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How does cAMP/Protein kinase A signaling lead to tumors in the adrenal cortex and other tissues?

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Abstract

The overwhelming majority of benign lesions of the adrenal cortex leading to Cushing syndrome are linked to one or another abnormality of the cAMP signaling pathway. A small number of both massive macronodular adrenocortical disease and cortisol-producing adenomas harbor somatic GNAS mutations. Micronodular adrenocortical hyperplasias are either pigmented (the classic form being that of primary pigmented nodular adrenocortical disease) or non-pigmented; Micronodular adrenocortical hyperplasias can be seen in the context of other conditions or isolated; for example, primary pigmented nodular adrenocortical disease usually occurs in the context of Carney complex, but isolated primary pigmented nodular adrenocortical disease has also been described. Both Carney complex and isolated primary pigmented nodular adrenocortical disease are caused by germline *PRKARIA* mutations; somatic mutations of this gene that regulates cAMP-dependent protein kinase are also found in cortisol-producing adenomas, and abnormalities of PKA are present in most cases of massive macronodular adrenocortical disease. Micronodular adrenocortical hyperplasias and some cortisol-producing adenomas are associated with phosphodiesterase 11A and 8B defects, coded, respectively, by the *PDE11A* and *PDE8B* genes. Mouse models of *Prkar1a* deficiency also show that increased cAMP signaling leads to tumors in adrenal cortex and other tissues. In this review, we summarize all recent data from ours and other laboratories, supporting the view that Wnt-signaling acts as an important mediator of tumorigenicity induced by abnormal PRKARIA function and aberrant cAMP signaling.

Keywords

adrenal cortex; protein kinase A; primary pigmented nodular adrenocortical disease; ACTH-independent macronodular adrenal hyperplasia; adrenal tumor; phosphodiesterase; Wnt signaling

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1.0 – Introduction

Cyclic adenosine monophosphate (cAMP) is produced following activation of adenylate cyclase downstream of G protein-coupled receptors. cAMP-dependent protein kinase (PKA), a serine/threonine kinase, is the main mediator of cAMP signaling, a ubiquitous signaling pathway that is conserved in all eukaryotes (Bossis and Stratakis, 2004). The PKA holoenzyme is a hetero-tetramer composed of two regulatory subunits (usually, but not always, identical), each bound to one catalytic subunit (Bossis and Stratakis, 2004; Skälhegg and Tasken, 2000). Four major regulatory (RI α , RI β , RII α , and RII β) and four catalytic (C α , C β , C γ and Prkx) subunits have been identified; type I PKA contains either regulatory subunit RI α or RI β in its structure; type II PKA contains either regulatory subunit RII α or RII β (Gamm et al., 1996; McKnight et al., 1988; Uhler and McKnight, 1987).

To bind and inhibit the catalytic subunit, RI α undergoes a dramatic conformational change in which the two cAMP-binding domains uncouple and wrap around the large lobe of the catalytic subunit (Kim et al., 2007). cAMP binding to the RI α subunits of PKA releases the catalytic subunits, which allows them to phosphorylate cytoplasmic targets and/or cAMP response element-binding (CREB) protein, resulting in activation of DNA transcription of cAMP-responsive element-containing genes (Bossis et al., 2004; Pearce et al., 2010). PKA system has a substantial capacity of self-regulation; over-expression of C α or C β in cell culture results in significant compensation by an increase in RI α protein (Uhler and McKnight, 1987). The cellular localization of PKA has a pivotal role in determining which substrates are phosphorylated and it is controlled by the multidomain scaffolding proteins known as A-kinase anchor proteins, which bind to the R subunits of PKA hetero-tetramers (Wong and Scott, 2004).

2.0 – Abnormalities of cAMP/PKA signaling in adrenal hyperplasias and tumors

PKA signaling has been extensively studied for many years, but only recently abnormalities of the PKA signaling pathway have been linked to tumor formation in endocrine tissues. This association was initially observed in McCune–Albright syndrome, which is caused by activating mutations in Gs α or GNAS (the product of the Gsp oncogene). GNAS activating mutations lead to constitutive stimulation of adenylate cyclase and PKA activation, and a variety of manifestations, including the classic triad of polyostotic fibrous dysplasia, café au lait skin pigmentation, and autonomous endocrine hyperfunction (Weinstein et al., 1991). The most frequent affected endocrine tissues are pituitary, ovarian and thyroid, but bilateral macronodular adrenocortical hyperplasia can also be found in the context of MAS (Lee et al., 1986; Stratakis and Kirschner, 1998). These and other molecular findings discussed below demonstrate the strong biological relevance of PKA activation in adrenal tumorigenesis.

The first demonstration of PKA involvement with human disease was the finding that inactivating mutations of the *PRKARIA* gene coding for the 1- α regulatory (RI α) subunit of protein kinase A (PKA) are responsible for Carney complex (CNC) in the majority of patients (Kirschner et al., 2000a; Kirschner et al., 2000b). CNC is a multiple neoplasia syndrome that is inherited in an autosomal dominant manner and is characterized by several types of skin tumors and pigmented lesions, myxomas, schwannomas, liver and other cancers, and endocrine neoplasms (Carney et al., 1985; Stratakis et al., 2001). In a recent review of 353 patients with CNC from 185 families, patients from all ethnicities and with a wide spectrum of clinical manifestations were described (Bertherat et al., 2009; Horvath et al., 2010; Stratakis et al., 2001). *PRKARIA* defects were found in 73% of these patients. Primary pigmented nodular adrenocortical disease (PPNAD) was the most common

endocrine tumor associated with CNC, occurring in 60% of the CNC patients (Bertherat et al., 2009). Isolated PPNAD was the only manifestation in 12% of patients carrying *PRKARIA* defects.

Among the remaining kindreds with micronodular adrenocortical hyperplasia (MAH) and no evidence of *PRKARIA* mutation, subgroups of patients were identified by clinical and histopathological criteria (Table 1) (Stratakis, 2009). Genetic defects in cAMP-binding phosphodiesterases (PDEs) have been described in isolated MAH. Five different *PDE11A* mutations were identified so far in patients with isolated MAH or PPNAD; three of them resulted in premature stop codon generation and the other two were single base substitutions in the catalytic domain of the protein, significantly affecting the ability of PDE11A to degrade cAMP *in vitro* (Horvath et al., 2006a; Horvath et al., 2006b). Two missense *PDE11A* substitutions, R804H and R867G, were also more frequent among patients with sporadic adrenocortical tumors (Horvath et al., 2006b). In addition, the chromosomal locus harboring the gene encoding phosphodiesterase 8B (*PDE8B*) was the second most likely region to be associated with a predisposition to isolated MAH (Horvath et al., 2006a). Sequencing of the *PDE8B*-coding regions identified a single base substitution (c.914A>T, p.H305P) in a young girl with Cushing syndrome (CS), which impaired the ability of the mutant protein to degrade cAMP (Horvath et al., 2008).

Adrenocorticotrophic hormone (ACTH)-independent macronodular adrenal hyperplasia, also known as massive macronodular adrenocortical disease is a rare cause of CS (Lacroix, 2009; Zhang et al., 2009). Cortisol production in ACTH-independent macronodular adrenal hyperplasia can be regulated by the aberrant expression of G-protein-coupled receptors others than ACTH (Lacroix et al., 2004; Lacroix et al., 2001). Somatic losses of the 17q22–24 region and PKA activity changes demonstrated that cyclic (c) AMP/protein kinase A (PKA) signaling is altered in ACTH-independent macronodular adrenal hyperplasia similarly to adrenal tumors with 17q losses or *PRKARIA* mutations (Bertherat et al., 2003; Bourdeau et al., 2006). Even more interesting was the finding that common adrenal lesions (i.e. adrenal adenomas) that did not harbor germline or somatic *GNAS*, *PRKARIA*, *PDE11A*, and *PDE8B* mutations and were associated with ACTH-independent CS had functional abnormalities of the cAMP signaling pathway, as shown by increased cAMP levels, decreased total PDE activity and/or increased PKA activity (Bimpaki et al., 2009b).

3.0 – PKA tumorigenicity: Evidence from mouse models and *in vitro* studies

3.1 – Mouse models of Carney complex

To characterize the basis for *PRKARIA*-associated tumorigenesis in an animal model, we generated mice carrying a floxed copy of exon 2 of the murine *Prkar1a* gene (Kirschner et al., 2005). *Prkar1a* haploinsufficiency in mice led to the development of tumors arising in cAMP-responsive tissues, such as the bone, Schwann and thyroid follicular cells. *Prkar1a*^{+/-} mice developed non-pigmented schwannomas and fibro-osseous bone lesions beginning around 6 months of age. Benign and malignant thyroid neoplasias were observed in 10% of older mice (Kirschner et al., 2005). Although the spectrum of tumors overlapped with what is seen in CNC patients, this mouse model did not present some of the most frequent CNC tumors, such as skin and heart myxomas, and pituitary adenomas (Kirschner et al., 2005).

A different mouse model that led to significantly higher *Prkar1a* down-regulation and consequently augmented cAMP signaling, produced a more severe phenotype with a significant decrease in the overall life-span (Griffin et al., 2004a; Griffin et al., 2004b). This transgenic mice carrying an antisense transgene for *Prkar1a* exon 2 (X2AS) under the control of a tetracycline responsive promoter developed thyroid follicular hyperplasia and

adenomas, adrenocortical hyperplasia and other CS features, including late onset weight gain and visceral adiposity (Griffin et al., 2004b). Then, a decrease in *Prkar1a* activity more than simple haploinsufficiency could generate a phenotype closer to CNC in various tissues.

Indeed, adrenal-cortex specific *Prkar1a* knockout mice induced autonomous adrenal hyperactivity and bilateral hyperplasia, both observed in human PPNAD, apparently caused by proliferation of cortisol-producing fetal adrenocortical cells induced by PKA dysregulation (Sahut-Barnola et al., 2010).

3.2 – Studies of cell lines with R1 α defects

The majority of human *PRKARIA* mutations result in premature stop codon caused by nonsense and frameshift changes. Mutant mRNAs containing a premature stop codon are unstable and undergo nonsense-mediated mRNA decay (Kirschner et al., 2000b; Robinson-White et al., 2003). Human and mouse studies showed that R1 α haploinsufficiency predisposed to tumor development (Kirschner et al., 2000a; Kirschner et al., 2005). R1 α -haploinsufficient cells had high basal and cAMP-stimulated PKA activity, activation of MAPK and mTOR pathways, and increased cell proliferation and survival (Mavrakis et al., 2006; Robinson-White et al., 2003; Robinson-White et al., 2006b). B-raf kinase, p-MEK1/2 and p-c-Myc overexpression were found in mutant-PRKAR1A lymphocytes (Robinson-White et al., 2006a).

Dysregulation of cyclins and E2F1 were key changes in the process of immortalization of *Prkar1a*^{-/-} mouse embryonic fibroblasts (Nadella and Kirschner, 2005) and transformed human adrenal *PRKARIA*-haploinsufficient cells (Nesterova et al., 2008). Up-regulation of D-type cyclins occurs independently of other pathways known to increase cyclin D levels (Nadella and Kirschner, 2005). Cyclin D1 can be directly phosphorylated by PKA and has three potential PKA phosphorylation sites (Sewing and Muller, 1994). In human adrenal cells harboring a *PRKARIA*-inactivating mutation, treatment with E2F1 siRNA led to a significant decrease in proliferation, whereas E2F4 down-regulation had no effect (Nesterova et al., 2008). Furthermore, it was demonstrated that inactivating mutations of *PRKARIA* observed in adrenocortical tumors affect SMAD3 function and inhibits apoptosis induced by TGF β (Ragazzon et al., 2009).

The first *PRKARIA* mutation associated with CNC and leading to an expressed protein, R1 α Δ 184-236, was found to increase CREB-phosphorylation in transfected cells (Groussin et al., 2002). This mutation was previously investigated in detail (Meoli et al., 2008). The functionality of seven missense (and, thus, expressed) substitutions in *PRKARIA* were also investigated (Greene et al., 2008). These missense *PRKARIA* defects (c.26G>A, c.178_348del171, c.220C>T, c.438A>T, c.547G>T, c.638C>A, c.865G>T), whose mRNAs escaped nonsense-mediated mRNA decay, increased PKA activity and PKA-specific activation due to decreased binding of R1 α to cAMP or the catalytic subunit, as well as conformational changes that prevented normal PKA function (Greene et al., 2008). Interestingly, patients with these expressed mutant R1 α proteins had generally a more severe and aggressive CNC phenotype (Bertherat et al., 2009; Greene et al., 2008; Groussin et al., 2002; Horvath et al., 2010; Meoli et al., 2008).

3.3 – *Prkar1a* haploinsufficiency as a generic tumorigenic signal

Mouse models such as those for neurofibromatosis type 1 and Peutz-Jeghers syndrome, diseases that are similar to CNC, failed to reproduce exactly or with the same intensity the respective human phenotypes until the mouse gene was knocked out in the *Trp53*^{+/-} background (Cichowski et al., 1999; Takeda et al., 2006; Vogel et al., 1999), even in the absence of any known involvement of p53 in the human tumors associated with these

diseases. The *Prkar1a* mouse model and *in vitro* studies demonstrated the significance of increased cAMP and/or PKA signaling to cause tumors and indicated that additional factors may be acting synergistically with *Prkar1a* haploinsufficiency (Kirschner et al., 2005; Nadella and Kirschner, 2005; Nesterova et al., 2008; Robinson-White et al., 2006b). In this context, we have recently investigated *Prkar1a* haploinsufficiency in the background of *Trp53* or *Rb1* haploinsufficiency (Almeida et al., 2010a). *Trp53*^{+/-} mice developed sarcomas in bone, a tissue frequently affected by *Prkar1a* haploinsufficiency in mice (Kirschner et al., 2005). Interestingly, *Prkar1a*^{+/-} *Trp53*^{+/-} mice exhibited significantly decreased survival and developed significantly more sarcomas than *Trp53*^{+/-} mice (Almeida et al., 2010a).

As stated above, the cell cycle factor E2F1 mediated proliferative effects of defective R1 α (Nesterova et al., 2008). E2F1 is a downstream effector of Rb and controls cell cycle progression (Johnson et al., 1993; Qin et al., 1994). *Rb1*^{+/-} mice developed intermediate lobe pituitary adenomas and thyroid C-cell hyperplasia or adenomas at high frequency (Jacks et al., 1992). Interestingly, *Rb1*^{+/-} *Prkar1a*^{+/-} mice exhibited overall reduced survival when compared to *Rb1*^{+/-} and *Prkar1a*^{+/-} mice. *Prkar1a*^{+/-} *Rb1*^{+/-} mice also developed more pituitary tumors and medullary thyroid carcinomas than *Rb1*^{+/-} mice (Almeida et al., 2010a) (Fig. 1).

Patients with CNC present skin pigmented lesions, myxomas, collagenomas and fibromas (Carney et al., 1985; Stratakis et al., 2001), but CNC mouse models did not lead to any skin tumors (Griffin et al., 2004a; Griffin et al., 2004b; Kirschner et al., 2005). However, *Prkar1a*^{+/-} mice developed more skin papillomas than wild-type animals after treatment with a skin carcinogenesis protocol (Fig. 2) (Almeida et al., 2010a). In these 3 different mouse models, *Prkar1a* haploinsufficiency did not affect the pattern of tumors, but it only increased their number and/or size. Therefore, *Prkar1a* haploinsufficiency act as a generic but relatively weak tumorigenic signal, which can cooperate with other tumor suppressors or tissue-specific factors or even chemicals to induce tumors.

3.4 – Effects of cAMP/PKA signaling dysregulation in bone

CNC patients also develop osteochondromyxomas, a congenital tumor microscopically characterized by benign-appearing polymorphic cells with few division figures arranged in sheets and lobules set in a myxomatous, cartilaginous, osseous, and hyaline fibrous matrix (Carney et al., 2001). Human osteochondromyxomas usually erode bone, infiltrating bony trabeculae and surrounding soft tissue (Carney et al., 2001). *Prkar1a*^{+/-} mice also developed bone lesions that were derived from cAMP-responsive osteogenic cells. Osteomyxomas in the caudal vertebrae were observed in 80% of the *Prkar1a*^{+/-} mice by 1 yr of age (Kirschner et al., 2005; Pavel et al., 2008).

Dysregulation of the catalytic subunits appears to be the most important mechanism for an increase in cAMP-responsive and total PKA activity detected in R1 α - haploinsufficient cells (Meoli et al., 2008). Indeed, our previous studies showed that *Prkaca* haploinsufficiency (*Prkaca*^{+/-}) abrogated the development of thyroid tumors and schwannomas in *Prkar1a*^{+/-} mice (Tsang et al., 2010). However, *Prkar1a*^{+/-} *Prkaca*^{+/-} mice developed more bone tumors, also starting at earlier ages and with a more aggressive behavior, occasionally infiltrating the periosteum (Tsang et al., 2010). Osteochondromyxomas, histologically similar to those bone tumors associated with CNC (Carney et al., 2001), were evidenced in 13% of *Prkar1a*^{+/-} *Prkaca*^{+/-} mice. Cartilaginous metaplasia, chondromas, osteochondromyxomas and osteochondrodysplasia were observed in marrow cavities of up to 1/3 of the long bones (femur, tibia) and in most of the vertebral bodies (up to 23% of the spinal column and 100% of the caudal vertebrae) of *Prkar1a*^{+/-} *Prkaca*^{+/-} mice (Tsang et al., 2010).

Tumor cells from *Prkar1a*^{+/-} *Prkaca*^{+/-} mice expressed early osteogenic and bone stromal cell markers, showed higher cAMP levels and PKA activity, that was mostly type II mediated, as well as an alternate pattern of catalytic subunit expression (Tsang et al., 2010). In addition, increased PKA led to inflammation-independent activation of caspase-1 via over-expression of the proto-oncogene (and early osteoblast factor) Ets-1 (Almeida et al., 2010b). High prostaglandin E2 levels in cells with defective PKA regulation increased cAMP levels and in turn activated Wnt signaling, like in other states of inappropriate PKA activity (Almeida et al., 2010b). Recently, *Prkar1a* gene deletion was pointed as a recurrent genetic trait for a molecularly distinct subclass of mouse osteosarcoma (Molyneux et al., 2010).

Taken together these data indicated the pivotal role of PKA for bone tumor formation. These data also pointed to the fact that *Prkar1a* deficiency in at least two tissues (bone and the adrenal cortex) leads to the proliferation of cells of fetal origin (Molyneux et al., 2010; Sahut-Barnola et al., 2010; Tsang et al., 2010). This is an important observation, as we consider the role of Wnt signaling in *Prkar1a*-defective cells.

4.0 – Wnt signaling as the ultimate effector of PKA activation

The Wnt signaling pathway is highly conserved in eukaryotes and controls cell proliferation and differentiation. Wnt signaling has also been implicated in the regulation of human embryonic stem cells. β -catenin functions as a transcriptional activator in conjunction with lymphoid enhancer factor/T-cell factor DNA binding proteins (Brembeck et al., 2006; Klaus and Birchmeier, 2008; Polakis, 2000). Mutations in members of the Wnt- β -catenin pathway occur in approximately 90% of colorectal cancers as well as in other cancer types, such as hepatocellular carcinomas or gastric cancers (Cottrell et al., 1992; Polakis, 2000; Powell et al., 1992). Somatic activating mutations of the *CTNNB1* gene are among the most frequent genetic defects identified both in adrenocortical adenomas and carcinomas (Tissier et al., 2005). In addition, somatic mutations of the *CTNNB1* gene have been found in adrenal tumors from patients with PPNAD, Carney complex and germline *PRKARIA*-inactivating mutations (Gaujoux et al., 2008; Tadjine et al., 2008).

Glycogen synthase kinase 3 β is a downstream target of cAMP/PKA signaling in steroidogenic cells (Roy et al., 2009). cAMP signaling via PKA and its target transcription factor CREB are required for Wnt-directed myogenic gene expression (Chen et al., 2005). Expression studies in PPNAD and ACTH-independent macronodular adrenal hyperplasias, a disorder associated with excess cAMP signaling (Hsiao et al., 2009), demonstrated the over-expression of genes that regulate or are part of the Wnt signaling pathway such as WNT1-inducible signaling pathway protein 2 (*WISP2*), *GSK3 β* and *CTNNB1* (Bourdeau et al., 2004; Horvath et al., 2006c) (Fig. 3).

A whole-genome transcriptome profiling of tumors produced by mouse models of *Prkar1a* haploinsufficiency identified Wnt signaling as the main pathway activated by abnormal cAMP signaling, along with cell cycle abnormalities (Almeida et al., 2010a). A significant enrichment of Wnt signaling pathway was found in *Prkar1a*^{+/-} *Trp53*^{+/-} sarcomas, *Prkar1a*^{+/-} *Rb1*^{+/-} pituitary and thyroid tumors, as well as in *Prkar1a*^{+/-} papillomas (Fig. 1 and 2). In addition, siRNA down-regulation of *Ctnnb1* and *WNT3* inhibited proliferation of human PPNAD cells and *Prkar1a*^{+/-} mouse embryonic fibroblasts, and arrested both cell lines at the G0/G1 phase of the cell cycle (Almeida et al., 2010a).

Recently, a microRNA profile analysis showed that cAMP and/or PKA via microRNA regulation affects the Wnt signaling pathway in both PPNAD (Iliopoulos et al., 2009) and ACTH-independent macronodular adrenal hyperplasia (Bimpaki et al., 2009a). miR-449 was one of the highest down-regulated microRNAs in PPNAD and is predicted to regulate

WISP2. Pharmacologic inhibition of PKA resulted in the up-regulation of miR-449 leading to the suppression of WISP2, demonstrating that miR-449 directly regulates WISP2 expression in PPNAD cells (Iliopoulos et al., 2009).

Up-regulation of *Wnt3*, *Wnt3a*, *Wnt7a*, *Wnt8a* and *Wnt8b* was also identified in bone tumors from *Prkar1a*^{+/-} *Prkaca*^{+/-} mice (Tsang et al., 2010). Bone lesions also showed down-regulation of Wnt signaling inhibitors, such as *Dkk1*. Activation of Wnt signaling was also demonstrated in other mouse model associated with cAMP/PKA dysregulation (Wilson et al., 2010). The *GNAS* R201C mutation in mice induced over-expression of Wnt and MAPK pathway target genes (Wilson et al., 2010).

5.0 – Concluding remarks

Aberrations of cAMP/PKA signaling are essential in the pathogenesis of benign cortisol-producing lesions of the adrenal cortex. *RIα* deficiency causes an increase in total cAMP-stimulated kinase activity and enhances other oncogenic pathways, such as MAPK and mTOR. In mouse models, *Prkar1a* haploinsufficiency acts as generic, synergistic and relatively weak tumorigenic signal that cooperate with other tumor suppressor gene defects or chemicals to induce tumors. Studies from our group and others also show the importance of PKA for bone and adrenal tumor development. Recent evidence suggests that the *Wnt* signaling pathway is an important mediator of defects or proliferation related to increased cAMP and/or PKA signaling and *R1α* haploinsufficiency (Fig. 3). Interestingly, it appears, at least in mice, that *Wnt*-signaling activation by abnormal PKA function leads to tumor formation from a particular population of tissue-specific cells that retain certain fetal characteristics and some degree of pluripotentiality.

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Abbreviations

cAMP	Cyclic adenosine monophosphate
CNC	Carney complex
CS	Cushing syndrome
CREB	cAMP response element-binding
MAH	micronodular adrenocortical hyperplasia
PDE	phosphodiesterases

PKA	protein kinase A
PPNAD	primary pigmented nodular adrenocortical disease
RIα	1- α regulatory

Figure 1. Pituitary (A) and thyroid (B) gland tumors in *Prkar1a*^{+/-} *Rb1*^{+/-} mice. *Prkar1a*^{+/-} *Rb1*^{+/-} mice also developed more pituitary tumors and medullary thyroid carcinomas than *Rb1*^{+/-} mice. Over-expression of β -catenin in pituitary (C) and thyroid (D) tumors from *Prkar1a*^{+/-} *Rb1*^{+/-} mice.

Figure 2.

A. *Prkar1a*^{+/-} mice developed significantly more skin papillomas than wild-type animals after treatment with a skin carcinogenesis protocol. **B.** Strong staining of Lrp5 and β-catenin in papillomas from *Prkar1a*^{+/-} mice.

Figure 3.

Schematic representation of Wnt signaling pathway activation in R1 α deficient tumor cells. Wnt signaling enrichment was demonstrated in human and mouse tumors associated with cAMP/PKA activation. Up-regulation of WNT3, WNT3A, WNT7 and WISP2 induces the activation of the β -catenin-dependent signaling (canonical pathway). Binding of Wnt ligands to the frizzled, LRP5, and LRP6 receptors inhibits the degradation of β -catenin cytoplasmic complex and leads to nuclear accumulation of β -catenin. *CTNNB1* mutations in adrenal tumors also promote β -catenin stabilization. miR-449 is one of the highest down-regulated microRNAs in PPNAD and regulates *WISP2* expression. WNT, Wingless-type MMTV integration site family; DKK, Dickkopf; WISP2, WNT1-inducible signaling pathway protein 2; LRP5, low-density lipoprotein receptor-related protein 5; DVL, Dishevelled; APC, activated protein C; GSK3 β , glycogen synthase kinase 3 beta; LEF/TCF, lymphoid enhancer factor/T-cell factor; PKA, protein kinase A; PPNAD, primary pigmented nodular adrenocortical disease.

Table 1
Types of micronodular adrenocortical disease/hyperplasia (associated with multiple adrenocortical nodules, mostly less than 1cm in diameter)

Disease	Epidemiology	Description	Genes
iPPNAD	Children and young adults	Microadenomatous hyperplasia with at least some pigment	<i>PRKARIA</i> , <i>PDE11A</i> , <i>PDE8B</i> , 2p16, others
cPPNAD	Children, young and middle aged adults with CNC	Microadenomatous hyperplasia with pigmentation and (mostly) internodular atrophy	<i>PRKARIA</i> , 2p16, others
MAH	Mostly children and young adults	Microadenomatous hyperplasia with internodular hyperplasia and limited or absent pigment	<i>PDE11A</i> , 2p12-p16, 5q, others

CNC, Carney complex; iPPNAD, isolated primary pigmented nodular adrenocortical disease; C-PPNAD, primary pigmented nodular adrenocortical disease associated with Carney complex; MAH, micronodular adrenal hyperplasia; PDE = phosphodiesterase; *PDE11A* = the gene for PDE11A; *PDE8B* = the gene for PDE8B. *PRKARIA* = protein kinase A regulatory subunit 1α.