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Review

New genes and/or molecular pathways associated with adrenal hyperplasias and related adrenocortical tumors

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ABSTRACT

Over the course of the last 10 years, we have studied the genetic and molecular mechanisms leading to disorders that affect the adrenal cortex, with emphasis on those that are developmental, hereditary and associated with adrenal hypoplasia or hyperplasia, multiple tumors and abnormalities in other endocrine glands. On the basis of this work, we propose an hypothesis on how adrenocortical tumors form and the importance of the cyclic AMP-dependent signaling pathway in this process. The regulatory subunit type 1-α (RIα) of protein kinase A (PKA) (the *PRKAR1A* gene) is mutated in most patients with Carney complex and primary pigmented nodular adrenocortical disease (PPNAD). Phosphodiesterase-11A (the *PDE11A* gene) and -8B (the *PDE8B* gene) mutations were found in patients with isolated adrenal hyperplasia and Cushing syndrome, as well in patients with PPNAD. PKA effects on tumor suppression and/or development and the cell cycle are becoming clear: PKA and/or cAMP act as a coordinator of growth and proliferation in the adrenal cortex. Mouse models in which the respective genes have been knocked out see m to support this notion. Genome-wide searches for other genes responsible for adrenal tumors and related diseases are ongoing; recent evidece of the involvement of the mitochondrial oxidation pathway in adrenocortical tumorigenesis is derived from our study of rare associations such as those of disorders predisposing to adrenomedullary and related tumors (Carney triad, the dyad of paragangliomas and gastric stromal sarcomas or Carney–Stratakis syndrome, hereditary leiomyomatosis and renal cancer syndrome) which appear to be associated with adrenocortical lesions.

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Contents

1. Introduction: adrenocortical tumorigenesis

Significant progress has been made through studies from our laboratory and other investigators on the understanding of molecular genetics of endocrine tumors such as adrenocortical tumors (ADT) (Stratakis, 2003). What was limited to *TP53* gene's involvement in adrenocortical cancer (ACC) in the early 1990s extends now from cAMP- to the *Wnt*-signaling pathways; a number of alterations have been detected at the genomic, transcript or protein levels (Bourdeau et al., 2004; Horvath et al., 2006a, 2006b, 2006c; Bornstein and Hornsby, 2005). The multi-step model of tumorigenesis that had been described in other tissues, and that we first proposed for ADTs (Stratakis, 2003), is now widely accepted (Bernard et al., 2003). The notion of studying, first, benign hyperplasias and "*cloning genes and identifying molecular pathways involved in the first steps of ADT formation*" that we suggested in the early 1990s has paid off with the identification of the cAMP/PKA-signaling pathway as the main molecular route that when defective leads to benign ADTs and hyperplasias; *Wnt*-signaling abnormalities (present also in some hyperplasias), growth factor expression

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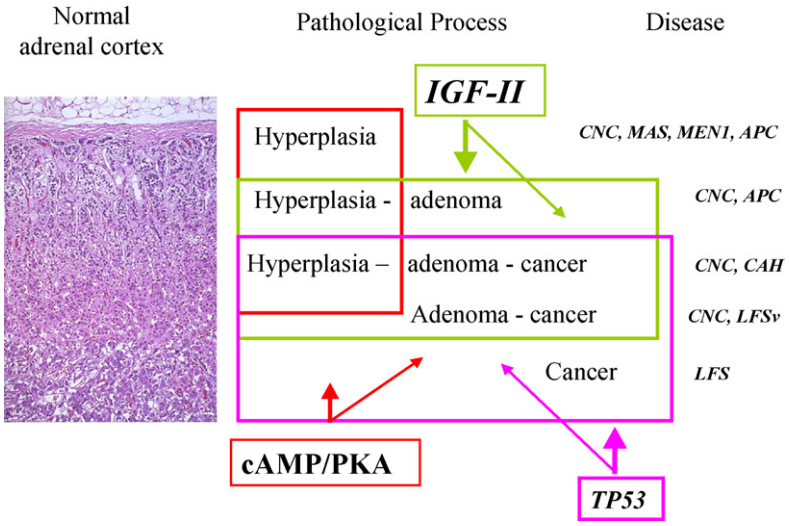

Fig. 1. Tumorigenesis in the adrenal cortex, like in other endocrine (i.e. pituitary) and non-endocrine tissues (i.e. colon) is subject to a multi-step process, although not all steps are detected in pathologic specimens, nor it is necessary for all steps to occur in all cell types. Both adenoma and cancer can form without first going through hyperplastic and adenomatous, respectively, transformation; the cAMP-signaling pathway leads to hyperplasias; IGF-II overexpression (and that of other growth factors) is associated with adenomas and cancer; defects of cell cycle genes (*TP53*, *CHEK2*) are the hallmark of both sporadic and familial adrenal cancer, but large benign adenomas can also harbor somatic *TP53* mutations. If the first "hit" is in *TP53*, such as is the case in LFS, cancer forms without need for any precursor steps; if, on the other hand, the first defect is in a gene of the cAMP-signaling pathway (as in CNC or MAS), the most likely outcome is hyperplasia and adenomas, which only rarely (if ever) progress to cancer. In support of this hypothesis, we and others reported somatic *Wnt*-signaling pathway abnormalities in adenomas formed in the context of hyperplasias; somatic *PRKAR1A* mutations also occur in sporadic adenomas that behave like those in CNC. Finally, chromosomal abnormalities are common in all ADTs and their complexity progresses with the severity of the pathology. Abbreviations: APC = Familial polyposis coli; CNC = Carney complex; CAH = congenital adrenal hyperplasia; LFS = Li-Fraumeni syndrome; LFSv = LFS variant (TP53CHEK2 "mild" mutations); MAS =M cCune-Albright syndrome; MEN 1 = menin - multiple endocrine neoplasia 1; PKA = protein kinase A.

(i.e. IGF-II, IGFBPs), and cell cycle gene defects (*TP53*, *CHEK2*) are associated with large adenomas and ACC (de Fraipont et al., 2005) (Fig. 1).

In our model, hyperplasias of the adrenal cortex remain the cornerstone of the elucidation of the molecular phenomena that culminate in cancer formation. This process does not necessarily precede linearly other steps (this may still happen rarely): one example of non-linear phenomena are those that happen in parallel in different cell types, such as the stroma and tumor cells. It is noteworthy that a recent autopsy study identified adrenal hyperplasia in 36% of the subjects studied (Saeger et al., 1998). It is for this reason, first, that we continue to focus on the clinical, genetic and molecular identification of bilateral adrenocortical hyperplasias (BAH): elucidation of all genetic defects leading to BAH will assist us in understanding the first (and apparently common) steps that adrenocortical tissue undertakes towards any pathology. The second reason is that unlike adenomas and cancer, BAHs are to a much larger extent inherited or more directly due to genetic causes (Bourdeau and Stratakis, 2002); this allows for the more efficient use of gene mapping tools that take advantage of positional approaches based on SNPs (Genome-wide association or GWA studies) and other family data in addition to classic cancer genetic techniques such as loss-of-heterozygosity (LOH). And, finally, BAHs associated with Cushing syndrome have a distinct biochemical phenotype of hypercortisolemia that we and others have striven to define with the development of proper testing; recently, we presented a tentative classification of all BAHs helped by the new genetic discoveries (Table 1) (Stratakis and Boikos, 2007).

2. Phosphodiesterases: extending the role of cAMP-signaling in adrenocortical tumors

In the year 2000, we identified the molecular cause of the most common among the micronodular BAHs, primary pigmented nodular adrenocortical disease (PPNAD) (Kirschner et al., 2000). PPNAD is characterized by small, pigmented nodules that are surrounded by mostly atrophic cortex in an otherwise normal-sized gland (Carney et al., 1985). Most cases of PPNAD, inherited or sporadic, are associated with Carney complex (CNC), a syndrome that causes abnormal skin and mucosal pigmentation in addition to a variety of tumors [myxomas of the skin, heart, breast and other sites, psammomatous melanotic schwannoma, growth hormone (GH)-producing pituitary adenomas, testicular Sertoli and Leydig cell neoplasms, thyroid tumors, breast adenomas, nevi, and, perhaps, colon and other cancers] (Stratakis et al., 2001). CNC shares features with multiple endocrine neoplasia (MEN) and lentiginosis syndromes, such as MEN 1 and Peutz-Jeghers syndrome (PJS), and other hamartomatoses (Marsh and Stratakis, 2001). By linkage analysis we and others identified two loci harboring genes for CNC on 2p16 (*CNC2*) and 17q22-24 (*CNC1*) (Stratakis et al., 2001). We then identified the *PRKAR1A* gene from the *CNC1* 17q22-24 locus (Kirschner et al., 2000). *PRKAR1A* encodes the regulatory subunit type 1A (R1α) of PKA, a molecule that is the main regulator of this cAMP-dependent protein kinase, one of the most important regulatory pathways in cellular signaling (Amieux and McKnight, 2002).

Following the identification of *PRKAR1A* gene mutations in PPNAD, we had, in addition to clinical and histopathological features, a molecular genetic marker that could be used to identify diagnostic subgroups of the BAH and CNC patients that we were working with (Table 1). This allowed us to identify significant genetic heterogeneity within patients that were previously thought to have one disease.

Before embarking on using the samples that were *PRKAR1A*-mutation-negative to search for other genes, we wanted to eliminate the possibility that genetic defects that would not be identifiable by sequencing were present. Fluorescent in situ hybridization (FISH) with bacterial artificial chromosome (BAC) probes containing the *PRKAR1A* gene did not show any large defects, but survey by restriction digestion and long-range PCR resulted in the identification of two chromosome 17q22-24 microdeletions among 36 *PRKAR1A*-mutation-negative unrelated kindreds (Horvath et al., 2008a,b). Based on this study, one would expect