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Mouse models of adrenocortical tumors

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ABSTRACT

The molecular basis of the organogenesis, homeostasis, and tumorigenesis of the adrenal cortex has been the subject of intense study for many decades. Specifically, characterization of tumor predisposition syndromes with adrenocortical manifestations and molecular profiling of sporadic adrenocortical tumors have led to the discovery of key molecular pathways that promote pathological adrenal growth. However, given the observational nature of such studies, several important questions regarding the molecular pathogenesis of adrenocortical tumors have remained. This review will summarize naturally occurring and genetically engineered mouse models that have provided novel tools to explore the molecular and cellular underpinnings of adrenocortical tumors. New paradigms of cancer initiation, maintenance, and progression that have emerged from this work will be discussed.

1. Introduction

Adrenal neoplasms are commonly diagnosed endocrine findings (Young, 2007). While the incidence of adrenocortical tumors (ACTs) is relatively high, affecting an estimated 3%–7% of the population, most are benign adenomas. However, even in non-malignant tumors, hormonal hyperfunction can lead to significant morbidity. In addition to benign adrenocortical adenomas (ACAs), malignant carcinomas may also arise in the adrenal cortex. These rare tumors, adrenocortical carcinomas (ACCs), are highly aggressive and routinely fatal, largely due to the high proportion of patients diagnosed at an advanced stage (Else et al., 2014). Surgical resection is therefore limited to a small cohort of ACC patients, and treatment is otherwise restricted to cytotoxic chemotherapy, radiation, and the adrenolytic drug mitotane. Given the prevalence and severity of ACAs and ACCs, respectively, the treatment and management of adrenal tumors remain a significant public health challenge.

Historically, mouse models have been essential for the study of adrenal tumorigenesis. Beginning in the early 1900s, discoveries in mice containing spontaneous or gonadectomy-induced adrenal tumors started to provide fundamental insights about cell growth and differentiation within the adrenal cortex. Later, new technologies allowed for the development of genetically modified models that focused on specific genes and pathways. These models have been and will continue to be critical for validating and interpreting the large amount of data emerging from the recent “omics” era. Finally, xenograft models in which human tumor tissue (primary or cell line-derived) is grown in immunocompromised mice have become more widely used in the adrenal field. These models are particularly well suited to study the heterogeneous nature of human ACCs. However, we will not address xenograft models further, as they will be discussed in greater detail elsewhere in this issue.

Here, we aim to provide a comprehensive overview of spontaneous and genetically modified mouse models of ACTs, including conditions of adrenal hyperplasia. We discuss the models in the context of the human pathology that they are most closely associated with, even though some models do not fully recapitulate the human disease. We describe key aspects of how each model was generated, the adrenal phenotype observed, and relevant implications for human health. Collectively, these models have provided valuable insights on the growth, differentiation, and transformation of the adrenal cortex, which are essential for the development of novel therapeutic strategies for the treatment of adrenal diseases.

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2. Mouse models of adrenocortical hyperplasia

Adrenocortical hyperplasia is a broad term that describes a group of conditions characterized by bilateral adrenal enlargement. Unlike the majority of ACTs, which are unilateral, monoclonal, and sporadic, the bilateral nature of hyperplasia is consistent with a polyclonal origin (Beuschlein et al., 1994; Diaz-Cano et al., 2000). Causes of hyperplasia include inherited genetic syndromes, sporadic/idiopathic forms, and overstimulation of the adrenal cortex by extrinsic factors such as adrenocorticotrophic hormone (ACTH) (Xing et al., 2015). Adrenocortical hyperplasia can be functionally classified as hormonally silent or actively producing steroids. Among the latter group, cortisol, aldosterone, and androgen-secreting forms have been documented (Ghayee et al., 2011; Piaditis et al., 2015; Stratakis and Kirschner, 1998). Although adrenocortical hyperplasia encompasses a heterogeneous group of diseases, specific molecular pathways are commonly dysregulated. In particular, most cases of cortisol-secreting hyperplasias have abnormal activation of the protein kinase A (PKA) pathway, while aldosterone-secreting forms are characterized by abnormal calcium-calmodulin dependent kinase (Ca^{2+}-CAMK) signaling (Stratakis, 2013; Zennaro et al., 2015). Physiologically, these molecular pathways are key regulators of cortisol and aldosterone production, respectively. In humans, adrenal hyperplasia can also occur in the context of rare multiple neoplasia syndromes, such as MEN1, Carney complex, adenomatous polyposis coli, or McCune-Albright syndrome (Lerario et al., 2014a). Several transgenic mouse models have been developed to recapitulate different types of human adrenocortical hyperplasia. In the following section, we summarize these models and discuss the molecular aspects of each that led to their adrenal manifestations (Table 1).

2.1. ACTH-dependent hyperplasia

As previously mentioned, chronic overstimulation of the adrenal glands by ACTH results in bilateral adrenal enlargement due to hyperplasia within the adrenal cortex. Among the causes of ACTH-dependent adrenocortical hyperplasia are Cushing's disease (ACTH-producing pituitary adenoma), ectopic ACTH syndrome, and a group of diseases broadly known as congenital adrenal hyperplasia (CAH) (Xing et al., 2015). CAH is characterized by inherited enzymatic defects in steroid hormone biosynthesis that result in cortisol deficiency. This loss of negative feedback on the hypothalamic–pituitary axis results in increased ACTH production, subsequent adrenal enlargement, and increased steroidogenic activity with accumulation of steroidogenic precursors. In humans, the most common form of CAH is 21-hydroxylase deficiency. Although mouse models of ACTH-dependent adrenal hyperplasia exist (Caron et al., 1997; Mullins et al., 2009; Riepe et al., 2005), they are beyond the scope of this review.

2.2. ACTH-independent hyperplasia

2.2.1. Aldosterone-producing hyperplasia

Primary aldosteronism, defined as autonomous aldosterone secretion by the adrenals, is the leading cause of secondary hypertension, with an estimated prevalence of ~10% among hypertensive patients (Zennaro et al., 2015). Causes of primary aldosteronism include aldosterone-producing adenomas (APA), bilateral sporadic adrenal hyperplasia, and familial hyperaldosteronism types I–III. The majority of cases are sporadic, since familial hyperaldosteronism is very rare. Furthermore, it has been demonstrated that abnormal activation of Ca^{2+}-CAMK signaling, which results in transcriptional activation of the aldosterone biosynthetic machinery, is the underlying molecular abnormality in both APAs and hyperplasias. Somatic and germline molecular defects in adrenal cells have been described in several ion-transporting membrane proteins, including potassium channels (*KCNJ5*), voltage-gated calcium channels (*CACNA1D* and *CACNA1H*), and Na^+/K^+ pumps (*ATP1A1* and *ATP2B3*). These defects ultimately lead to increased cytoplasmic Ca^{2+} and abnormal activation of CAMK, causing autonomous secretion of aldosterone and cell proliferation. Mouse models that recapitulate these defects have contributed substantially towards our understanding of how the regulatory mechanisms of calcium homeostasis become disrupted.

2.2.1.1. TASK1/TASK3 mouse models. TASK1 (KCNK3) and TASK3 (KCNK9) are two-pore domain potassium channels that play an important role in the maintenance of the highly polarized state of the cell membrane of adrenocortical cells. These channels form heterodimers that allow a high transmembrane background potassium conductance, which maintains an electronegative gradient across the cell membrane. The activity of these channels is inhibited by angiotensin-II, which binds to its AT1 receptor and promotes

Table 1
Summary of current genetically modified mouse models of adrenal hyperplasia.

Model	Gene	Promoter/ Driver	Phenotype	Reference
Task1 KO	*Kcnk3*	Whole-body KO	Severe hyperaldosteronism. Disruption of normal zonation with ectopic expression of *Cyp11b2* in the zG.	Heitzmann et al. (2008)
Task3 KO	*Kcnk9*	Whole-body KO	Mild autonomous aldosterone production in adult animals. Severe hyperaldosteronism in newborn mice with autonomous corticosterone and progesterone secretion.	Guagliardo et al. (2012), Bandulik et al. (2013)
Task1; Task3 KO	*Kcnk3*/ *Kcnk9*	Whole-body KO	Severe hyperaldosteronism. No zonation defect.	Davies et al. (2008)
Prkar1a$^{2\Delta/+}$	*Prkar1a*	EIIA-Cre (ubiquitous)	Spectrum of tumors highly overlapping with human CC. No adrenal phenotype.	Kirschner et al. (2005)
rTA/X2AS	*Prkar1a*	Tet-Off system	Spectrum of tumors highly overlapping with human CC. Persistence of the X-zone in males and females. Nodular cortical changes. Autonomous corticosterone secretion.	Griffin et al. (2004)
AdKO	*Prkar1a*	0.5 *Akr1b7*-Cre	Autonomous corticosterone secretion. Expansion of X-zone derived aberrant progenitor-like cells that ultimately occupy the entire cortex.	Sahut-Barnola et al. (2010)
Men1$^{+/-}$	*Men1*	Whole-body KO	Spectrum of tumors highly overlapping with human MEN1. Adrenal hyperplasia to adenoma to carcinoma evolution.	Crabtree et al. (2001), Bertolino, et al. (2003), Loffler et al. (2007), Harding et. al (2009)

Abbreviations: zG, zona glomerulosa; CC, Carney complex; MEN1, Multiple endocrine neoplasia type 1; Prkar1a, Protein kinase cAMP-dependent regulatory subunit type I alpha; Kcnk3, Potassium channel subfamily K member 3; Kcnk9, Potassium channel subfamily K member 9, beta 1; Akr1b7, aldo-keto reductase family 1, member b7.