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Review

Regulation of zonation and homeostasis in the adrenal cortex

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ABSTRACT

The adult adrenal cortex is organized into concentric zones, each specialized to produce distinct steroid hormones. Cellular composition of the cortex is highly dynamic and subject to diverse signaling controls. Cortical homeostasis and regeneration rely on centripetal migration of steroidogenic cells from the outer to the inner cortex, which is accompanied by direct conversion of zona glomerulosa (zG) into zona fasciculata (zF) cells. Given the important impact of tissue structure and growth on steroidogenic function, it is essential to understand the mechanisms governing adrenal zonation and homeostasis. Towards this end, we review the distinctions between each zone by highlighting their morphological and ultra-structural features, discuss key signaling pathways influencing zonal identity, and evaluate current evidence for long-term self-renewing stem cells in the adult cortex. Finally, we review data supporting zG-to-zF transdifferentiation/direct conversion as a major mechanism of adult cortical renewal.

1. Introduction

The adult adrenal cortex is a major site of steroid hormone production in mammals. It is composed of concentric zones of steroidogenic cells surrounding the chromaffin cells of the adrenal medulla (Gallo-Payet and Battista, 2014; Yates et al., 2013). Each zone of the cortex produces distinct steroid hormones that affect a variety of physiological functions. The outer layer, the zona glomerulosa (zG) makes up about 15% of the cortex and produces aldosterone, a mineralocorticoid whose major function is to regulate intravascular volume through sodium retention and thereby controls blood pressure. Aldosterone excess in pathophysiological conditions such as primary aldosteronism can cause irreversible cardiovascular damage and ultimately lead to multi-system dysfunction (Galati et al., 2013; Magill, 2014). The inner layer, the zona fasciculata (zF), roughly eight times the size of the zG, synthesizes glucocorticoids, which have diverse effects on immunity, metabolism, development and behavior. In humans, some non-human primates (e.g., rhesus macaques, marmosets), ferrets and the spiny mouse, a third layer, the zona reticularis (zR) lies between the zF and the medulla and produces androgens (Pihlajoki et al., 2015). While traditional laboratory mice lack a true zR, a temporary zone, designated the X-zone, has been identified and is believed to be a remnant of the fetal adrenal cortex (Morohashi and Zubair, 2011).

Embryonic development of the adrenal gland is relatively well understood (Xing et al., 2015). At E9.0 in the mouse, a group of cells in the coelomic epithelium become committed to the adrenogonadal lineage by expressing *Steroidogenic factor 1* (*Sf1*). These cells then delaminate into the underlying mesenchyme and form the adrenogonadal primordium (AGP). At E10.5, a subset of AGP cells marked by *Sf1-Fetal Adrenal Enhancer (FAdE)* enhancer activity separates out to form the fetal adrenal anlagen. At around E12.5, neural crest cells migrate into the fetal adrenal and become precursors of the medulla. The fetal cortex starts to regress at E14.5 as the definitive cortex emerges beneath the newly formed capsule. Lineage tracing studies have shown that the definitive cortex arises from the fetal cortex and later on gives rise to the adult cortex (Wood et al., 2013; Zubair et al., 2008).

Proper control of steroidogenic function in the adult adrenal cortex relies not only on appropriate endocrine signaling but also on the integrity of tissue structure and homeostasis (Gallo-Payet and Battista, 2014). Disruption of zonation and homeostasis has been implicated in many adrenal diseases such as primary

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aldosteronism, cortisol-producing adenomas, primary pigmented nodular adrenocortical disease (PPNAD), congenital adrenal hyper- and hypoplasia and adrenocortical carcinoma (Walczak and Hammer, 2014). However, the cellular and molecular mechanisms that maintain normal tissue homeostasis in the adult cortex remain poorly understood. Hence, this review highlights our current knowledge of adult adrenocortical homeostasis and zonation, with an emphasis on 1) adrenal morphology and ultrastructure, 2) signaling pathways important for control of zonation, 3) evidence for adrenocortical stem cells and 4) transdifferentiation/direct conversion between differentiated cells.

2. Adrenal zonation: morphology and ultrastructure

The adrenal cortex is an epithelial tissue enveloped in a mesenchymal capsule. As part of an epithelial structure, adrenocortical cells express epithelial markers such as laminin I and cytokeratins, markers of the basement membrane such as type IV collagen and a diverse array of laminin-associated integrin subunits (i.e., alpha 3, beta 1) (Campbell et al., 2003; Otis et al., 2007; Virtanen et al., 2003; Miettinen et al., 1985). However, in contrast to classical epithelial tissues (e.g., as found in the intestine) adrenocortical cells do not express the epithelial cell marker E-cadherin, but instead express N-cadherin, generally thought to be a neuronal marker (Tsuchiya et al., 2006). Morphologically, the cortical zones demonstrate clear differences in their cellular structure and organization. For instance, cells in the zG are arranged in discrete cellular clusters, referred to as glomeruli, which are surrounded by basement membrane proteins and a capillary network extending from the capsule (Otis et al., 2007). Cells in each glomerulus are densely packed, possess little cytoplasm and present with apposition of large membrane domains. Electron microscopic analysis reveals the presence of narrow gap junctions and a limited number of lipid droplets and mitochondria with lamelliform cristae. In addition, rough endoplasmic reticulum is more abundant than the smooth endoplasmic reticulum (Black et al., 1979; Friend and Gilula, 1972; Nussdorfer, 1980). Notably, the structure of the zG is highly conserved among many species (Nussdorfer, 1980). Along with a morphological identity, zG cells possess a particular molecular signature and can be identified by the presence of patches of Cyp11b2 (Aldosterone Synthase, AS)-expressing cells (Fig. 1) (Walczak et al., 2014) and by the expression of Disabled homolog 2 (Dab2; Romero et al., 2007), Protein delta homolog 1 (Dlk1; Halder et al., 1998) and β-catenin (Figs. 1 and 3), (as discussed below) (Eberhart and Argani, 2001; Walczak et al., 2014). Given the strong association between the region of β-catenin positivity and the morphologically identifiable zG, it is tempting to speculate that β-catenin may promote a transcriptional program that leads to the distinct zG morphology.

The cells in the zF, which produce glucocorticoids in response to the adrenocorticotropic hormone (ACTH), have strikingly different morphological and ultra-structural features. zF cells are organized in cord-like structures flanked by fenestrated blood vessels, which facilitate the rapid exchange of hormones between the circulation and steroidogenic cells. Cells in the zF are larger and less densely packed than those in the zG and possess a well developed smooth endoplasmic reticulum, large gap junctions, many lipid droplets, and mitochondria characterized by tubule-vesicular cristae (Black et al., 1979; Nussdorfer, 1980). zF cells are commonly identified by the expression of Cyp11b1 (11-beta hydroxylase, 11β-OH; Gomez-Sanchez et al., 2014) and Akr1b7 (aldo-keto reductase family 1, member B7) (Aigueperse et al., 1999).

The cells of the zR produce dehydroepiandrostenedione (DHEA) and the sulfated derivative DHEA-S during fetal life and upon adrenarche in humans, some non-human primates, ferrets and the spiny mouse (Pihlajoki et al., 2015). zR cells display similar characteristics to zF cells, though they tend to exhibit less lipid droplets, more lysosomes and lipofuscin pigment granules (Rhodin, 1971). The X-zone in mice represents a transient, fetal-derived region enriched in eosinophilic cells located between the zF and the cortical-medullary boundary (Morohashi and Zubair, 2011). The cells of the X-zone are smaller than zF cells, with varied degrees of cytoplasmic density and display diverse mitochondrial shapes endowed mainly with tubular cristae (Sato, 1968). The X-zone seems to be linked to catabolism of progesterone (Hershkovitz et al., 2007).

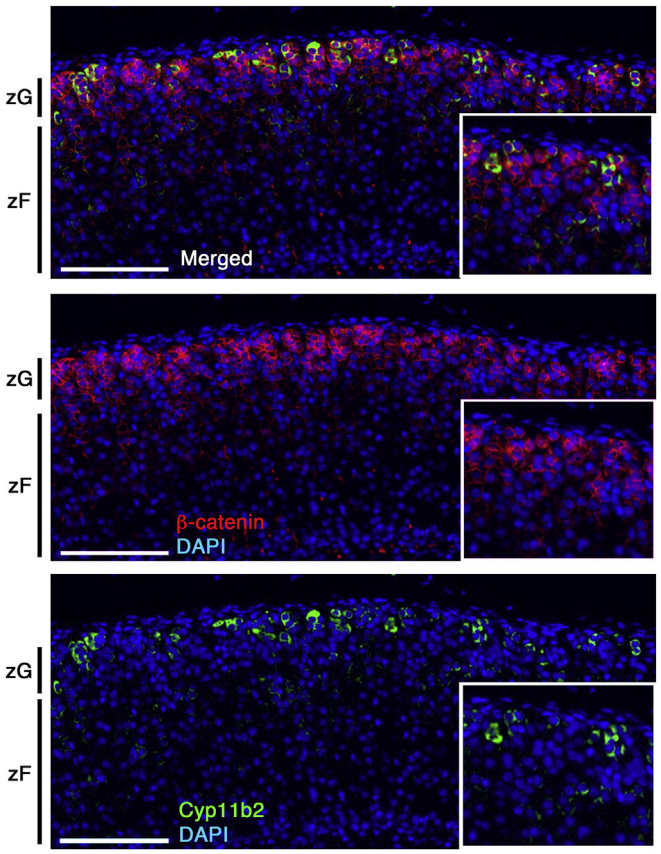

Fig. 1. Cyp11b2 is expressed in a patchy fashion in the zG. Immunofluorescent staining of paraffin sections from a wild-type adrenal cortex. The expression pattern of Cyp11b2 is restricted to the putative zG region, marked by β-catenin. However, not all β-catenin positive cells also express Cyp11b2, indicating that Wnt/β-catenin signaling is likely necessary but not sufficient for the synthesis of aldosterone. Insets are magnified details of the larger image. Scale bar: 100 μm.

The existence of morphologically different zones in the adrenal cortex, without the presence of physical barriers between them, suggests the presence of molecular cues that tightly control the identity of each zone. In the following section we will review data that implicate several signaling pathways in the regulation of zonation.

3. Signaling pathways and zonation

Over the past 15 years, significant advances have led to an increased understanding of how Angiotensin II (AngII), potassium ions (K^+) and ACTH, as well as their corresponding downstream signaling pathways, contribute to zonation. Important progress has also been made regarding the role of the canonical Wnt/β-catenin signaling pathway in maintaining proper zonation. In addition,