour as single cells. In a recent study, nuclear and/or cytoplasmic accumulation of β-catenin was observed in 100% of the ACP cases analysed ($n=72$); however, mutations in exon 3, which harbours the most common mutations in *CTNNB1*, were identified in only 52% of the ACP samples (Preda *et al*. 2015). The proportion of cells that accumulates nucleo-cytoplasmic β-catenin in the form of clusters or single cells is highly variable between tumours. This variability may account for the broad range of discovery of *CTNNB1* mutations in human ACP (16–100%), because in tumours with a low percentage of β-catenin-accumulating cells, the identification of the mutant allele could be missed in the sequencing data. Several studies have used laser capture microdissection (LCM) to separate and analyse specific tumour cell compartments to investigate which ones actually carry the mutation. Sekine *et al*. (2002) reported the presence of the same *CTNNB1* mutation in cell clusters and the epithelial tumour tissue that surrounds them in two ACP samples. In contrast, similar LCM analyses by Holsken *et al*. (2009) revealed the presence of different *CTNNB1* mutations inside and outside of the clusters in four out of the eight human ACPs analysed, which suggests the existence of tumour heterogeneity and supports a polyclonal origin of the tumours.

More recently, exome sequencing of 12 human ACP samples has been performed. *CTNNB1* mutations were found in 11 of the samples (Brastianos *et al*. 2014). Only samples with a minimum of 10% tumour cells were used in that study. The allelic frequencies of the *CTNNB1* mutations were found to be very low, which suggests that human ACPs are non-clonal. Only when the allelic frequencies were adjusted by a computational method that is used for assessing cancer cell fraction were the allelic frequencies high enough to conclude that ACPs may be clonal. Whether this computed adjustment revealed the clonality of the β-catenin-accumulating clusters and not that of the whole tumour is a possibility that is not discussed in that paper. As previously described (Andoniadou *et al*. 2013), data from the adult mouse model have demonstrated that cluster cells are clonal and are derived from $Sox2^+$ pituitary stem cells, which suggests that they may represent the clonal population that was inferred in the human study (Brastianos *et al*. 2014). Further biological studies are needed to address the clonality of human ACP by performing thorough molecular analyses of specific cell populations, for instance, analyses of isolated cells with flow cytometry or LCM, as well as single-cell sequencing analyses.

Childhood-onset ACPs are likely to be developmental tumours

The molecular and genetic studies that used both embryonic and adult ACP mouse models discussed earlier in this review revealed compelling evidence that these genetically engineered mouse models are excellent tools for understanding the aetiology and pathogenesis of human tumours. In addition, these and a recent xenograft model (Stache *et al*. 2014) represent a platform for testing novel treatments in pre-clinical studies.

As mentioned earlier in this review, there are some histological features of human ACP that have not been observed in the mouse models, such as calcification and the presence of nodules of anucleated ghost cells with brightly eosinophilic cytoplasm that has been termed wet keratin. These regressive changes may require years, rather than months, to develop, and they therefore may represent species-specific differences resulting from the varied periods of time required for tumour establishment. The location of the tumours also varies between mice and humans. In humans, some tumours develop in the suprasellar region, in direct contact with and often infiltrating the hypothalamus. In contrast, mouse tumours are located within the sellar region (i.e. in contact with the basisphenoid bone) and do not infiltrate the hypothalamus. In this regard, it is important to note that pituitary morphogenesis is different between mice and humans. In both human and mouse embryos, the precursors of the anterior pituitary, which is contained

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in RP, are initially in contact with the overlying hypothalamic primordium, and their descendants must migrate into the sellar region to form the anterior pituitary. The primordium of the posterior pituitary (i.e. infundibulum) is initially embedded within the hypothalamus and moves downwards to its final position, which is adjacent to the anterior pituitary within the sella turcica. However, because the pituitary stalk is markedly
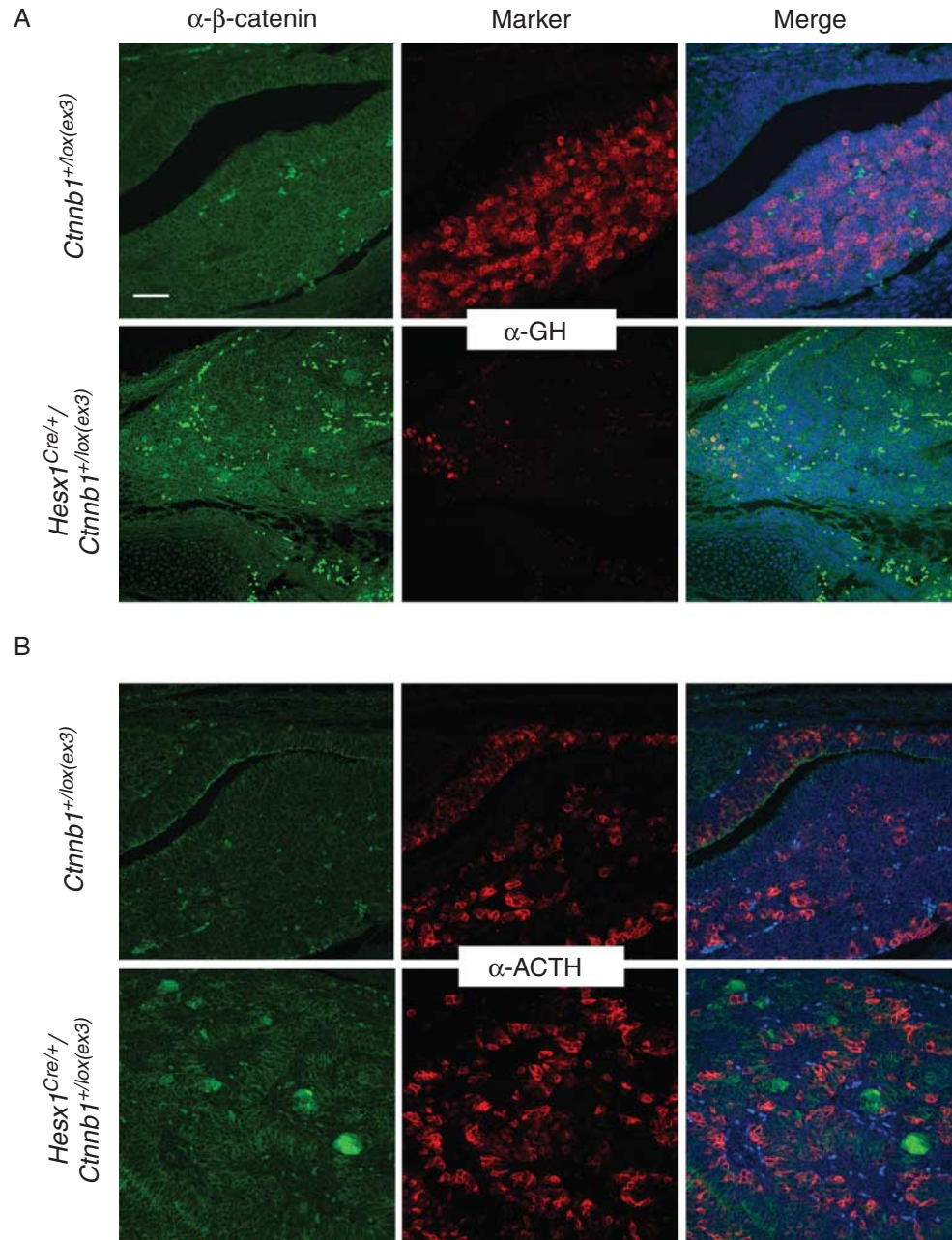
Figure 5

Terminal differentiation of growth hormone (GH)-producing cells is severely disrupted in the pre-tumoural pituitary of the embryonic ACP mouse model. Double immunostaining with either β-catenin antibody (green) or terminal differentiation markers (red) on 18.5 dpc WT or *Hesx1$^{Cre/+}$/Ctnnb1$^{+/lox(ex3)}$* pituitary sections. Numbers of somatotrophs (GH-positive cells) (A) but not melanotrophs or corticotrophs (ACTH-positive cells) (B) are drastically reduced in the mouse ACP model relative to the control. Note that β-catenin-accumulating cell clusters do not express GH or ACTH (merge image). Bright fluorescent cells are red blood cells. Reproduced, with permission, from Gaston-Massuet C, Andoniadou CL, Signore M, Jayakody SA, Charolidi N, Kyeyune R, Vernay B, Jacques TS, Taketo MM, Le Tissier P, *et al.* (2011) Increased Wingless (Wnt) signaling in pituitary progenitor/stem cells gives rise to pituitary tumors in mice and humans. *PNAS* **108** 11482–11487.

longer in human embryos than in mice embryos, pituitary cells must migrate further during morphogenesis, and migration defects are therefore more likely to occur (Larsen 2001). Indeed, developmental defects in these morphogenetic movements lead to pituitary stalk interruption syndrome in humans, which is characterised by the presence of a thin or absent pituitary stalk and is commonly associated with a hypoplastic or aplastic anterior pituitary or an ectopic posterior pituitary (e.g. a pituitary that is attached to the floor of the third ventricle, optic chiasm or median eminence; Abernethy 1998, Wang et al. 2014). Taking into account the anatomical differences in the length of the pituitary stalk as well as the more pronounced cephalic flexure (Xue et al. 2013) of human embryos, it is possible that tumour epithelial cells (e.g. clusters) could be trapped in the suprasellar region near the hypothalamus, where they could eventually give rise to a tumour with little or no sellar component at all. This may also explain the rare occurrence of CP, which can develop in the posterior fossa (Aquilina et al. 2006).

An implication derived from the embryonic mouse model is that human ACP, and childhood-onset ACP in particular, is likely to be a developmental disorder. This is based on the remarkable similarities between human and mouse clusters, not only morphologically but also at the molecular level, which suggests that they represent equivalent structures in mouse and human tumours. In fact, whole-transcriptome gene expression profiling of mouse cluster cells has revealed numerous genes/pathways that are dysregulated in mouse tumours and also in human tumours (Andoniadou et al. 2012). Analyses of mouse embryos at different stages of development have revealed that β-catenin-accumulating cell clusters with a similar gene expression profile to that of the human clusters are present in the pituitary gland of the embryonic ACP model beginning in early gestation (Fig. 2; Gaston-Massuet et al. 2011). Therefore, this suggests the possibility that CTNNB1 mutations may also occur during gestation, and clusters may develop during embryonic or fetal stages in children with ACP. This 'pre-tumoural' pituitary, which contains β-catenin-accumulating clusters, could be present in newborns before the appearance of any tumour mass, which takes longer to develop. In fact, fetal ACP was recently diagnosed in an 18-week human fetus (Kostadinov et al. 2014).

This hypothesis is supported by clinical evidence. In a retrospective analysis of 90 children diagnosed with ACP (median age at diagnosis 8.3 years), Muller et al. (2004) reported a significant decrease in height score at 10–12 months of age that persisted until the diagnosis of ACP.

The reasons that underlie these growth defects remain to be determined in humans. Of note, somatotroph differentiation is clearly disrupted in the embryonic ACP model, possibly because of the paracrine activities of the clusters and the over-activation of the Wnt/β-catenin signalling pathway, which results in a significant reduction in somatotroph numbers in the pituitary gland by the end of gestation (Fig. 5; Olson et al. 2006, Gaston-Massuet et al. 2011). It could be hypothesised that the growth delay reported by Muller et al. could be a result of growth hormone deficiency in the children that eventually develop ACP. It is tempting to speculate that the adult mouse ACP model, in which adult Sox2$^+$ stem cells are targeted to express oncogenic β-catenin, may represent the murine form of adult-onset ACP. Further characterisation of childhood vs adult ACP in mice and humans is required to explore this hypothesis.

Conclusion

Basic studies in mouse models and human tumours have provided important insights into the aetiology and pathogenesis of human ACP. The discovery of genes that are up-regulated in these aggressive tumours will expedite the development of treatments, such as using specific inhibitors that are already being used in the clinic for other human conditions against pathogenic pathways. The efficacy of these inhibitors could and should be tested in rigorous and well-designed pre-clinical studies in mouse ACP models, and their mechanisms of action should be investigated further in vivo. These studies must be carried out in order to select those drugs that are most likely to work efficiently in human clinical trials, so that great disappointments and potentially serious side effects can be avoided (Begley & Ellis 2012, Mak et al. 2014). This research will also help discover and validate biomarkers with predictive prognostic value for tumour evolution, identify patients that are likely to benefit from specific treatments, and monitor treatment efficacy. Overall, basic studies have paved the way for translational research, which is expected to result in more efficient patient management.

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