E Pluribus Unum? The Main Protein Kinase A Catalytic Subunit (PRKACA), a Likely Oncogene, and Cortisol-Producing Tumors

Constantine A. Stratakis

Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health (NIH); Section on Endocrinology and Genetics, Program on Developmental Endocrinology and Genetics, NICHD, NIH; and Inter-Institute Pediatric Endocrinology Training Program, NIH, Bethesda, Maryland 20892

Evidence has been accumulating for years that increased cAMP signaling (Figure 1) is the culprit behind the development of most benign cortisol-producing tumors of the adrenal gland (1, 2). First, GNAS1 mutations in McCune-Albright syndrome (MAS) were responsible for cortisol-producing tumors in the context of an otherwise normal adrenal gland in toddlers with MAS and Cushing syndrome (CS) (3). Then, a rare disease, massive macronodular adrenocortical disease (MMAD) or ACTH-independent macronodular adrenocortical hyperplasia (AIMAH), now aptly renamed as primary macronodular hyperplasia (PMAH) (4), was found to be linked to the ectopic expression of G protein-coupled receptors (GPCRs) (5, 6). And we found mutations of the main cAMP receptor in all cells, PRKAR1A, the gene that codes for the regulatory subunit type 1α (R1α) of the cAMP-dependent protein kinase or protein kinase A (PKA) to cause another rare form of cortisol-producing hyperplasia: primary pigmented nodular adrenocortical disease (PPNAD) is caused in both its isolated form and the type associated with Carney complex, a multiple endocrine neoplasia syndrome, by PRKAR1A-inactivating mutations (7, 8). However, proof that increased cAMP signaling is in fact behind the common CPA came out (finally) this year, first with our work in the *New England Journal of Medicine* (21), and then with confirmatory data from three other groups (22–24) showing somatic mutations of the main catalytic subunit of PKA, a serine-threonine kinase (STK), PRKACA (coding for the Cα/H9251 protein) in CPAs from patients with CS from various ethnic groups. The publications attracted significant attention as is evident

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.
Copyright © 2014 by the Endocrine Society
Received August 25, 2014. Accepted August 25, 2014.
For article see page E2093

from editorials and other coverage (25–27). In this issue of the JCEM, Di Dalmazi et al (28) present the first follow-up data after the four original reports of PRKACA mutations (21–24). Di Dalmazi et al (28) studied a large population of patients from many centers around Europe and identified somatic PRKACA mutations in 22 of 64 samples from patients with CS, all with CPAs (34%). As in the previous studies, no mutations were found in peripheral DNA or in the samples from other tumors, including nonfunctioning adrenal tumors and MMAD/AIMAH/PMAH.

What do these data show? In brief, fairly specific PRKACA mutations, such as the c.617A>C (p. Leu206Arg), are present in adrenocortical tumor tissue only. These mutations are somatic and confined to a single adrenal gland (and thus presumably occurring late in life) are present in a location of Cα protein that is responsible for binding to the regulatory subunit(s) (21, 28). They thus lead to activation of PKA by making the catalytic subunit of the enzyme largely inaccessible to its main controller and binding partner, the “receptor” for cAMP in the cell, the PKA regulatory subunit(s) (Figure 1).

The cAMP-dependent protein kinase or PKA is a tetramer (Figure 1) that consists of two inactive catalytic subunits and two regulatory subunits (29). The main regulatory subunit of PKA is PRKAR1A (coding for the R1α protein; there are three others—PRKAR1B, PRKAR2A, and PRKAR2B), and its main catalytic subunit is PRKACA (Ca; there are three more—PRKACB, PRKACG, and PRKX). In the inactive tetramer, each molecule of Ca is bound by a molecule of R1α. The two heterodimers are then held together by the two inactive regulatory subunits mainly. When two molecules of cAMP bind to each regulatory subunit, the tetramer dissolves and the catalytic subunits are free to phosphorylate downstream targets. cAMP levels are regulated by PDEs. PDE11A and PDEBB mutations predispose to all types of cortisol-producing lesions of the adrenal cortex. Ectopic overexpression of GPCRs lead to nodular forms of hyperplasia, whereas GNAS1 and PRKAR1A mutations lead to nodular and micronodular BAHs, respectively (A), and rarely to CPAs (C). Mutations of PRKAR1A also lead to Carney complex (B). PRKACA mutations lead to CPAs (C). Finally, there are a number of cortisol-producing lesions that are caused by yet unidentified genetic defects; three examples of such lesions from respective patients (two children and one adult with CS) are shown in panel D.

Figure 1. The involvement of cAMP signaling defects in CS is shown on this diagram. GPCRs that act through GNAS1 (Gsα), in response to a ligand hormone, activate adenylate cyclase to produce cAMP. cAMP mediates most of its effects through the cAMP-dependent PKA. PKA is a tetramer that consists of two inactive catalytic subunits and two regulatory subunits. The main regulatory subunit of PKA is PRKAR1A, and its main catalytic subunit is PRKACA; in the inactive tetramer, each catalytic subunit molecule is bound by a molecule of regulatory subunit. The two heterodimers are held together by the two inactive regulatory subunits. When two molecules of cAMP bind to each regulatory subunit, the tetramer dissolves, and the catalytic subunits are free to phosphorylate downstream targets. cAMP levels are regulated by PDEs. PDE11A and PDEBB mutations predispose to all types of cortisol-producing lesions of the adrenal cortex. Ectopic overexpression of GPCRs lead to nodular forms of hyperplasia, whereas GNAS1 and PRKAR1A mutations lead to nodular and micronodular BAHs, respectively (A), and rarely to CPAs (C). Mutations of PRKAR1A also lead to Carney complex (B). PRKACA mutations lead to CPAs (C). Finally, there are a number of cortisol-producing lesions that are caused by yet unidentified genetic defects; three examples of such lesions from respective patients (two children and one adult with CS) are shown in panel D.
in Figure 1 and discussed above, for years work was pointing to the PKA tetramer and its main catalytic subunit, \( \alpha \), as a candidate. “The truth is simple.” Out of many components of the GPCR/GNAS1/cAMP signaling pathway, one really counts — its ultimate responder, the PKA catalytic subunit. As Heraclitus said “ἐκ πάντων ἐν καὶ ἐκ ἕνος πάντα” (e pluribus unum), and PRKACA is this one molecule in cAMP signaling that should have been looked at earlier. After all, PRKACA mutations that were predicted to lose interaction with the regulatory subunit while maintaining enzymatic activity had been studied in vitro in the early 1990s (35, 36). Additional PRKACA sequences that had similar effects (also in vitro) were described in 2009 (37), and the first PRKACA mutations in humans with predicted functional effects were reported in 2012 (38).

But is it really so simple? Was it totally missed all these years? As Oscar Wilde said, “The truth is rarely pure and never simple.” In fact, the first study to ever question the presence of PRKACA mutations in endocrine tumors was conducted by Esapa and Harris (39) as early as 1999. But this investigation produced negative results despite studying a large collection of thyroid and pituitary tumors, lesions in which one would have predicted PRKACA mutations based on their harboring GNAS1 mutations frequently. In my laboratory, we had in fact also looked for PRKACA mutations in PPNA, MMAD/AIMAH/PMAH, and related conditions but found none (21). Then, in 2012, we reported (in a grant renewal application) the identification of a copy number variant of chromosome 19p that led to a gain of one extra copy of the PRKACA gene (located on 19p13.12) in a mother-son pair affected by what was a form of bilateral adrenocortical hyperplasia (BAH) intermediate between PPNA and MMAD/AIMAH/PMAH (2) and a single individual with what had been called PPNA, albeit slightly different than the one seen in the context of Carney complex (C. A. Stratakis, unpublished data). In the following 2 years, we found two more patients with BAH and CS at a young age with copy number variant gains that included the 19p locus of the PRKACA gene (21), and the evidence became stronger that increased genetic dosage of this cAMP-responsive STK at germline could lead to adrenocortical lesions and hypercortisolism. The identification of somatic PRKACA mutations in CPAs by Beuschlein et al (21) closed the loop in what is now a quite amazing story that is not true (at least not so far) for any other “endocrine” gene involved in tumor formation; PRKACA is relatively unique in that it causes a rare form of BAH leading to CS in children and young adults when it is present as a germline defect associated with increased genetic dosage of chromosome 19p13.12 material. It then causes the relatively common CPA leading to CS in older age when it acquires mutations in the somatic state, isolated in the adrenal cortex of one gland, and allowing it to mediate STK activity in response to cAMP but not confined by the shackles of a PKA regulatory subunit. It all appears to be a matter of dosage of PKA catalytic activity, and the adrenal cortex appears to be exquisitely sensitive to it, a hypothesis that requires testing in animal models (Figure 2). It is also likely that the Leu206Arg PRKACA mutation (and the other mutations identified so far) is not compatible with life in human embryos, as is true for GNAS1 mutations leading to MAS that are only present in the somatic state (40). This would be expected from a gene with a ubiquitous presence and an activity that is so, apparently, tightly controlled by cAMP and the PKA regulatory subunits, but it remains to be proven.
So is PRKACA an oncogene, like GNAS1? It certainly will not be the first STK that has an oncogenic function. Recent data showing that recurrent DNAJB1-PRKACA chimeric transcripts are present in fibrolamellar hepatocellular carcinoma suggest that this is so (41). Mice with Prkar1a defects develop liver tumors (42, 43), and patients with Carney complex develop hepatocellular carcinoma (albeit not fibrolamellar) (44). At least one of our patients with germline PRKACA copy number gain (21) has developed breast cancer at a young age (C. A. Stratakis, unpublished data), and PRKACA appears to be involved in the regulation of apoptosis in breast cancer cells (45).

Like GNAS1, PRKACA and the other PKA catalytic subunits have tumorigenic (and other) roles in bone in both humans and mice (46, 47).

There are, therefore, many questions to be answered as a consequence of these discoveries—from the adenocortical sensitivity to PRKACA dosage (and cAMP?) effects to whether PRKACA is an oncogene in the adrenal, liver, breast, bone, and possibly other tissues. Animal models will be helpful in this quest. Complete knockout is not compatible with life in most mice, but incredibly, few animals in certain backgrounds survive birth to die later in life (48); the Prkar1a-haploinsufficient mouse does not have a phenotype other than mildly decreased bone mineral density (47, 48). Its adenocortical function has not been studied specifically. And what is the role of the other PKA catalytic subunits? We recently showed that gain of PRKACB is involved in a phenotype that is similar to Carney complex (49), and studies in mice attest to PRKACB’s involvement in at least some coordination with PRKACA’s function (47, 48, 50).

Perhaps the most important outcome of these studies is that a new pathway, other than steroidogenesis or the glucocorticoid receptor, emerges as a possible therapeutic target for patients with CPAs and/or other forms of hypercortisolism and CS. Inhibitors of cAMP signaling and/or PKA and its catalytic subunits exist in vivo (PKA inhibitor) (51, 52), and it should not be hard to test small molecules that would mimic the endogenous PKA inhibitors or identify new ones that would target the adrenal Ca overactivity in patients with CS. This is truly good news for a field of endocrinology that is in dire need of good medications without the side effects of ketoconazole, mifepristone, or identify new ones that would target the adrenal Cα regulatory subunit in patients with the Carney complex. Nat Genet. 2000;26:89–92.


Acknowledgments

Address all correspondence and requests for reprints to: Dr Constantine Stratakis, CRC-Room 1-3330, East Laboratories, Building 10-CRC, 10 Center Drive, Section on Endocrinology & Genetics/Program in Developmental Endocrinology and Genetics, Eunice Kennedy Shriver National Institute of Child Health & Human Development, National Institutes of Health, Bethesda, MD 20892. E-mail: stratak@mail.nih.gov.

This work was supported by the Intramural Research Program of the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Disclosure Summary: The author has nothing to declare.

References


