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Protein kinase A defects and cortisol-producing adrenal tumors

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Abstract

Purpose of review—Cushing syndrome caused by cortisol-producing adrenal adenomas is a rare condition, associated with high morbidity due to weight gain, diabetes mellitus, osteoporosis, hypertension, muscle weakness, mood disturbance, etc. The first gene to be identified as causative of Cushing syndrome was PRKAR1A. We present an update on protein kinase A (PKA) defects and Cushing syndrome.

Recent findings—The cyclic AMP-dependent PKA catalytic subunit alpha (*PRKACA*) hotspot point mutation (c.617A>C [p.Leu206Arg]), leading to an increase of basal protein kinase A (PKA) activity, and formation of cortisol-producing adenoma has been frequently shown to cause the most common form of Adrenocorticotropic hormone-independent Cushing syndrome.

Summary—Somatic *PRKACA* mutations have been found in up to 50% of patients with adrenal adenomas. Germline *PRKACA* amplification was also seen in bilateral adrenal hyperplasias. *PRKACA* activation was associated with higher cortisol levels, smaller tumor size and overt Cushing syndrome. This breakthrough is expected to improve our understanding of how PKA defects lead to Cushing syndrome and may spearhead the development of new, molecularly designed therapies.

Keywords

PRKAR1A; PRKACA; PRKACB; PKA; Cushing syndrome; cortisol-producing adrenal adenoma; adrenal tumor

Introduction

Adrenal tumors are found increasingly more commonly due to the number of imaging studies obtained in the general population [1]. A small fraction of these tumors are producing hormones. Endogenous overproduction of cortisol by the adrenal adenomas may cause Cushing syndrome, associated with significant morbidity [2]. Adrenal Cushing syndrome is often missed, given that some of the patients have a subclinical or even cyclical

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Conflicts of interest

The authors have no conflicts of interest.

Cushing syndrome. Overt Cushing syndrome is diagnosed in patients with significant metabolic abnormalities, such as weight gain, diabetes mellitus, hypertension, osteoporosis, etc.

Genetic mutations, causing cortisol-producing tumors have been suspected for years, but only few genetic defects had been discovered until recently. Patients with McCune-Albright syndrome have somatic mutations in Guanine Nucleotide Binding Protein, Alpha Stimulating (GNAS)1 in their adrenal glands causing cortisol overproduction due to a unique form of bilateral adrenocortical hyperplasia (BAH) or single adenomas. Cushing syndrome caused by primary pigmented nodular adrenocortical disease (PPNAD) is due to mutations in cyclic AMP (cAMP)-dependent protein kinase (PKA) type 1 alpha regulatory subunit (*PRKAR1A)* [3]. Recently, mutations in the armadillo repeat containing 5 *(ARMC5),* a tumor-suppressor gene, were found to be the cause of primary macronodular adrenal hyperplasia (PMAH), formerly known as Adrenocorticotropic hormone-independent macronodular adrenal hyperplasia (AIMAH) [4-8]. Finally, phosphodiestarases (PDE) *PDE8B* and *PDE11A* may also play an important role in the formation of cortisol-producing adenomas [9-11] or even cancer [12].

Cyclic AMP signaling pathway

The cAMP/protein kinase A (PKA) pathway is crucial for the function of the adrenal gland [13, 14] (Figure 1). Corticotropin (ACTH) binds to its G protein-coupled transmembrane receptor (*MC2R*), leading to the synthesis of cAMP by adenylate cyclase [15]. cAMP, acting as a secondary messenger, targets tetramer PKA. The latter is a cAMP-dependent serine-kinase, consisting of two regulatory (with *PRKAR1A* being the main one) and two inactive catalytic subunits. cAMP binds to the regulatory subunits, dissolves the tetramer, thus enabling catalytic subunits to phosphorylate a number of targets, and activate transcription of the genes.

The role of the cAMP pathway in adrenal tumors associated with Cushing syndrome has been repeatedly shown. For example, inactivating mutations in *PRKAR1A* in patients with PPNAD lead to inactivation of R1α, which allows Cα to be uninhibited [16], resulting in PKA activation.

The *PRKAR1A* gene, Carney complex, and PPNAD

Carney complex is a multiple endocrine neoplasia syndrome, inherited in an autosomal dominant pattern [17, 18]. Patients have PPNAD, as well as other endocrine neoplasms, such as pituitary adenoma and/or hyperplasia, thyroid and gonadal tumors. It is also associated with many non-endocrine lesions, such as spotty skin pigmentation (also known as “lentiginosis”), myxomas and schwannomas [17, 19]. PPNAD, which causes an often indolent form of adrenal Cushing syndrome, is the most common endocrine manifestation of Carney complex. Most of the affected patients present as children or young adults. Patients have a rather unique “paradoxical” rise of cortisol after the high-dose dexamethasone part of the Liddle's test [18, 20].

There are two genetic loci (17q22-24 and 2p16), which are associated with this complex. Mutations in the *PRKAR1A* gene were first recognized in families with linkage to the 17q22-24 locus [21]. Approximately two thirds of the patients affected by Carney complex have *PRKAR1A* mutations, while no gene has been identified at the 2p16 locus to-date [22]. *PRKAR1A* is likely to be a tumor suppressor gene, since the allelic loss of the wild-type allele is often seen in patients with Carney complex [3, 21]. To date more than 126 *PRKAR1A* mutations have been described. A publicly available database is available online, and continuously updated by our team (http://prkar1a.nichd.nih.gov/hmdb/mutations.html). Most of these mutations encompass small deletions and single base substitutions, or open reading frame rearrangements, with a few large deletions reported [23]. Importantly, an association between Carney complex and adrenal cancer, was recently described in patients with *PRKAR1A* mutations [24, 25]. Other cancerous associations, such as hepatocellular carcinoma [26] and pancreatic malignancies have been reported [27].

PRKACA defects and Cushing syndrome

Recently, Beuschlein *et al.* identified somatic activating mutations of the main catalytic subunit of PKA, *PRKACA*, in unilateral cortisol-producing adrenal tumors, causing overt Cushing syndrome [28]; this discovery was confirmed by others [29-34]. These groups performed whole exome DNA sequencing of the available tumors and leukocyte DNA, and identified a recurring hotspot point *PRKACA* mutation (c.617A>C, also known as c. 617T>G), resulting in arginine substitution of amino acid 206 (Leu206Arg).

Beuschlein at al. studied 139 patients with adrenal adenomas, adrenocortical carcinomas and ACTH-independent primary adrenal hyperplasias [28]. These patients were screened for *PDE8B, PDE11A, PRKAR1A* mutations and were found to be negative. The researchers identified mutations in *PRKACA* in 8 of the 10 originally screened unilateral cortisol-producing adenomas, with a majority (7 patients) having the c.617A>C, p.Leu206Arg mutation, whereas one had the insertion located at c.595_596CAC, Leu199_Cys200insTrp. The p.Leu206Arg mutation is located in a highly conservative core of the interaction between the regulatory (RIIβ) and catalytic subunits of PKA. Importantly, these investigators described the clinical phenotype of these patients: 37 % of the studied cohort had overt Cushing syndrome due to a unilateral cortisol-producing adenoma with a *PRKACA* mutation. These patients had a higher index of disease severity, as shown by increased urinary free cortisol and late-night serum cortisol levels. These findings were correlated with expression levels of the steroidogenic enzymes that were higher in tissues with *PRKACA* mutations. Patients with germline copy number gain of the *PRKACA* locus on chromosome 19p had BAH.

Shortly after the initial report, we described in detail the three pathologic phenotypes of BAH of the previously reported [28] five patients with germline *PRKACA* copy number gain. Three patients had a disease that looked like PPNAD plus extranodular cortical atrophy and mild intra- and extracapsular extension of adrenocortical cells, whereas others had cortical hyperplasia and capsular and extracapsular micronodular cortical hyperplasia [34].

The described hotspot mutations [28] may interfere with the creation of a stable PKA molecule and render the mutant Cα subunits constitutively active [35]. Cao and collaborators performed whole exome sequencing of 49 tumors and RNA sequencing of 44 tumors, including cortisol-producing adenomas, carcinomas and AIMAH [29]. They identified an even higher rate of somatic *PRKACA* mutations: 69.2% (27 out of 39). They published a hotspot c.617T>G, resulting in Leu205Arg substitution. It should be noted that this is the same mutation that was reported by Beuschlein *et al*. [28]: apparently, Cao et al. used a different amino acid numbering system, where initiating methionine is counted as residue zero (and not one), thus reporting Leu205Arg *vs.* Leu206Arg [36]. Interestingly, they found no statistical significant differences in serum cortisol, plasma ACTH, and urinary free cortisol levels, when compared to the patients without *PRKACA* mutations. The researchers performed functional studies (gain of function mutation in 293T cells), and found that overexpression of Leu205Arg mutants increases phosphorylation of PKA derivatives relative to the wild type [29]. In particular, the *PRKACA* Leu205Arg mutation induced phosphorylation of the cAMP response element binding protein (CREB), confirming the hypothesis that Leu205Arg mutation may enhance PKA activity [28].

In the same issue of the journal *Science*, Sato and colleagues independently reported finding the identical Leu206Arg hotspot mutation in *PRKACA* [32], as the cause of Cushing syndrome in patients with adrenal tumors. The researchers performed whole exome sequencing on eight adrenal tumors, and found 50% of them had the mentioned mutations. Moreover, they screened 57 follow-up cases and found that 24 of them had the *PRKACA* Leu206Arg somatic mutations. The affected patients had a smaller tumor size ($p=0.00005$) and higher levels of serum cortisol after 1 mg dexamethasone suppression test ($p=0.0026$) [32], reporting similar findings to others [31]. They also expressed wild-type *PRKACA* and the Leu206Arg in Human Embryonic Kidney 293 cells in which they showed that the Leu206Arg *PRKACA* mutant did not interact with the PKA regulatory subunits. The wild-type and mock *PRKACA*-transduced cells demonstrated enhanced PKA activity and increased CREB phosphorylation, irrespective of forskolin treatment [32]. Sato et al also detected *GNAS* mutations in 16.9% of the studied cohort.

Goh et al., reported the results of exome sequencing of cortisol-producing tumors from 25 patients (22 with adrenocortical adenoma and 3 with adrenocortical carcinoma) [31]. They found *PRKACA* heterozygous somatic mutations (c.617A>C [p.Leu206Arg]) in 6 patients. Similarly to the original study [28], they discovered a higher steroidogenic enzymatic activity in the adrenal tissue with *PRKACA* mutations, thus causing tumor development and endogenous Cushing syndrome. Patients with mutant adrenal adenomas were younger and had smaller tumors, associated with overt Cushing syndrome.

Di Dalmazi and colleagues investigated 149 frozen tumor samples from nine European medical centers, paired with leucocyte DNA and patients clinical and biochemical data, when available [30]. Similarly to the previous investigators, they found mutations of exon 7 of *PRKACA* gene in 34% studied samples, associated with Cushing syndrome. In addition to previously described missense mutation c.617A>C (p.Leu206Arg) (18 out of 22 patients), they found two novel mutations in another 4 patients (c.600_601insGTG/ p.Cys200_Gly201insVal and c.639C>G+c.638_640insATTATCCTGAGG/p.Ser213Arg

+p.Leu212_Lys214insIle-Ile-Leu-Arg). No germline *PRKACA* mutations were identified. The authors found higher levels of serum cortisol after dexamethasone testing, and smaller size of cortisol-producing adenomas.

Recently, Nakajima et al. screened tumors of 13 patients in Japan, and found recurrent somatic mutations of the PRKACA gene, p.L206R (c.617T>G) in 23% of patients with overt Cushing syndrome [33].

The *PRKACB* gene and Carney complex

We recently found that genomic amplification of the *PRKACB* locus may lead to Carney Complex without any *PRKAR1A* mutations [37]. Forlino *et al*. described a single case of a 19-year-old female patient presenting with acromegaly and lentigines, who also had myxomas, but no Cushing syndrome. This patient had somatic copy number gain of the chromosome 1p31.1 *PRKACB* locus, leading to an increase of the PKA catalytic subunit Cβ expression and higher PKA activity in the patient's cells. A mouse model, carrying a transgene for human *PRKACB*, had an increase in growth hormone secretion [37].

Conclusions

Somatic mutations in *PRKACA* may be a frequent cause of Cushing syndrome in patients with adrenal adenomas, while BAH may be caused by *PRKAR1A* or *PRKACA* defects. *PRKACA* mutations that lead to Cushing syndrome prevent the binding of the catalytic to the regulatory subunits, thus causing constitutive and cAMP-independent activation of PKA, and eventually, “autonomous” overproduction of cortisol. It is possible that larger tumors produce cortisol less efficiently, whereas smaller tumors may be more steroidogenic. This exciting discovery has been well covered in the scientific literature [36, 38-44], is expected to lead to a number of questions: Do *PRKACA* gene mutations cause tumors and cancers in other organs (breast, colon, liver, pituitary, etc.), since cAMP/PKA is instrumental in virtually every tissue in the human body? Does mutation in other PKA catalytic subunits (Cβ, Cγ, and PRKX) cause adrenal adenomas and/or hyperplasia? Moody and colleagues reported increased *PRKACA* expression in trastuzumab-resistant breast cancer, suggesting that the cAMP/PKA pathway may be stimulated in some breast cancers [45]. Fibrolamellar hepatocellular carcinoma was found to be due to a recurrent *DnaJ* homolog subfamily B member 1 **(***DNAJB1*)-*PRKACA* chimeric transcript [46]. Parathyroid hormone receptor (PTHR1) and parathyroid related protein (PTHrP) may promote tumor invasion and proliferation in osteosarcoma, via enhanced PKA signaling [47]. Vitali et al. showed that cAMP surges DNA synthesis and cyclin D1 expression in somatotropinomas, and abolishes in prolactinomas and non-functioning pituitary tumors [48].

The new genetics of Cushing syndrome may assist clinicians to provide appropriate counseling and in their decision-making regarding medical and/or surgical intervention. These findings may also lead to the development of individualized pharmacological treatment(s) for Cushing syndrome and other disease associated with PKA defects.

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Abbreviations

PRKACA	protein kinase catalytic subunit alpha
PRKACB	protein kinase catalytic subunit beta
PRKAR1A	protein kinase type 1 alpha regulatory subunit
PPNAD	primary pigmented nodular adrenal dysplasia
cAMP	cyclic AMP
ARMC5	armadillo repeat containing 5
AIMAH	ACTH-independent macronodular adrenal hyperplasia
BAH	bilateral adrenocortical hyperplasia
PMAH	primary macronodular adrenal hyperplasia
PDE	phosphodiesterase
PKA	protein kinase A
CREB	response element binding protein
PRKACB	protein kinase catalytic subunit beta
DNAJB1	DnaJ homolog subfamily B member 1
PTHR1	Parathyroid hormone receptor
PTHrP	parathyroid related protein

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Key points

1. Somatic mutations of the main catalytic subunit of PKA, a serine-threonine kinase, *PRKACA*, may cause cortisol-producing adenomas and Adrenocorticotropic hormone-independent Cushing syndrome.
2. Germline duplications of the *PRKACA* may result in bilateral adrenal hyperplasia.
3. Patients with *PRKACA* defects may have smaller tumors and more severe forms of Cushing syndrome.
4. The cAMP/PKA pathway is a potential target for molecularly designed therapies of Cushing syndrome.

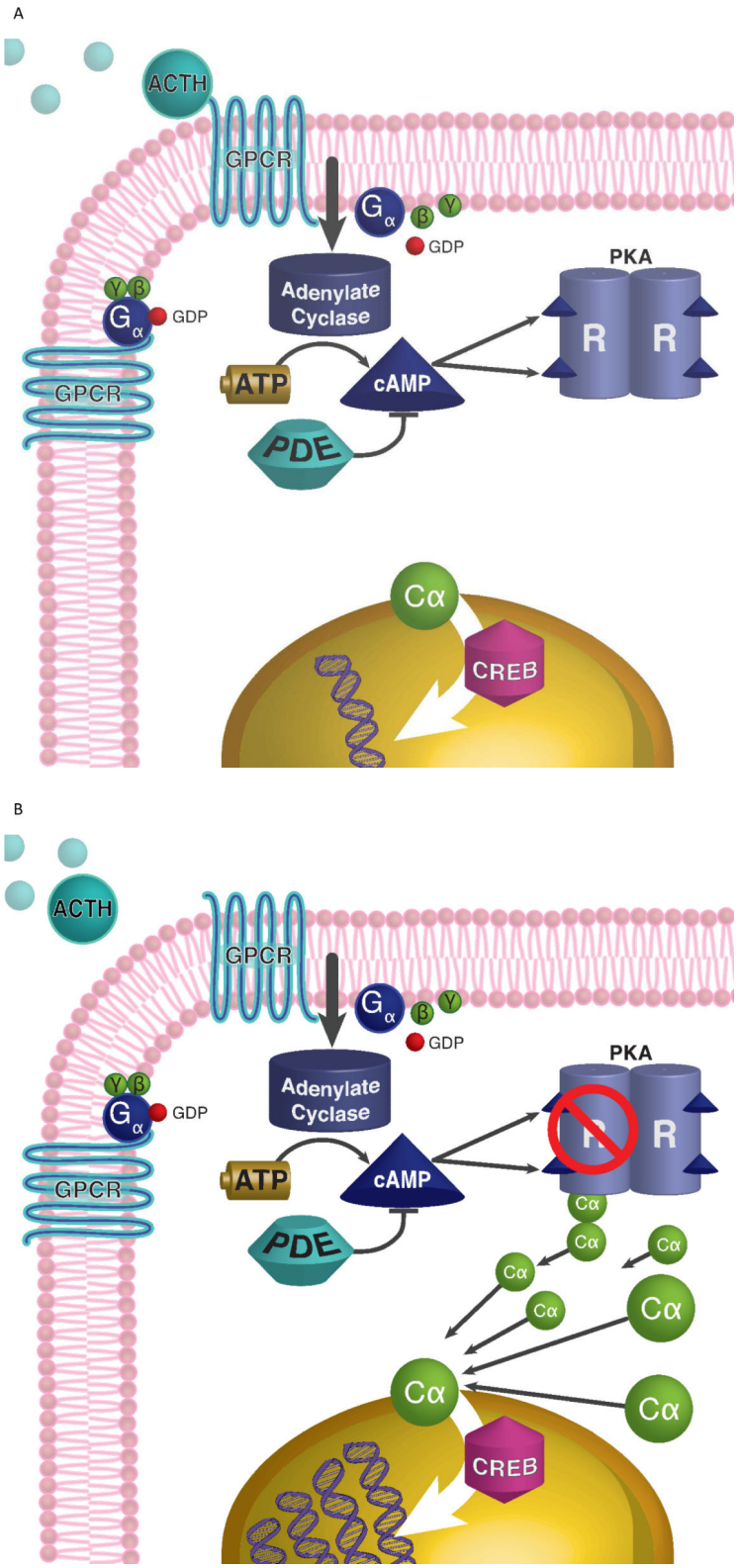

Figure 1.

Protein Kinase A signaling and adrenal Cushing syndrome. **A**. ACTH binds to its 7-transmembrane G-protein coupled receptor (GPCR), which activates Gsα protein and stimulates adenylate cyclase to generate cAMP (from ATP). PKA is a tetrameric enzyme, composed by 2 regulatory (R) and 2 catalytic (C) subunits, and is activated by an increase of the intracellular cAMP concentration: cAMP molecules bind to the R subunits which then set free the C subunits, resulting in phosphorylation of transcription factor cAMP response element binding protein (CREB) and other target molecules. Phosphodiesterases (PDEs), like PDE11A and PDE8B, bind cAMP and decrease its levels. **B**. Increased PKA activity in the setting of somatic *PRKACA* mutations in patients with cortisol-producing adenomas is due to the lack of the mutant *PRKACA*'s binding to the R subunits, or due to excess C subunits caused by copy number gain of the gene coding for it.