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The cAMP pathway and the control of adrenocortical development and growth

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Abstract

In the last 10 years, extensive studies showed that the cAMP pathway is deregulated in patients suffering from adrenocortical tumours, and particularly in primary pigmented nodular adrenocortical disease (PPNAD). Here we describe how evidence arising from the analysis of patients' data, mouse models and in vitro experiments, have shed light on the cAMP pathway as a central player in adrenal physiopathology. We also show how novel data generated from mouse models may point to new targets for potential therapies.

Keywords

cAMP; PKA; Adrenal; Pathology

1. Introduction

1.1. Histological and functional introduction to the adrenal gland

The adult adrenal gland is an encapsulated organ with two major compartments: the medulla, and the cortex. The medulla forms the core of the gland and is essentially composed of neuroendocrine cells, the chromaffin cells that mainly produce catecholamines (adrenaline/noradrenaline). The cortex itself is subdivided in three histologically and functionally different zones. The outer *Zona glomerulosa* (where cells are organised in glomeruli) (ZG) is the main site for production of aldosterone. Its endocrine activity is controlled by the renin/angiotensin system and is implicated in the long-term regulation of blood pressure. The intermediate *Zona fasciculata* (where cells are organised in tightly packed cords) (ZF) is responsible for producing glucocorticoids (mainly cortisol in humans) under the control of the adrenocorticotrophic hormone (ACTH). Finally, the inner cortical zone, which is in contact with the medulla, is called *Zona reticularis* (its cells form a kind of net pattern) (ZR). This zone, which is only found in higher primates, produces androgens, mainly DHEA/DHEAS, and develops in the first years of life in humans (reviewed in (Auchus, 2011)).

Whereas chromaffin cells are derived from neuroblasts that migrate to the adrenal primordium during development, all mature cortical cells originate from the foetal cortex within the adrenal (Zubair et al., 2009, 2008). This foetal cortex also gives rise to the foetal zone, a large transitory zone located in the inner cortex, that essentially produces DHEA/DHEAS during foetal life, and that progressively disappears in post-natal stages. This progressive atrophy is concomitant with the growth of the mature cortex arising from the persistent definitive/transitional zone (Spencer et al., 1999). This post-natal development and later maintenance of the cortex depends on progenitor cells that are located in the subcapsular region of the gland (King et al., 2009).

In mice, adrenal development is roughly comparable with human. However, corticosterone but not cortisol is produced from the *Z. fasciculata*. This results from absence of Cyp17 expression in adult rodent adrenals (Keeney et al., 1995). Therefore, DHEA cannot be synthesized and no *Z. reticularis* can be distinguished. The X-zone, which can be considered as the mouse foetal cortical zone, remains present after birth. It disappears through a massive wave of apoptosis during puberty in males, or during the first pregnancy in females (Beuschlein et al., 2003; Holmes and Dickson, 1971). In nulliparous females, the X-zone is maintained for several months, and eventually regresses.

1.2. Role and description of the cAMP pathway in adrenocortical function

The main role of the cAMP pathway in the adrenal is associated with control of cortisol production. MC2R, the receptor of the pituitary adrenocorticotrophic hormone (ACTH), is strongly expressed in ZF cells, and to a lower extent in ZG cells. MC2R is a G protein-coupled receptor. When activated by ACTH, it induces adenylate cyclase activity *via* Gs α , increasing the intracellular levels of the secondary messenger cAMP. In turn, this rise induces cAMP-dependent protein kinase A (PKA) activation whose targets ultimately stimulate cortisol production and release. PKA is a heterotetramer composed of two catalytic subunits (C) endowed with serine/threonine kinase activity. These are associated with a dimer of regulatory subunits (R) that are the targets of cAMP. Eight genes encode the four C subunits, C α , β , γ and the most recently characterized Prkx (Zimmermann et al., 1999), and the four R subunits, RI α and β , RII α and β (Tasken et al., 1997). In the absence of cAMP, the C kinase activity is repressed by interaction with the R subunits. Binding of cAMP molecules to the R subunits induces a conformational change and their dissociation from the PKA tetramer ultimately leading to the release of fully active C subunits (Kim et al., 2007, 2005). Phosphorylated downstream targets of PKA include the CREB protein (cAMP response element-binding), a transcriptional factor inducing transcription of genes whose products are involved in steroidogenesis, such as the steroidogenic acute regulatory protein StAR. Interestingly, StAR, which is responsible for the limiting step of cholesterol transport into the mitochondria, is also directly activated by PKA phosphorylation (Arakane et al., 1997).

Inactivation of PKA catalytic activity follows the termination of adenylate cyclase stimulation. Excess cAMP is degraded by phosphodiesterases (PDE), allowing the PKA inactive tetramer to reform.

1.3. cAMP pathway deregulation and its link to human adrenal pathologies

Cushing's syndrome is the result of excessive production and release of cortisol. The two main causes of this excessive cortisol production are ACTH secreting pituitary adenomas, or autonomous activation of the adrenal cortex itself. In the last 10 years, particular emphasis has been put on ACTH-independent Cushing's syndromes. Autonomous activation of ZF has been linked to induction of the cAMP/PKA pathway resulting from alterations occurring at different levels of signal transduction: ectopic expression of illegitimate G protein

coupled receptors, activating mutations of the *GNAS* gene (encoding G α subunit), inactivating mutations of genes encoding RI α (*PRKARIA*) or encoding phosphodiesterases (*PDE11A4* and *PDE8B*).

Adrenal overactivity has been associated with tumour formation, raising the question of a possible role of the cAMP pathway in adrenal tumourigenesis. Indeed, presence of ectopically expressed G-protein coupled receptors (Lacroix et al., 2010) or *GNAS* mutations (Fragoso et al., 2003) were found in ACTH-independent macronodular adrenal hyperplasia (AIMAH). Both *PRKARIA* mutations (Bertherat et al., 2009; Kirschner et al., 2000) and *PDE* mutations (Horvath et al., 2006a; Horvath et al., 2008) have been associated with isolated micronodular adrenocortical disease, notably with PPNAD. Loss of heterozygosity on the wild-type allele was associated with *PRKARIA* and *PDE11A4* inactivating mutations. Consequently, the corresponding wild-type proteins are absent from the tumours (Groussin et al., 2002; Horvath et al., 2006a). Strikingly, in many of the patients displaying *PRKARIA* mutations, PPNAD was detected as part of Carney complex. This pathology is characterised by tumour growth in the skin and heart but also in many endocrine glands in which the tumours induce overactivity. Moreover, altered cAMP signalling, somatic *PRKARIA* mutations and somatic losses in *PRKARIA* locus have all been reported in sporadic adrenocortical adenomas and carcinomas (Bertherat et al., 2003). These observations suggest that *PRKARIA* is a good candidate tumour-suppressor in a number of tissues, including the adrenal.

To try to demonstrate the role of the cAMP pathway members, and particularly *PRKARIA* in normal and pathological adrenal growth and development, various animal models have been developed in the last 10 years.

2. Impact of cAMP pathway activity on the adrenal: from little to plenty of activity

2.1. Loss of function of cAMP pathway in the adrenal

Two mouse models are particularly relevant to the study of the role of the PKA pathway in adrenocortical development and maintenance: *Mc2r* knock-out mice, where no ACTH-Receptor is produced by adrenocortical cells (Chida et al., 2007), and *Pomc* knock-outs, where no ACTH is produced, but MC2R is present (Coll et al., 2004; Karpac et al., 2005). Both gene inactivation strategies failed to induce adrenal defects during foetal development or the neonatal period but later induced a progressive atrophy of the ZF. Interestingly, ACTH supplementation of POMC-null mice restored adrenal size, cortex morphology and glucocorticoid secretion through a hypertrophic response of the ZF cells (Coll et al., 2004). In contrast, transplantation of *Pomc*^{-/-} adrenals in wild-type animals restored the hyperplastic response (Karpac et al., 2005). These data suggest that POMC peptides are dispensable for foetal adrenal development but are required for postnatal proliferation and maintenance of adrenal structures. Importantly, atrophy of MC2R-null adrenals was not accounted for by a decrease in the number of ZF cells (Chida et al., 2007). This could indicate that in addition to its innocuity on adrenal development, absence of ACTH-dependent activation of the cAMP pathway does not impair the establishment of a normal stock of progenitor cells. Thus, the progressive ZF atrophy could be interpreted either as a differentiation defect, where progenitors would not be recruited in the absence of cAMP signalling, or as a possible increase in ZF cells death rate that would not be compensated for by a basal recruitment of progenitors. Of note, as in the first hypothesis, the progenitors are supposed to be subcapsular cells that do not express the ACTH receptor. The observed defect should thus result from an indirect mechanism.

These mouse phenotypes reflect what is seen in human with Familial Glucocorticoid deficiency type 1, a disease mostly resulting from mutations in *MC2R* that impair its trafficking to the membrane (Chung et al., 2008). This shows that, overall, lack of cAMP pathway activity in adrenal does not induce developmental defects, but that this activity is required postnatally, to produce cortisol and to maintain the structure of the ZF.

2.2. Gain-of-function of the cAMP pathway in adrenal cells

At the other end, different models were designed to reproduce hyperactivity of the cAMP pathway in the adrenal.

One strategy was to recapitulate ectopic expression of G protein-coupled receptors (GPCRs) found in some AIMAH using xenotransplantation models of bovine adrenocortical cells, transduced with viral expression vectors (Mazzuco et al., 2006a,b). These xenografted mice developed benign tumours, indicating that ectopic expression of a single GPCR was sufficient to induce phenotypic changes leading to Cushing's syndrome and tumour transformation. However, mechanisms leading to tumour formation are difficult to assess in transplantation models and thus participation of cAMP-dependent *vs*-independent pathways in cell transformation mediated by GPCR remains to be established.

Another strategy was to decrease the degradation of cAMP by reducing phosphodiesterase activity. Both *PDE8B* and *PDE11A4* have been found mutated in some patients developing adrenal hyperplasia, in association with loss of heterozygosity at the chromosomal region containing the *PDE11A4* gene (Horvath et al., 2006a). In *Pde8b* KO mice, mild hypercortisosteronemia was detected in adults, but no tumour growth was shown. This could suggest that increased PKA activity is not sufficient to promote developmental defects or tumour growth, even though it is sufficient to induce a Cushing's syndrome. It is consistent with the human data where *PDE11A* mutations were considered as phenotype modifiers of already pre-existing conditions resulting from *PRKARIA* mutations (especially as an enhancer of phenotype in males) (Libe et al., 2011). Similarly, *PDE8B* variants seemed to predispose to adrenal defects, but were not clearly shown to promote adrenal tumour formation (Horvath et al., 2006a, 2008).

A third strategy targeted *Prkar1a*, the most promising tumour-suppressor candidate of the pathway. Total *Prkar1a* KO mice have severe heart development defects causing their death at E9.5 (Amieux et al., 2002). Haplo-insufficiency of *Prkar1a* induces tumour growth in thyroid, Schwann cells, bone, and liver but not in the adrenal. These observations showed that *Prkar1a* was a tumour-suppressor gene in many tissues, but evidence was lacking for its specific role in the adrenal (Kirschner et al., 2005; Veugelers et al., 2004). A more severe loss of RI α protein was achieved by an antisense strategy, which resulted in decreases up to 70% in some tissues (Griffin et al., 2004). Although the decrease in RI α protein in the adrenal did not reach 50%, half of the transgenic mice presented with adrenal hyperplasia. Interestingly, histological defects suggesting that foetal X-zone was maintained were observed. This indicated that some of the phenotypes could result from developmental alterations.

In order to overcome the previous technological limitations, we generated an adrenocortical-specific KO of *Prkar1a* (AdKO) (Sahut-Barnola et al., 2010). In our system, genetic ablation occurs in adrenocortical cells at E14.5 and targets all cortical subtypes in adults (Lambert-Langlais et al., 2009). This targeted KO induced the appearance of a PPNAD-like syndrome in mice, with the development of an age- and sex-dependent Cushing's syndrome (males adrenals were much less affected by the KO, which correlates with the human pathology that develops more frequently in women (Bertherat et al., 2009)), and subsequent tumour formation. Interestingly, this was correlated with the growth of a population of large cells at

the medullocortical boundary that expressed both X-zone (20 α HSD) (HersHKovitz et al., 2007) and ZF (Akr1b7) specific markers (Aigueperse et al., 2001). Centrifugal extension of this foetal-like zone coincided with atrophy of the normal ZF. A possible hypothesis for this ZF atrophy could be that continuous and autonomous production of corticosterone repressed the production of ACTH by a regulatory feedback loop. Interestingly, plasma ACTH levels were mildly decreased in this mouse model even though were not blunted (Sahut-Barnola et al., 2010). Therefore, autonomous corticosterone production could result in a “*Pomc* KO-like” or “*Mc2r* KO-like” phenotype in the ZF, indicating that in AdKO mice, cells of the “normal” ZF and/or progenitor recruitment could still be partially dependent on ACTH release by the pituitary. To elucidate the aetiology of the phenomenon resulting in ZF atrophy, expression of progenitor markers was quantified in AdKO mice. These were not altered (Sahut-Barnola et al., 2010), which was reminiscent of *Pomc/Mc2r* KO mice. We also tested potential decay of ZF cells in AdKO mice. In AdKO mice, adrenocortical cells displayed increased resistance to apoptosis (Sahut-Barnola et al., 2010). However, we detected high β -galactosidase activity in the inner ZF cells lining the hypertrophic cells of the foetal-like zone, a phenomenon that is typically associated with senescence (Fig. 1). Hence, ZF atrophy may result from abnormally fast ageing of cells in the adrenal-specific *Prkar1a* KO mouse model.

Interestingly, AdKO mouse model is globally reminiscent of the situation in PPNAD patients' tissues. In that case, the nodules that form the tumours completely lack *PRKARIA* following loss of heterozygosity. They are also made of very large cells surrounded by atrophic internodular ZF tissue, these combined defects resulting in a hyperplastic but normal-sized adrenal. Similar to AdKO cells, they express high levels of the foetal marker inhibin α , suggesting that a developmental defect could also be involved in the establishment of the pathology in human.

3. Origin of the tumour cells in the mouse adrenal cortex lacking Rla

3.1. Are tumour cells, foetal-like cells with adult markers or adult-like cells with foetal markers?

Recent studies on FAdE, the foetal adrenal enhancer of the *Sf1* gene, have shown that all adrenocortical cells, from the subcapsular region to the X-zone, initially arise from cells where the FAdE is active (Zubair et al., 2008). Then, from E14 onwards FAdE activity is maintained in the cells that will give rise to the future X-zone, but is turned off in the precursors of the definitive cortical zone. This suggests that there is not one, but that there are two different populations of progenitors, one for the X-zone, and one for the definitive adult zone.

Because of their position and the markers that they express, foetal-like cells emerging from the innermost cortex of AdKO adrenals seem to be derived not from adult progenitors, but from foetal cells. This would imply that first, there is actually a population of foetal progenitors that lies at the medulla/cortex boundary, and second, that for some reason, this population is stimulated and proliferates to produce the tumour expansion that is seen in the AdKO adrenal. Several arguments are in favour of this model.

First, in adult male mice, castration induces the appearance of a secondary X-zone (Hirokawa and Ishikawa, 1975). Given that primary X-zone cells segregate from adult cortical cells early in development (Zubair et al., 2006), it seems logical to explain this phenomenon by reactivation of a dormant progenitor population lying in the innermost cortex. Second, normal ZF cells in AdKO model seem to enter senescence (Fig. 1). It is thus unlikely that they are later recruited as precursors of the tumour cells. Third, in mice where activating mutation of β -catenin is targeted in the adrenal cortex (Δ Cat mice), tumour

growth first occurs at the juxtamedullary region and results in invasion of the medulla. Only later on does the tumour develop from the subcapsular region where adult cortical progenitors are found (Berthon et al., 2010). This would indicate that there really is a population of dormant precursors that lie in the inner cortex of the adult adrenal (Fig. 2).

3.2. How could foetal-like cells display tumour potential in AdKO mice?

Two non-mutually exclusive mechanisms can account for recruitment of foetal-like cells for tumour formation: repression of an inhibitory mechanism, which prevents their recruitment in a normal setting, or activation of their recruitment. In AdKO mice, adrenocortical cells displayed increased resistance to apoptosis, indicating that a phenomenon of protection against death signals was likely to be involved in tumour growth (Sahut-Barnola et al., 2010). Interestingly, apoptosis is the normal mechanism of clearance of cells in the X-zone (Beuschlein et al., 2003). Resistance to apoptotic signals could favour the growth of foetal-like cells in an environment that would normally be hostile for them. Resistance could be the direct result of ectopic activation of the cAMP pathway in X-zone cells. Indeed, normal X-zone cells being devoid of MC2R, they are insensitive to ACTH, the main activator of cAMP pathway in adrenal cortex. However, other pathways are likely to be involved in the tumour process.

4. Molecular mechanisms of adrenal tumour formation in mice lacking R1 α

4.1. The TGF β pathway

Many convergent data indicate that TGF β signalling could be an essential actor in the process of tumour growth in the adrenal. First, Beuschlein et al. demonstrated that activin is able to induce specific apoptosis of X-zone cells (Beuschlein et al., 2003), an observation that correlated with the mechanisms implicated in foetal zone regression in human (Spencer et al., 1999). Activin receptors and Smad2, a critical mediator of the activin/TGF β intracellular signalling, were strongly expressed in the X-zone. In a context of high incidence of adrenal tumours arising from the inner zone of the cortex, actual development of tumours negatively correlated with levels of Smad2. Interestingly, down-regulation of *PRKARIA* in H295 cells that are derived from adrenocortical cancer induced resistance to apoptosis. This has been attributed to a severe decrease in SMAD3 protein levels, SMAD3 having the same role as SMAD2 in the TGF β signalling pathway (Ragazzon et al., 2009). Finally, in AdKO mice, overexpression of inhibin α and follistatin, both inhibitory ligands of activin, was found, and correlatively, inhibin α was detected at high levels in the cells forming the tumours in PPNAD patients' tissues (Sahut-Barnola et al., 2010). Taken together, these data show that down-regulation of the gene coding the R1 α subunit of PKA decreases the activity of a pathway which is implicated in adrenal foetal zone clearance.

However, TGF β pathway downregulation could be related to the inhibition of a repressive system rather than to real promotion of tumourigenesis. At least two other pathways known to have strong relevance in tumour growth and in cancer were also found altered in PPNAD tissues.

4.2. The Wnt/ β -catenin pathway

The Wnt/ β -catenin signalling pathway is widely implicated in the control of cell proliferation and differentiation, deregulation of this pathway being found in many cancers. Targeting the early loss of *Ctnnb1* gene (encoding β -catenin) in developing adrenocortical cells induces complete failure of adrenal at birth, while lowering the number of cells with β -catenin inactivation only affects cortical cell renewal in adults (Kim et al., 2008). This nicely shows that, as opposed to cAMP/PKA pathway, Wnt/ β -catenin pathway is essential to both adrenal development and growth. Activating mutations of the *CTNNB1* gene were

frequently detected in both adrenocortical adenomas and carcinomas (Bonnet et al., 2011; Gaujoux et al., 2011; Tissier et al., 2005). This strongly argues for an oncogenic role of β -catenin in the adrenal cortex. This was definitively proven by the Δ Cat mouse model, where adrenocortical specific expression of a constitutively active β -catenin induced hyperplasia and carcinogenesis in late stages. Interestingly, *CTNNB1* gene somatic mutations have been found in adrenal tumours developing secondarily in patients with PPNAD, as well as in patients suffering from Carney complex (Gaujoux et al., 2008; Tadjine et al., 2008). This could suggest that in rare cases, cAMP pathway overactivation by favouring cell survival could also favour appearance of secondary genetic hits targeting β -catenin in order to promote growth of a more aggressive tumour. A more frequent related event is the overexpression of genes related to the Wnt/ β -catenin signalling pathway such as WNT1-inducible signalling pathway protein 2 (*WISP2*), *GSK3 β* and *CTNNB1*. These were detected in PPNAD tissues and other adrenal hyperplasia related to excessive cAMP signalling (Bourdeau et al., 2004; Horvath et al., 2006b). This regulation defect may depend on some microRNAs (Bimpaki et al., 2010; Iliopoulos et al., 2009). Overall, increased activity of cAMP/PKA signalling pathway is at the origin of adrenocortical tumour growth, and its positive effect on Wnt/ β -catenin signalling could either be the founder step of tumour growth or a way to reinforce an already existing tumour potential of adrenocortical cells.

4.3. The mTOR signalling pathway

In two papers, Mavrikakis et al., showed that mTOR is activated in cells of the nodules that lack *PRKARIA* in PPNAD tissues. In this context, R1 α seems to behave as a regulatory subunit for mTOR (Mavrikakis et al., 2006, 2007). Deregulation of mTOR pathway is found in many tumours (Grozinsky-Glasberg and Shimon, 2010; Zoncu et al., 2011). Therefore, increased mTOR activity through loss of R1 α could support adrenocortical tumour formation (Doghman et al., 2010). In this model, R1 α dependent deregulation of mTOR would be completely independent of catalytic PKA activity. The possibility that R1 α could exert activities independently of PKA is intriguing. This could account for the more penetrant tumour promoting activity of *PRKARIA* mutations compared with *PDE* mutations that would preferentially impact PKA catalytic activity (Section 2.2.). Interestingly, as in PPNAD, tumour cells in AdKO mice were hypertrophic, which is a hallmark of mTOR activation. Further analysis of the role of mTOR pathway in adrenocortical tumourigenesis is in progress in AdKO mouse model.

5. From mouse models back to human development/pathology

5.1. Prkar1a as a tumour suppressor in mice

What was suspected in human, where up to 80% of the PPNAD cases were related to *PRKARIA* inactivating mutations (Bertherat et al., 2009), has been confirmed in mice. Mice heterozygous for *Prkar1a* developed a Carney-like complex, and ablation of R1 α in adrenocortical cells induced tumourigenesis. However, mouse models show that tumour development arises from the innermost cortex, and correlates with appearance of foetal markers that were retrospectively found in human samples.

5.2. Questions about the origin of the PPNAD tumour in mouse and human

Data from AdKO mice strongly suggest the existence of a population of dormant adrenocortical progenitors in the juxtamedullary part of the cortex. The fact that in tumours from Δ Cat mice (Berthon et al., 2010) the same apparent mobilisation of inner progenitors occurs upon β -catenin activation strongly reinforces this hypothesis (Section 3.1.). More interestingly, these potential progenitors could differentiate into ectopic ZG under the influence of Wnt/ β -catenin signalling (Δ Cat model), or pseudo ZF (foetal-like zone with ZF

markers) under RI α ablation-dependent increased cAMP/PKA signalling (AdKO model). This indicates that these progenitors have plasticity in their possible fate (Fig. 2).

In human, ZR arises in the inner cortex at 5–7 years of age, in a process called the adrenarche (Havelock et al., 2004). ZR thus appears after regression of the foetal zone, but interestingly shares the same DHEA/DHEAS steroid production activity. Previous data indicated that ZR probably arises from transdifferentiation of ZF cells. The localisation of the ectopic zone in AdKO mice suggested that it could represent a pseudo-ZR. ZR is characterised by DHEA/DHEAS production that requires the presence of CYP17, but is also favoured by low 3 β HSD expression (3 β HSD competes for pregnenolone, the substrate CYP17 metabolizes into 17-hydroxypregnenolone) and high CYTB5 levels (that facilitates the 17–20 lyase activity of CYP17 to transform 17-hydroxypregnenolone into DHEA) (Dharia et al., 2004; Mapes et al., 1999; Narasaka et al., 2001; Nguyen et al., 2009). We did not detect circulating or intra-adrenal DHEA/DHEAS in AdKO mice (Sahut-Barnola personal communication). However, Cyp17 was expressed and functional in the foetal-like cells, which were indeed able to produce cortisol (Sahut-Barnola et al., 2010). Altogether these data also suggest that mouse Cyp17 may have low lyase activity. Moreover, both low 3 β HSD and high Cytb5 levels were expressed in AdKO mice when compared to WT (Fig. 3). This situation is reminiscent of both human foetal zone and human adult ZR (Dharia et al., 2004; Narasaka et al., 2001). Furthermore, these findings in mice have to be compared to recent data obtained from the study of marmoset. In this primate, no ZR can be detected in males on the basis of histological and functional criteria (Pattison et al., 2005). However, ZR as revealed by Cytb5 expression and adrenal DHEA production can be detected in adult females. Surprisingly, appearance of this ZR depends on social status: dominant ovulatory females show a faint Cytb5 staining, which is increased in anovulatory subordinate females. Ovariectomized marmosets display an even stronger and expanded staining (Pattison et al., 2007). This suggests that there is a population of “ZR progenitors” that can be mobilized in adult marmosets, depending on gender and ovulatory status. Alternatively, this expansion could also occur by a transdifferentiation of ZF cells. The mouse model could simply differ from primates or it could suggest alternative possibilities that should be considered in future studies.

5.3. How to improve mice models? A matter of microenvironment?

Mice models with strict *Prkar1a* haploinsufficiency were unable to develop ACTH-independent Cushing’s syndrome. The antisense strategy resulting in a loss of 50% of RI α protein in adrenal cells was able to promote defects in the inner cortex of the adrenal (Section 2.2.). Total *Prkar1a* knockout in the population of adrenocortical steroidogenic cells (AdKO) induced clear hyperplasia, foetal characters resurgence, gender predilection and adult cortex atrophy. However, in addition to all of these characters, other criteria found in PPNAD in human were absent in mouse models. Indeed, hallmarks of Wnt/ β -catenin pathway activation were frequent in PPNAD (Gaujoux et al., 2008; Tadjine et al., 2008), but were never observed in any of the mouse models. Another evident criterion is the nodular aspect of the dysplasia in PPNAD patients, which contrasts with the emergence of a new adrenocortical zone in mouse models. Also, the neuroendocrine differentiation of the cells composing the nodules in human (Stratakis et al., 1999) was never detected in mice. Finally, we could not show paradoxical response to dexamethasone in AdKO mice, even though this is one of the diagnostic factors in patients (Stratakis et al., 2001). Indeed, although dexamethasone should result in repression of the HPA axis and subsequent reduction in plasmatic cortisol levels, PPNAD patients respond to this treatment by increased cortisol production. This paradoxical response is attributed to overexpression and abnormal PKA-coupling of the Glucocorticoid Receptor (GR) within the nodules (Bourdeau et al., 2003; Louiset et al., 2009). These four defects were not found in any of the mouse models where

RI α levels were altered in the adrenal. However in contrast with patients, most mouse models had decreased *Prkar1a* expression but not complete loss of RI α in all the cell-types that compose the adrenal. On the contrary, the AdKO model exhibited complete loss of *Prkar1a* but this was restricted to the steroidogenic cells of the cortex. In the only model in which some loss of heterozygosity (and consequently complete knockout of *Prkar1a*) was detected in some adrenal cells, nodular organisation was observed within the hyperplastic area (Griffin et al., 2004). One hypothesis that could account for these observations is that the loss of *Prkar1a* in steroidogenic cells is directly responsible for all the phenotypes that were described in the AdKO model. Under this hypothesis, other phenomena that were detected in PPNAD, i.e. the nodular shape of the tumours, neuroendocrine differentiation, ectopic GR expression and paradoxical response to dexamethasone would be dependent on *Prkar1a* haploinsufficiency in the other cell-types residing within the adrenal. To address this question, we are currently analysing mice in which genetic ablation of RI α in steroidogenic cells is combined with haploinsufficiency in all other cell types within the adrenal. This should recreate an accurate picture of the microenvironment found in the context of the human pathology.

References

- Aigueperse C, Val P, Pacot C, Darne C, Lalli E, Sassone-Corsi P, Veyssiere G, Jean C, Martinez A. SF-1 (steroidogenic factor-1), C/EBP β (CCAAT/enhancer binding protein), and ubiquitous transcription factors NF1 (nuclear factor 1) and Sp1 (selective promoter factor 1) are required for regulation of the mouse aldose reductase-like gene (AKR1B7) expression in adrenocortical cells. *Mol. Endocrinol.* 2001; 15:93–111. [PubMed: 11145742]
- Amieux PS, Howe DG, Knickerbocker H, Lee DC, Su T, Laszlo GS, Idzerda RL, McKnight GS. Increased basal cAMP-dependent protein kinase activity inhibits the formation of mesoderm-derived structures in the developing mouse embryo. *J. Biol. Chem.* 2002; 277:27294–27304. [PubMed: 12004056]
- Arakane F, King SR, Du Y, Kallen CB, Walsh LP, Watari H, Stocco DM, Strauss JF 3rd. Phosphorylation of steroidogenic acute regulatory protein (StAR) modulates its steroidogenic activity. *J. Biol. Chem.* 1997; 272:32656–32662. [PubMed: 9405483]
- Auchus RJ. The physiology and biochemistry of adrenarche. *Endocr. Dev.* 2011; 20:20–27. [PubMed: 21164255]
- Bertherat J, Groussin L, Sandrini F, Matyakhina L, Bei T, Stergiopoulos S, Papageorgiou T, Bourdeau I, Kirschner LS, Vincent-Dejean C, Perlemoine K, Gicquel C, Bertagna X, Stratakis CA. Molecular and functional analysis of PRKAR1A and its locus (17q22–24) in sporadic adrenocortical tumors: 17q losses, somatic mutations, and protein kinase A expression and activity. *Cancer. Res.* 2003; 63:5308–5319. [PubMed: 14500362]
- Bertherat J, Horvath A, Groussin L, Grabar S, Boikos S, Cazabat L, Libe R, Rene-Corail F, Stergiopoulos S, Bourdeau I, Bei T, Clauser E, Calender A, Kirschner LS, Bertagna X, Carney JA, Stratakis CA. Mutations in regulatory subunit type 1A of cyclic adenosine 5'-monophosphate-dependent protein kinase (PRKAR1A): phenotype analysis in 353 patients and 80 different genotypes. *J. Clin. Endocrinol. Metab.* 2009; 94:2085–2091. [PubMed: 19293268]
- Berthon A, Sahut-Barnola I, Lambert-Langlais S, de Joussineau C, Damon-Soubeyrand C, Louiset E, Taketo MM, Tissier F, Bertherat J, Lefrancois-Martinez AM, Martinez A, Val P. Constitutive beta-catenin activation induces adrenal hyperplasia and promotes adrenal cancer development. *Hum. Mol. Genet.* 2010; 19:1561–1576. [PubMed: 20106872]
- Beuschlein F, Looyenga BD, Bleasdale SE, Mutch C, Bavers DL, Parlow AF, Nilson JH, Hammer GD. Activin induces x-zone apoptosis that inhibits luteinizing hormone-dependent adrenocortical tumor formation in inhibin-deficient mice. *Mol. Cell. Biol.* 2003; 23:3951–3964. [PubMed: 12748296]
- Bimpaki EI, Iliopoulos D, Moraitis A, Stratakis CA. MicroRNA signature in massive macronodular adrenocortical disease and implications for adrenocortical tumorigenesis. *Clin. Endocrinol. (Oxf).* 2010; 72:744–751. [PubMed: 19849700]

- Bonnet S, Gaujoux S, Launay P, Baudry C, Chokri I, Ragazzon B, Libe R, Rene-Corail F, Audebourg A, Vacher-Lavenu MC, Groussin L, Bertagna X, Dousset B, Bertherat J, Tissier F. Wnt/beta-catenin pathway activation in adrenocortical adenomas is frequently due to somatic CTNNB1-activating mutations, which are associated with larger and nonsecreting tumors: a study in cortisol-secreting and -nonsecreting tumors. *J. Clin. Endocrinol. Metab.* 2011; 96:E419–E426. [PubMed: 21084400]
- Bourdeau I, Antonini SR, Lacroix A, Kirschner LS, Matyakhina L, Lorang D, Libutti SK, Stratakis CA. Gene array analysis of macronodular adrenal hyperplasia confirms clinical heterogeneity and identifies several candidate genes as molecular mediators. *Oncogene.* 2004; 23:1575–1585. [PubMed: 14767469]
- Bourdeau I, Lacroix A, Schurch W, Caron P, Antakly T, Stratakis CA. Primary pigmented nodular adrenocortical disease: paradoxical responses of cortisol secretion to dexamethasone occur in vitro and are associated with increased expression of the glucocorticoid receptor. *J. Clin. Endocrinol. Metab.* 2003; 88:3931–3937. [PubMed: 12915689]
- Chida D, Nakagawa S, Nagai S, Sagara H, Katsumata H, Imaki T, Suzuki H, Mitani F, Ogishima T, Shimizu C, Kotaki H, Kakuta S, Sudo K, Koike T, Kubo M, Iwakura Y. Melanocortin 2 receptor is required for adrenal gland development, steroidogenesis, and neonatal gluconeogenesis. *Proc. Natl. Acad. Sci. USA.* 2007; 104:18205–18210. [PubMed: 17989225]
- Chung TT, Webb TR, Chan LF, Cooray SN, Metherell LA, King PJ, Chapple JP, Clark AJ. The majority of adrenocorticotropin receptor (melanocortin 2 receptor) mutations found in familial glucocorticoid deficiency type 1 lead to defective trafficking of the receptor to the cell surface. *J. Clin. Endocrinol. Metab.* 2008; 93:4948–4954. [PubMed: 18840636]
- Coll AP, Challis BG, Yeo GS, Snell K, Piper SJ, Halsall D, Thresher RR, O’Rahilly S. The effects of proopiomelanocortin deficiency on murine adrenal development and responsiveness to adrenocorticotropin. *Endocrinology.* 2004; 145:4721–4727. [PubMed: 15231703]
- Dharia S, Slane A, Jian M, Conner M, Conley AJ, Parker CR Jr. Colocalization of P450c17 and cytochrome b5 in androgen-synthesizing tissues of the human. *Biol. Reprod.* 2004; 71:83–88. [PubMed: 14985252]
- Doghman M, El Wakil A, Cardinaud B, Thomas E, Wang J, Zhao W, Peralta-Del Valle MH, Figueiredo BC, Zambetti GP, Lalli E. Regulation of insulin-like growth factor-mammalian target of rapamycin signaling by microRNA in childhood adrenocortical tumors. *Cancer Res.* 2010; 70:4666–4675. [PubMed: 20484036]
- Fragoso MC, Domenice S, Latronico AC, Martin RM, Pereira MA, Zerbini MC, Lucon AM, Mendonca BB. Cushing’s syndrome secondary to adrenocorticotropin-independent macronodular adrenocortical hyperplasia due to activating mutations of GNAS1 gene. *J. Clin. Endocrinol. Metab.* 2003; 88:2147–2151. [PubMed: 12727968]
- Gaujoux S, Grabar S, Fassnacht M, Ragazzon B, Launay P, Libe R, Chokri I, Audebourg A, Royer B, Sbiera S, Vacher-Lavenu MC, Dousset B, Bertagna X, Allolio B, Bertherat J, Tissier F. Beta-catenin activation is associated with specific clinical and pathologic characteristics and a poor outcome in adrenocortical carcinoma. *Clin. Cancer Res.* 2011; 17:328–336. [PubMed: 21088256]
- Gaujoux S, Tissier F, Groussin L, Libe R, Ragazzon B, Launay P, Audebourg A, Dousset B, Bertagna X, Bertherat J. 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.* 2008; 93:4135–4140. [PubMed: 18647815]
- Griffin KJ, Kirschner LS, Matyakhina L, Stergiopoulos SG, Robinson-White A, Lenherr SM, Weinberg FD, Claflin ES, Batista D, Bourdeau I, Voutetakis A, Sandrini F, Meoli EM, Bauer AJ, Cho-Chung YS, Bornstein SR, Carney JA, Stratakis CA. A transgenic mouse bearing an antisense construct of regulatory subunit type 1A of protein kinase A develops endocrine and other tumours: comparison with Carney complex and other PRKAR1A induced lesions. *J. Med. Genet.* 2004; 41:923–931. [PubMed: 15591278]
- Groussin L, Kirschner LS, Vincent-Dejean C, Perlemoine K, Jullian E, Delemer B, Zacharieva S, Pignatelli D, Carney JA, Luton JP, Bertagna X, Stratakis CA, Bertherat J. Molecular analysis of the cyclic AMP-dependent protein kinase A (PKA) regulatory subunit 1A (PRKAR1A) gene in patients with Carney complex and primary pigmented nodular adrenocortical disease (PPNAD) reveals novel mutations and clues for pathophysiology: augmented PKA signaling is associated

- with adrenal tumorigenesis in PPNAD. *Am. J. Hum. Genet.* 2002; 71:1433–1442. [PubMed: 12424709]
- Grozinsky-Glasberg S, Shimon I. The potential role of mTOR inhibitors in the treatment of endocrine tumors. *J. Endocrinol. Invest.* 2010; 33:276–281. [PubMed: 20503485]
- Havelock JC, Auchus RJ, Rainey WE. The rise in adrenal androgen biosynthesis: adrenarche. *Semin. Reprod. Med.* 2004; 22:337–347. [PubMed: 15635501]
- HersHKovitz L, Beuschlein F, Klammer S, Krup M, Weinstein Y. Adrenal 20alpha-hydroxysteroid dehydrogenase in the mouse catabolizes progesterone and 11-deoxycorticosterone and is restricted to the X-zone. *Endocrinology.* 2007; 148:976–988. [PubMed: 17122075]
- Hirokawa N, Ishikawa H. Electron microscopic observations on the castration-induced X zone in the adrenal cortex of male mice. *Cell Tissue Res.* 1975; 162:119–130. [PubMed: 1175217]
- Holmes PV, Dickson AD. X-zone degeneration in the adrenal glands of adult and immature female mice. *J. Anat.* 1971; 108:159–168. [PubMed: 4322581]
- Horvath A, Boikos S, Giatzakis C, Robinson-White A, Groussin L, Griffin KJ, Stein E, Levine E, Delimpasi G, Hsiao HP, Keil M, Heyerdahl S, Matyakhina L, Libe R, Fratticci A, Kirschner LS, Cramer K, Gaillard RC, Bertagna X, Carney JA, Bertherat J, Bossis I, Stratakis CA. A genome-wide scan identifies mutations in the gene encoding phosphodiesterase 11A4 (PDE11A) in individuals with adrenocortical hyperplasia. *Nat. Genet.* 2006a; 38:794–800. [PubMed: 16767104]
- Horvath A, Giatzakis C, Tsang K, Greene E, Osorio P, Boikos S, Libe R, Patronas Y, Robinson-White A, Remmers E, Bertherat J, Nesterova M, Stratakis CA. A cAMP-specific phosphodiesterase (PDE8B) that is mutated in adrenal hyperplasia is expressed widely in human and mouse tissues: a novel PDE8B isoform in human adrenal cortex. *Eur. J. Hum. Genet.* 2008; 16:1245–1253. [PubMed: 18431404]
- Horvath A, Mathyakina L, Vong Q, Baxendale V, Pang AL, Chan WY, Stratakis CA. Serial analysis of gene expression in adrenocortical hyperplasia caused by a germline PRKARIA mutation. *J. Clin. Endocrinol. Metab.* 2006b; 91:584–596. [PubMed: 16278264]
- Iliopoulos D, Bimpaki EI, Nesterova M, Stratakis CA. MicroRNA signature of primary pigmented nodular adrenocortical disease: clinical correlations and regulation of Wnt signaling. *Cancer Res.* 2009; 69:3278–3282. [PubMed: 19351815]
- Karpac J, Ostwald D, Bui S, Hunnewell P, Shankar M, Hochgeschwender U. Development, maintenance, and function of the adrenal gland in early postnatal proopiomelanocortin-null mutant mice. *Endocrinology.* 2005; 146:2555–2562. [PubMed: 15731356]
- Keeney DS, Jenkins CM, Waterman MR. Developmentally regulated expression of adrenal 17 alpha-hydroxylase cytochrome P450 in the mouse embryo. *Endocrinology.* 1995; 136:4872–4879. [PubMed: 7588219]
- Kim AC, Reuter AL, Zubair M, Else T, Serecky K, Bingham NC, Lavery GG, Parker KL, Hammer GD. Targeted disruption of beta-catenin in Sf1-expressing cells impairs development and maintenance of the adrenal cortex. *Development.* 2008; 135:2593–2602. [PubMed: 18599507]
- Kim C, Cheng CY, Saldanha SA, Taylor SS. PKA-I holoenzyme structure reveals a mechanism for cAMP-dependent activation. *Cell.* 2007; 130:1032–1043. [PubMed: 17889648]
- Kim C, Xuong NH, Taylor SS. Crystal structure of a complex between the catalytic and regulatory (RIalpha) subunits of PKA. *Science.* 2005; 307:690–696. [PubMed: 15692043]
- King P, Paul A, Laufer E. Shh signaling regulates adrenocortical development and identifies progenitors of steroidogenic lineages. *Proc. Natl. Acad. Sci. USA.* 2009; 106:21185–21190. [PubMed: 19955443]
- Kirschner LS, Carney JA, Pack SD, Taymans SE, Giatzakis C, Cho YS, Cho-Chung YS, Stratakis CA. Mutations of the gene encoding the protein kinase A type I-alpha regulatory subunit in patients with the Carney complex. *Nat. Genet.* 2000; 26:89–92. [PubMed: 10973256]
- Kirschner LS, Kusewitt DF, Matyakhina L, Towns WH 2nd, Carney JA, Westphal H, Stratakis CA. A mouse model for the Carney complex tumor syndrome develops neoplasia in cyclic AMP-responsive tissues. *Cancer Res.* 2005; 65:4506–4514. [PubMed: 15930266]
- Lacroix A, Bourdeau I, Lampron A, Mazzucco TL, Tremblay J, Hamet P. Aberrant G-protein coupled receptor expression in relation to adrenocortical overfunction. *Clin. Endocrinol. (Oxf).* 2010; 73:1–15. [PubMed: 19719763]

- Lambert-Langlais S, Val P, Guyot S, Ragazzon B, Sahut-Barnola I, De Haze A, Lefrancois-Martinez AM, Martinez A. A transgenic mouse line with specific Cre recombinase expression in the adrenal cortex. *Mol. Cell Endocrinol.* 2009; 300:197–204. [PubMed: 19041690]
- Libe R, Horvath A, Vezzosi D, Fratticci A, Coste J, Perlemoine K, Ragazzon B, Guillaud-Bataille M, Groussin L, Clauser E, Raffin-Sanson ML, Siegel J, Moran J, Drori-Herishanu L, Faucz FR, Lodish M, Nesterova M, Bertagna X, Bertherat J, Stratakis CA. Frequent phosphodiesterase 11A gene (PDE11A) defects in patients with Carney complex (CNC) caused by PRKAR1A mutations: PDE11A may contribute to adrenal and testicular tumors in CNC as a modifier of the phenotype. *J. Clin. Endocrinol. Metab.* 2011; 96:E208–E214. [PubMed: 21047926]
- Louisset E, Stratakis CA, Perraudin V, Griffin KJ, Libe R, Cabrol S, Feve B, Young J, Groussin L, Bertherat J, Lefebvre H. The paradoxical increase in cortisol secretion induced by dexamethasone in primary pigmented nodular adrenocortical disease involves a glucocorticoid receptor-mediated effect of dexamethasone on protein kinase A catalytic subunits. *J. Clin. Endocrinol. Metab.* 2009; 94:2406–2413. [PubMed: 19383776]
- Mapes S, Corbin CJ, Tarantal A, Conley A. The primate adrenal *Zona reticularis* is defined by expression of cytochrome b5, 17 α -hydroxylase/17, 20-lyase cytochrome P450 (P450c17) and NADPH-cytochrome P450 reductase (reductase) but not 3 β -hydroxysteroid dehydrogenase/ δ 5–4 isomerase (3 β -HSD). *J. Clin. Endocrinol. Metab.* 1999; 84:3382–3385. [PubMed: 10487714]
- Mavrikis M, Lippincott-Schwartz J, Stratakis CA, Bossis I. Depletion of type IA regulatory subunit (RI α) of protein kinase A (PKA) in mammalian cells and tissues activates mTOR and causes autophagic deficiency. *Hum. Mol. Genet.* 2006; 15:2962–2971. [PubMed: 16963469]
- Mavrikis M, Lippincott-Schwartz J, Stratakis CA, Bossis I. MTOR kinase and the regulatory subunit of protein kinase A (PRKAR1A) spatially and functionally interact during autophagosome maturation. *Autophagy.* 2007; 3:151–153. [PubMed: 17204847]
- Mazzuco TL, Chabre O, Feige JJ, Thomas M. Aberrant expression of human luteinizing hormone receptor by adrenocortical cells is sufficient to provoke both hyperplasia and Cushing's syndrome features. *J. Clin. Endocrinol. Metab.* 2006a; 91:196–203. [PubMed: 16249277]
- Mazzuco TL, Chabre O, Sturm N, Feige JJ, Thomas M. Ectopic expression of the gastric inhibitory polypeptide receptor gene is a sufficient genetic event to induce benign adrenocortical tumor in a xenotransplantation model. *Endocrinology.* 2006b; 147:782–790. [PubMed: 16254030]
- Narasaka T, Suzuki T, Moriya T, Sasano H. Temporal and spatial distribution of corticosteroidogenic enzymes immunoreactivity in developing human adrenal. *Mol. Cell Endocrinol.* 2001; 174:111–120. [PubMed: 11306177]
- Nguyen AD, Corbin CJ, Pattison JC, Bird IM, Conley AJ. The developmental increase in adrenocortical 17, 20-lyase activity (biochemical adrenarche) is driven primarily by increasing cytochrome b5 in neonatal rhesus macaques. *Endocrinology.* 2009; 150:1748–1756. [PubMed: 19036885]
- Pattison JC, Abbott DH, Saltzman W, Nguyen AD, Henderson G, Jing H, Pryce CR, Allen AJ, Conley AJ, Bird IM. Male marmoset monkeys express an adrenal fetal zone at birth, but not a *Zona reticularis* in adulthood. *Endocrinology.* 2005; 146:365–374. [PubMed: 15459122]
- Pattison JC, Saltzman W, Abbott DH, Hogan BK, Nguyen AD, Husen B, Einspanier A, Conley AJ, Bird IM. Gender and gonadal status differences in *Zona reticularis* expression in marmoset monkey adrenals: Cytochrome b5 localization with respect to cytochrome P450 17, 20-lyase activity. *Mol. Cell Endocrinol.* 2007; 265–266:93–101.
- Ragazzon B, Cazabat L, Rizk-Rabin M, Assie G, Groussin L, Fierrard H, Perlemoine K, Martinez A, Bertherat J. Inactivation of the Carney complex gene 1 (protein kinase A regulatory subunit 1A) inhibits SMAD3 expression and TGF β -stimulated apoptosis in adrenocortical cells. *Cancer Res.* 2009; 69:7278–7284. [PubMed: 19738044]
- Sahut-Barnola I, de Joussineau C, Val P, Lambert-Langlais S, Damon C, Lefrancois-Martinez AM, Pointud JC, Marceau G, Sapin V, Tissier F, Ragazzon B, Bertherat J, Kirschner LS, Stratakis CA, Martinez A. Cushing's syndrome and fetal features resurgence in adrenal cortex-specific Prkar1a knockout mice. *PLoS Genet.* 2010; 6:e1000980. [PubMed: 20548949]

- Spencer SJ, Mesiano S, Lee JY, Jaffe RB. Proliferation and apoptosis in the human adrenal cortex during the fetal and perinatal periods: implications for growth and remodeling. *J. Clin. Endocrinol. Metab.* 1999; 84:1110–1115. [PubMed: 10084603]
- Stratakis CA, Carney JA, Kirschner LS, Willenberg HS, Brauer S, Ehrhart-Bornstein M, Bornstein SR. Synaptophysin immunoreactivity in primary pigmented nodular adrenocortical disease: neuroendocrine properties of tumors associated with Carney complex. *J. Clin. Endocrinol. Metab.* 1999; 84:1122–1128. [PubMed: 10084605]
- Stratakis CA, Kirschner LS, Carney JA. Clinical and molecular features of the Carney complex: diagnostic criteria and recommendations for patient evaluation. *J. Clin. Endocrinol. Metab.* 2001; 86:4041–4046. [PubMed: 11549623]
- Tadjine M, Lampron A, Ouadi L, Horvath A, Stratakis CA, Bourdeau I. Detection of somatic beta-catenin mutations in primary pigmented nodular adrenocortical disease (PPNAD). *Clin. Endocrinol. (Oxf)*. 2008; 69:367–373. [PubMed: 18419788]
- Tasken K, Skalhegg BS, Tasken KA, Solberg R, Knutsen HK, Levy FO, Sandberg M, Orstavik S, Larsen T, Johansen AK, Vang T, Schrader HP, Reinton NT, Torgersen KM, Hansson V, Jahnsen T. Structure, function, and regulation of human cAMP-dependent protein kinases. *Adv. Second Messenger Phosphoprotein Res.* 1997; 31:191–204. [PubMed: 9344252]
- Tissier F, Cavard C, Groussin L, Perlempine K, Fumey G, Hagnere AM, Rene-Corail F, Jullian E, Gicquel C, Bertagna X, Vacher-Lavenu MC, Perret C, Bertherat J. 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.* 2005; 65:7622–7627. [PubMed: 16140927]
- Veugeliers M, Wilkes D, Burton K, McDermott DA, Song Y, Goldstein MM, La Perle K, Vaughan CJ, O'Hagan A, Bennett KR, Meyer BJ, Legius E, Karttunen M, Norio R, Kaariainen H, Lavyne M, Neau JP, Richter G, Kirali K, Farnsworth A, Stapleton K, Morelli P, Takanashi Y, Bamforth JS, Eitelberger F, Noszian I, Manfroi W, Powers J, Mochizuki Y, Imai T, Ko GT, Driscoll DA, Goldmuntz E, Edelberg JM, Collins A, Eccles D, Irvine AD, McKnight GS, Basson CT. Comparative PRKAR1A genotype-phenotype analyses in humans with Carney complex and prkar1a haploinsufficient mice. *Proc. Natl. Acad. Sci. USA.* 2004; 101:14222–14227. [PubMed: 15371594]
- Zimmermann B, Chiorini JA, Ma Y, Kotin RM, Herberg FW. PrKX is a novel catalytic subunit of the cAMP-dependent protein kinase regulated by the regulatory subunit type I. *J. Biol. Chem.* 1999; 274:5370–5378. [PubMed: 10026146]
- Zoncu R, Efeyan A, Sabatini DM. MTOR: from growth signal integration to cancer, diabetes and ageing. *Nat. Rev. Mol. Cell Biol.* 2011; 12:21–35. [PubMed: 21157483]
- Zubair M, Ishihara S, Oka S, Okumura K, Morohashi K. Two-step regulation of Ad4BP/SF-1 gene transcription during fetal adrenal development: initiation by a Hox-Pbx1-Prep1 complex and maintenance via autoregulation by Ad4BP/SF-1. *Mol. Cell Biol.* 2006; 26:4111–4121. [PubMed: 16705164]
- Zubair M, Oka S, Parker KL, Morohashi K. Transgenic expression of Ad4BP/SF-1 in fetal adrenal progenitor cells leads to ectopic adrenal formation. *Mol. Endocrinol.* 2009; 23:1657–1667. [PubMed: 19628584]
- Zubair M, Parker KL, Morohashi K. Developmental links between the fetal and adult zones of the adrenal cortex revealed by lineage tracing. *Mol. Cell Biol.* 2008; 28:7030–7040. [PubMed: 18809574]

Fig. 1. Senescence-associated β -galactosidase staining in adrenals from 14-month-old females in WT and AdKO mice. C, cortex; XL, X-like zone; M, medulla; HE, β bars, 50 μ m.

Fig. 2.

Dysregulation of PKA-Wnt signalling pathways influences zonal identities and promotes tumours. Loss of $R1\alpha$ in adrenocortical cells (AdKO) leads to the resurgence, the expansion and the centrifugal differentiation of foetal-like cells that acquire *fasciculata* markers, concomitantly to an atrophy of *fasciculata* cells in the adult cortex. AdKO mice develop morbid hyperplasia causing ACTH-independent Cushing syndrome reminiscent of PPnAD in human (Sahut-Barnola et al., 2010). Constitutive activation of β -catenin in adrenocortical cells (Δ Cat) leads to expansion of tumours that originate from both the outer cortex and the medullo-cortical boundary. There, tumourigenic cells expand centripetally and differentiate into ectopic *glomerulosa* to develop into Conn adenomas, at the expense of both *fasciculata* cells and medulla. Eventually, these benign adenomas may progress into carcinomas (Berthon et al., 2010). P, adult cortex progenitors; P?, hypothetical foetal cortex progenitors; Ca, capsule; G, Zona glomerulosa; F, Zona fasciculata; X, X-zone

Fig. 3.

Cytb5 and 3β HSD expression in WT and AdKO adrenals. A–B, Immunodetection of 3β HSD (A) and *Cytb5* (B) in female adrenals. In 18-month-old AdKO mice, full expansion of foetal-like cortex (low 3β HSD staining) is revealed. Remains of the atrophic adult cortex, displaying higher 3β HSD staining, is delineated by dotted lines. C, Quantitative representation (RT-qPCR) of *Cytb5* mRNA levels in adrenals of 12-month-old females. D, Levels of *Cytb5* and 3β HSD were quantified by western blotting in adrenals of 12-month-old females. ** $P < 0.01$. C, cortex; X_L, X-like zone; M, medulla; HE, haematoxylin and eosin; Scale bars, 50 μ m.