Aldosterone-Producing Adenomas
Mining for Genes
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Primary aldosteronism is the most common form of secondary hypertension and is present in about 8% to 10% of patients with hypertension. In primary aldosteronism, aldosterone secretion is excessive and relatively autonomous of the normal regulatory mechanisms. It was originally described by Jerome Conn from the University of Michigan and is thought to be due to an aldosterone-producing adenoma (APA) causing hypertension and hypokalemic alkalosis. Primary aldosteronism is now recognized to include a spectrum of disorders from unilateral APA to bilateral zona glomerulosa (ZG) hyperplasia, or idiopathic hyperaldosteronism. The diagnosis of all forms of primary aldosteronism is based on the finding of elevated levels of aldosterone and suppressed renin secretion. Differentiation between unilateral and bilateral aldosteronism is done by a combination of adrenal imaging (computed tomography or MRI) and bilateral adrenal sampling.

Diagnosis of an APA is attractive because surgical therapy with unilateral adrenalectomy should cure the disease, and mechanistically, APA would suggest a single etiology of a benign neoplastic transformation of cells of the ZG producing aldosterone, regardless of the molecular mechanism. However, the histological characteristics of most adrenal tumors classified as APA are heterogeneous when one considers the surrounding adrenal cortex and include varying proportions of 4 different types of cells: clear cells with large vacuolated lipid-laden cytoplasm and central round nuclei similar to zona fasciculata-like cells; lipid-poor ZG-like cells; compact eosinophilic cells similar to those of the zona reticularis; and cells with cytological features of both ZG and zona fasciculata cells, designated hybrid cells. The lipid-laden fasciculata-like cells usually predominate, giving the tumors a characteristic golden yellow color, but some have more glomerulosa-like characteristics. However, the histological picture is far more complex, as most patients with APA also have hyperplasia of the rest of the adrenal ZG (40%) or hyperplastic nodules of ZG cells within the ZG (56%). In situ hybridization for the aldosterone synthase enzyme expression was done to demonstrate that the dominant nodule in 22 of 27 patients with the diagnosis of an adenoma was functionally significant. In 14 of these patients, significant but lower aldosterone synthase expression was also found in other areas of the ZG, and adrenalectomy resulted in a cure. However, in one of these patients with high aldosterone synthase expression outside the dominant adenoma, the syndrome recurred after adrenalectomy. Two patients in whom the expression was in smaller nodules rather than the dominant one were also not cured by adrenalectomy. APAs have been separated into the more common adenomas that are angiotensin II nonresponsive and 10% to 30% that are angiotensin II responsive, but from a diagnostic point of view they are very similar. Definition of an APA requires the presence of a unilateral adrenal mass and the lateralization of aldosterone secretion by adrenal vein sampling, as well as a therapeutic response to adrenalectomy. A high ratio of aldosterone to cortisol in the effluent from the affected, compared with unaffected, adrenal is the hallmark for diagnosis, although the exact ratio for diagnosis has been controversial, as most often aldosterone production from the unaffected adrenal is not completely suppressed. Unfortunately, a therapeutic response to adrenalectomy is frequently partial, yet some cases of clear bilateral disease have responded to unilateral adrenalectomy. Half to two thirds of APA patients treated surgically continue to require antihypertensive medications, albeit at lower doses or with fewer drugs. Partial surgical response in APA might depend on whether patients are selected on the basis of adrenal vein sampling, extent of contralateral aldosterone suppression, duration and severity of the hypertension, or age of the patient.

This preamble indicates that the diagnosis of APA is not simple and that the tumors comprise a number of disparate histological and pathological entities. Several studies in the literature have attempted to determine the molecular characteristics of adrenal adenomas by using transcriptome analysis to identify expressed genes that participate in the pathogenesis of the adenoma. The regulated genes have differed greatly between studies; in one study even aldosterone synthase was not found to be upregulated in the supposed APA of some patients. Such discrepant results might be due to patient selection, use of insufficiently stringent criteria for lateralization of aldosterone secretion, or inclusion of dominant nodules that did not express aldosterone synthase, rather than smaller nodules which were likely to be producing the aldosterone, as shown by other studies. A few studies have searched for G-protein–coupled receptor expression not normally present in adrenal gland that might be responsible for aberrant responses, and although expression is frequently present in APA, the response of patients to the hormone ligands has not been studied in most cases.
The genomic analysis study reported in this issue by Williams et al\textsuperscript{10} used adenoma tissues from a group of patients who had been homogeneously selected following rigorous diagnostic procedures, including strict criteria for adrenal venous sampling interpretation and postadrenalectomy evaluation, although suppression of aldosterone production in the contralateral adrenal was not discussed. Oligonucleotide microarrays were performed for transcriptional screening of 8 APA compared with 3 normal adrenals. Using strict interpretation criteria, they found that 53 genes were differentially expressed in APA compared with normal adrenals, of which 33 were upregulated and 20 were downregulated. Four of the most regulated genes were studied further by real-time RT-PCR in 19 patients, including some of the original patients used in the microarray studies. The most upregulated gene was teratocarcinoma-derived growth factor 1 (TDGF-1), also called Cripto-1, which was elevated in 15 of 19 APA patients, with an average above increase of 21.4-fold, but there was great heterogeneity in the degree of expression. The expression of the gene was not increased in other adenalar adrenals of different etiologies, but these control groups were small. The second most common regulated gene was that for tumor protein D52, present in the quantitative RT-PCR results of 14 of 19 patients. In a study similar to one previously reported, these 2 genes were not among those found regulated in the microarray; the reason is not clear, but the microarray platforms used were different.\textsuperscript{7} Real-time RT-PCR of those cases would be of great interest to determine whether the findings are common in APA.

An important aspect of this study is that the functional role of TDGF-1 was addressed. Transfection of a TDGF-1 plasmid into the adrenal carcinoma cell line H295R resulted in a 3.8-fold increase in aldosterone secretion without sensitizing the cells to the effect of angiotensin II, as is the case in classical adrenomas. TDGF-1 activates Akt signaling pathway, and inhibitors of phosphatidylinositol 3-kinase inhibited the increase in TDGF-1-mediated aldosterone secretion.\textsuperscript{10} TDGF-1 is a membrane glycoprotein with a glycosylphosphatidylinositol anchor that is an essential coreceptor for Nodal and required for Nodal signaling.\textsuperscript{11} Nodal is a member of the transforming growth factor-\beta superfamily that plays essential role in mesoderm formation. TDGF-1 contains 2 functional domains that play roles in Nodal signaling, a truncated epidermal growth factor-like repeat and the cysteine-rich domain. TDGF-1 is also a coreceptor that interacts with other factors, including activin, Lefty, and Temo3regulin-1 and can activate the Ras/Raf/mitogen-activated protein kinase and the phosphatidylinositol 3-kinase/Akt pathways independently of Nodal signaling. Single-point mutations of Thr72 in TDGF-1 result in loss of the coreceptor function with nodal but have no effect on mitogen-activated protein kinase signaling.\textsuperscript{12} TDGF-1 is both anchored to the plasma membrane and secreted. It has growth stimulatory properties on tumor cells of many kinds, supports cell survival and protects or induces apoptosis depending on the context, and inhibits differentiation of some cells. TDGF-1 acts as a growth factor and inhibits apoptosis in H295R cells, and it is overexpressed in and increases the metastatic potential of multiple malignant epithelial tumors, including breast, pancreas, colon, and prostate cancer.\textsuperscript{10} It has been best studied in mammary cells in culture. In addition to activities that may contribute to metastatic progression of tumors, TDGF-1 is essential for early embryonic development and is required for cardiogenesis.\textsuperscript{11} Although endogenous TDGF-1 expression in the H295R adrenal carcinoma cell line was very low, transfection of TDGF-1 cDNA resulted in stimulation of aldosterone secretion by H295R cells. One unusual finding in the study of Williams et al\textsuperscript{10} was that expression of TDGF-1 in the patient adenoma cells was nuclear while it was expressed in the cytoplasm of the surrounding ZG cells. The explanation is not apparent, but one can speculate that because TDGF-1 is a coreceptor for nodal, activin, and other proteins, interaction with another, as-yet- unidentified protein might result in the localization of TDGF-1 in the nucleus, where its function remains unknown.

In conclusion, tumors in a large proportion of stringently selected patients with APAs express several interesting genes, one of which, TDGF-1, stimulates aldosterone secretion independent of angiotensin II stimulation. This is a first step in understanding the molecular pathogenesis of APA, but details remain very incomplete.

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