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Regulation of the adrenocortical stem cell niche: implications for disease

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Abstract

Stem cells are endowed with the potential for self-renewal and multipotency. Pluripotent embryonic stem cells have an early role in the formation of the three germ layers (ectoderm, mesoderm and endoderm), whereas adult tissue stem cells and progenitor cells are critical mediators of organ homeostasis. The adrenal cortex is an exceptionally dynamic endocrine organ that is homeostatically maintained by paracrine and endocrine signals throughout postnatal life. In the past decade, much has been learned about the stem and progenitor cells of the adrenal cortex and the multiple roles that these cell populations have in normal development and homeostasis of the adrenal gland and in adrenal diseases. In this Review, we discuss the evidence for the presence of adrenocortical stem cells, as well as the various signalling molecules and transcriptional networks that are critical for the embryological establishment and postnatal maintenance of this vital population of cells. The implications of these pathways and cells in the pathophysiology of disease are also addressed.

Introduction

The adrenal cortex produces different corticosteroid hormones necessary for human life. The organ is subdivided into discrete histological and functional steroidogenic cell layers under the control of distinct endocrine signals. Despite the fairly concentric zonation of these layers under normal physiological conditions, dynamic centripetal ‘streaming’ of adrenocortical cells occurs throughout life. Adrenocortical cells proliferate under the capsule and are displaced centripetally until they undergo apoptosis at the adrenocortical–medullary boundary. Maintenance of adrenal volume and function presumably necessitates replenishment of steroidogenic cells from a pool of somatic stem and progenitor cells. Pluripotent embryonic stem cells have an early role in the formation of the three germ layers (ectoderm, mesoderm and endoderm), whereas somatic stem cells are responsible for postdevelopmental and homeostatic tissue maintenance of most organs.[1] Such cells are

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described as long-lived, slow-cycling and clonogenic cells, and simultaneously possess the abilities of self-renewal and terminal differentiation. Whereas stem cells retain the capacity to proliferate indefinitely, their daughter progenitor cells are more committed in lineage and are thought to possess limited replicative potential.[2]

In this Review, we discuss the current knowledge on the establishment and maintenance of adrenocortical stem and progenitor cells. We first discuss basic adrenal biology and detail evidence for the presence of adrenocortical cells with stem or progenitor-like capacities. We then describe the process of adrenal development, postnatal tissue maintenance and the various origins and descendants of adrenocortical cell lineages. We summarize how adrenal organogenesis and postnatal homeostasis are regulated by a large array of signalling molecules, including combinatorial inputs from distinct paracrine signalling pathways and the endocrine system. Clinical consequences of stem cell failure and unmitigated activation of associated paracrine signalling pathways are also discussed.

Adrenal anatomy and function

The adrenal gland is composed of two discrete endocrine organs with distinct embryological origins. The inner adrenal medulla, formed from the neural crest, produces catecholamines that are mediators of the ‘fight-or-flight’ response. The outer adrenal cortex, derived from the intermediate mesoderm, is the primary site of corticosteroid biosynthesis. The organization of the adrenal cortex was first described in 1866 by Julius Arnold, whose nomenclature remains in use today.[3] The adrenal cortex is subdivided into three separate histological and functional zones, each responsible for the production of steroid hormones that mediate different aspects of stress response and homeostasis. The outermost layer, the zona glomerulosa, is composed of cellular rosettes that secrete the mineralocorticoid aldosterone, which contributes to maintenance of electrolyte balance, under the control of serum potassium levels and the renin–angiotensin–aldosterone system (RAAS). When stimulated by the hypothalamic–pituitary–adrenal (HPA) axis, the middle zona fasciculata produces glucocorticoids (cortisol in humans and corticosterone in mice) to facilitate the mobilization of energy stores in response to stress (real or perceived threats to body integrity). The innermost zona reticularis contains a network of cells that synthesize androstenedione and dehydroepiandrosterone that are precursors to sex steroid hormones. The developmental establishment of the adrenal cortex occurs similarly in most mammals,[4] yet zonal differences exist between species. Whereas humans and primates have the three adrenocortical zones described above, rodents lack the zona reticularis.

Evidence for adrenocortical stem cells

Many studies have provided evidence for the existence of adrenocortical cells with stem-like and progenitor-like capacities. Undifferentiated adrenocortical cells with limited steroidogenic capacity have been described across mammalian species. In mice and humans, the outermost layer of the adrenal cortex, the zona glomerulosa, contains differentiated aldosterone-producing cells intermingled with clusters of undifferentiated cells.[5,6] In rats, an undifferentiated zone exists between the zona glomerulosa and the zona fasciculata, and is referred to as the zona intermedia.[7] In the adrenal glands of the common seal (*Phoca*

vitulina vitulina), groupings of large rounded cells that have been described as ‘adrenocortical blastema’ (undifferentiated cells capable of adrenocortical regeneration) are found adjacent to trabeculae of connective tissue extending from the capsule.[8] These cell clusters are likely to represent homologous groups of adrenocortical stem and progenitor cells in the different species.

The continued proliferative capacity of the adult adrenal gland to maintain adrenal volume and function throughout life is consistent with the presence of a stem-like population of cells. In experiments performed as early as the 1930s, complete restoration of adrenocortical mass was observed within 6 weeks after adrenal enucleation (removal of most of the cortex and medulla that leaves behind just the capsule and the underlying outermost cortical layer),[9] which is consistent with clonal expansion and differentiation of a peripheral stem and progenitor cell population. More recent studies demonstrated that when primary cultures of bovine adrenocortical cells were transplanted underneath the kidney capsules of adrenalectomized immunocompromised mice, functional vascularized steroid-secreting adrenocortical tissue was reconstituted.[10,11] Under normal homeostatic conditions, cells proliferate in the outermost layers of the adrenal cortex and capsule. Tritiated thymidine and bromodeoxyuridine (BrdU) pulse–chase experiments in rodents have established that cells labelled in the periphery of the adrenal cortex are centripetally displaced until they reach the cortical–medullary boundary where they undergo apoptosis.[12–15] Observations of chimaeric transgenic mice expressing a reporter gene in a radial pattern of columns throughout the adrenal cortex[16–18] support such a clonal relationship between peripheral stem cells and differentiated adrenocortical cells. Seminal studies performed over 70 years ago also demonstrated that capsular cells give rise to adrenocortical cells. In pulse–chase experiments utilizing trypan blue injections, labelled capsular cells became evident within the adrenal cortex following a 2–30 day chase period.[19] Genetic experiments within the past decade have defined the clonal relationship between inner adrenocortical cells and overlying capsular and subcapsular cells (see discussion below and Figure 1).

Adrenal development and homeostasis

Overview of adrenal organogenesis

Cells destined to become adrenocortical cells originate from the coelomic epithelium and condense to form a shared embryological structure, the adrenogonadal primordium (AGP), that is the precursor of the steroidogenic organs, the gonads and the adrenal glands. The AGP appears around 3–4 weeks postconception in humans and embryonic day (E) 9.0 in mice.[20–22] Establishment of the AGP is followed by its separation into adrenal and gonadal primordia, with subsequent mesenchymal cell encapsulation of the adrenal primordium (the ‘fetal adrenal gland’) by 9 weeks postconception in humans or E12.5 in mice. Concomitant with encapsulation of the fetal adrenal gland, sympathoadrenal precursors destined to become the adrenal medulla begin migrating into the fetal adrenal gland from the neural crest.[4,23] Following encapsulation, the developing adrenal gland grows owing to the emergence of definitive or ‘adult’ adrenocortical cells between the fetal gland and the capsule while the fetal adrenocortical cells begin to regress (Figure 1). At the time of birth, the majority of the adrenal cortex is comprised of definitive adrenocortical cells, whereas

only a small ring of the fetal adrenocortical cells remains.[4] This remaining layer of fetal adrenocortical cells regresses shortly after birth in humans, but does not disappear until puberty in male mice and during the first pregnancy in female mice.

Postnatal growth and homeostasis

Definitive adrenocortical cells emerge during late embryogenesis and zonation of the adrenal cortex into distinct steroidogenic layers is completed in the perinatal period with the onset of specific expression of *Cyp11b2* in the zona glomerulosa.[24] In humans and some nonhuman primates, the zona reticularis emerges at the onset of adrenarche, an early step in sexual maturation that is initiated by androgen secretion from the adrenal cortex.[25] Homeostasis of the postnatal adrenal gland is maintained by the balance between cell proliferation at the outer cortex, centripetal migration of differentiating cells, and apoptosis of cells at the cortical–medullary boundary (Figure 2). As discussed above, some of the first studies of adrenal gland biology in the early 1930s observed that adult adrenal glands contain a subset of peripheral cells with continual proliferative potential and the capacity to regenerate adrenocortical tissue. Data published in 2013 confirmed and expanded these earlier observations by showing that newly formed, proliferating cells (as seen with BrdU labelling) in the outer adult adrenal cortex do not colocalize with steroidogenic markers, but become differentiated as they are centripetally displaced.[15] Additionally, a small fraction of label-retaining cells remains in the capsule and subcapsular cortex 23 weeks after a BrdU pulse.[15] This finding is consistent with the presence of a slowly cycling or fairly quiescent stem cell population. These data further support the idea that homeostatic adrenocortical growth is maintained by a persistent population of undifferentiated cells existing in both the capsule and the outer adrenal cortex.

Adrenocortical stem cell lineages

The search for adrenocortical stem and progenitor cells has revealed very complex cell lineage relationships in which the adrenal capsule provides precursors of adult adrenocortical cells and that at least some capsular cells are derived from fetal adrenal cells of the adrenal primordium (Figure 1).

Fetal adrenal cells give rise to adult cells

AGP formation and subsequent adrenal gland development is critically dependent on the expression of *Nr5a1*, which encodes the nuclear receptor steroidogenic factor 1 (SF-1, also known as adrenal 4-binding protein or steroid hormone receptor Ad4BP). Genetic loss of *Nr5a1* or transcriptional regulators that activate *Nr5a1* expression, such as those encoded by *Pbx1*,[26] *Wt1*[27,28] and *Cited2*,[29] results in complete AGP or adrenal gland agenesis (Table 1).[21] In addition to its presence in the adrenal glands and gonads, SF-1 expression is found in nonsteroidogenic tissues such as the ventromedial hypothalamus,[30] pituitary gonadotropes[31] and the spleen.[32] Distinct tissue-specific enhancers of the *Nr5a1* gene mediate expression of SF-1 in these organs. Analysis of the transcriptional regulation of *Nr5a1* identified a fetal adrenal enhancer (*FAdE*), whose activity is solely restricted to fetal adrenal cells. Activation of *FAdE* is initiated by complexes of transcription factors (the homeobox proteins PBX1, PKNOX1 and HOX) and later maintained in an autoregulatory

manner by SF-1.[33] Lineage-tracing experiments utilizing *FAdE*-driven Cre recombinase (*FAdE-Ad4bp-Cre*) revealed that *FAdE*-driven-*Ad4bp*-expressing cells were precursors of most, if not all, SF-1-positive (SF-1$^+$) cells in the adult adrenal cortex.[34] Interestingly, experiments using a *FAdE-CreERT2* inducible allele demonstrated that only *FAdE*-driven-*Ad4bp*-expressing cells that were marked prior to fetal adrenal encapsulation could be precursors of adult adrenal cells. When induction takes place on or after E14.5, *FAdE*-derived adult adrenocortical cells were no longer detected.[34] These data suggest that an initial population of fetal adrenal cells contributes to the lineage of adult adrenocortical cells, a process that occurs exclusively during early embryonic life.

Capsular cells give rise to cortical cells

Trypan-blue lineage tracing experiments from the early 20th century were the first to suggest that adrenocortical cells are derived from the overlying SF-1-negative (SF-1$^-$) mesenchymal capsule.[19] Within the past decade, data from three independent laboratories studying the Hedgehog pathway in the adrenal gland also provided genetic evidence for this phenomenon. A family of secreted Hedgehog molecules, consisting of sonic hedgehog (SHH), desert hedgehog (DHH) and indian hedgehog (IHH), exists in mammals. These ligands bind to the cell-surface receptor protein patched homolog 1 (PTC1). In the absence of Hedgehog ligands, PTC1 inhibits the positive signal transducer smoothened homolog (SMO), allowing proteolytic processing of GLI transcription factors, which generates transcriptional corepressor forms of these proteins. Hedgehog binding to PTC1 relieves inhibition of SMO, which results in activation of downstream signalling that inhibits proteolysis of GLI proteins. Hedgehog target genes, including *Gli1* and *Ptch1* (which encodes PTC1), are induced by active GLI transcription factors and are important for feedback regulation of this signalling pathway.[35] In the adrenal gland, SHH is expressed in peripheral SF-1$^+$ adrenocortical cells from E12.5 onward (Figure 3).[36–38] These cells rarely colocalize with steroido-genic markers during development or postnatal life and remain fairly undifferentiated.[36] The SHH protein secreted by SF-1$^+$ cells acts upon SF-1$^-$ cells that reside within an inner layer of the adrenal capsule; these cells express *Smo*, *Ptch1* and *Gli1*.[36–38] Cell lineage analysis with mice expressing the *ROSA26-YFP* reporter construct crossed with mice harbouring a Cre recombinase replacing the endogenous *Shh* locus (*Shh^{gfpcre}:R26-YFP*) revealed that, postnatally, all cells of the cortex (but not of the capsule or medulla) were derived from *Shh*-expressing fetal cells.[36] Further experiments utilizing mice with an inducible *Shh*-Cre allele crossed with mice expressing the *ROSA26-mT/mG* reporter (*Shh-CreT2:R26-mR/mG* mice) showed that shortly after labelling, *Shh*-expressing cells remain restricted to the peripheral cortex.[36] Over time, these cells and their descendants form clonal, radial stripes that extend deep into the cortex and colocalize with steroidogenic cells of both the zona glomerulosa and the zona fasciculata. In addition to *Shh*-expressing cells, the lineage of *Gli1*$^+$ (SHH-receiving) cells was also investigated. Employing an inducible *Gli1-CreERT2* allele, investigators demonstrated that during adrenal development SF-1$^-$;*Gli1*$^+$ capsular cells give rise to SF-1$^+$;*Gli1*$^-$ undifferentiated SHH$^+$ cells, as well as to SF-1$^+$;*Gli1*$^-$ steroidogenic cells.[36] Postnatal lineage tracing experiments had similar results.[36,39] Collectively, these data suggest that SF-1$^-$;*Gli1*$^+$ capsular cells are stem cells or precursor cells of SF-1$^+$;SHH$^+$ undifferentiated progenitors

and differentiated steroidogenic cells during adrenal organogenesis and postnatal maintenance.

Pathological stress can engage different pools of stem cells and precursor cells to participate in tissue regeneration in a variety of organ systems.[40] During normal maintenance of the adult adrenal cortex, mesenchymal capsular cells expressing Wilms tumour protein homolog (WT1) can contribute to the steroidogenic lineage, albeit infrequently.[41] However, engagement of the $WT1^+$ capsular population is more robust following gonadectomy. Using *Wt1-CreERT2: ROSA*$^{mT/mG}$ mice, investigators demonstrated that cords of GFP^+ descendants of $WT1^+$ cells give rise to $WT1^-$;$SF\text{-}1^+$ cells that express the gonadal genes *Cyp17a1* and luteinizing hormone/choriogonadotropin receptor (*Lhcgr*).[41] These data indicate that the adrenal capsule contains several populations of multipotent adrenocortical stem and progenitor cells that have the capacity to differentiate into adrenal-like or gonadal-like cells in response to activation of the HPA versus the hypothalamic–pituitary–gonadal axes.

Fetal adrenal cells become capsular stem cells

Data discussed above indicate that both fetal adrenal cells expressing *Nr5a1* under the control of the *FAdE* enhancer and $SF\text{-}1^-$ capsular cells are precursors of definitive adrenocortical cells. A unifying model integrating these observations would predict that a subpopulation of fetal adrenal cells could contribute to the forming capsule and switch off *Nr5a1* expression.[42] These cells or their descendants would express *Gli1* in the capsule and give rise to adult adrenocortical cells expressing *Nr5a1* under the control of the definitive enhancer as demonstrated previously.[36] Research at our laboratory has provided experimental evidence supporting this model. To investigate whether fetal adrenal cells in which *FAdE* is activated give rise to capsular cells, *FAdE-Ad4bp-Cre* mice were crossed with mice expressing the *ROSA26-tdTomato/eGFP* reporter (generating *FAdE-Ad4bp-Cre:R26R*$^{mT/mG}$ mice) and transgenic adrenal glands were evaluated at E18.5 and adulthood. In *FAdE-Ad4bp-Cre:R26R*$^{mT/mG}$ adrenal glands, GFP^+ descendants from *FAdE*-driven-*Ad4bp*-expressing cells colocalized with steroidogenic cells of the adult adrenal cortex as observed previously.[34] Of note, a subset the $SF\text{-}1^-$ capsular cells also expressed GFP.[39] When *FAdE-Ad4bp-Cre:R26R*$^{mT/mG}$ mice were bred with mice expressing the *Gli1-LacZ* reporter, *FAdE-Ad4bp-Cre:R26R*$^{mT/mG}$*:Gli1-LacZ* adrenal glands contained capsular cells that coexpressed GFP and LacZ.[39] These data collectively demonstrate that SF-1 expression is extinguished in the capsular descendants of fetal adrenal cells, which contribute to the population of $SF\text{-}1^-$;$Gli1^+$ capsular cells (Figure 1). The data unify the seemingly conflicting reports on the cell lineage relationships of adult adrenocortical precursors.

Zonation and lineage conversion

Definitive adrenocortical cells arise during embryogenesis, but zonation of the adrenal cortex into distinct steroidogenic layers occurs perinatally. The pattern of zonation is fairly constant under normal physiological conditions but the process of centripetal displacement suggests that each cell transits through various compartments of the adrenal cortex throughout its limited lifespan. In 1883, Max Gottschau proposed the centripetal migration model in which progenitor cells in the peripheral cortex of the adrenal gland first

differentiate into mineralocorticoid-producing cells in the zona glomerulosa and upon centripetal migration transform into glucocorticoid-producing cells of the zona fasciculata.[43] On the basis of the disparate effects of hypophysectomy on the zona glomerulosa and zona fasciculata (broadening of the zona glomerulosa with atrophy of the zona fasciculata), along with the distinct functions of the steroids produced from these zones (previously discussed), a separate model hypothesized that each steroidogenic layer contains its own progenitor pool.[44] Although tritiated thymidine, BrdU and trypan blue pulse–chase experiments support the centripetal migration model, none of these studies directly tested lineage conversion of a zona glomerulosa cell to a zona fasciculata cell. Genetic evidence for this conversion was provided in 2013 by cell-lineage-tracing experiments in mice harbouring a Cre recombinase gene inserted at the *Cyp11b2* locus, in which *Cre* was expressed only in terminally differentiated zona glomerulosa cells (AS^{Cre} mice).[45] When these mice were crossed with mice expressing the $R26R^{mT/mG}$ reporter, GFP^+ cells appeared in the zona glomerulosa from the outset of *Cyp11b2* expression and eventually populated the entire zona glomerulosa.[45] Of note, *Cyp11b1*-expressing cells in the zona fasciculata (which do not express *Cyp11b2*) also became GFP^+ over time, indicating that these cells underwent lineage conversion from zona glomerulosa cells. In 12-week-old mice, nearly the entire cortex was GFP^+.[45] This phenomenon was also observed under forced homeostatic maintenance of the cortex, during the process of regeneration following dexamethasone-induced atrophy of the zona fasciculata; 8 weeks following withdrawal of dexamethasone, $AS^{+/Cre}$:$R26R^{+/mTmG}$ mice exhibited GFP^+ cells in the zona fasciculata,[45] indicating lineage conversion of zona glomerulosa cells to zona fasciculata cells still occurred under this homeostatic paradigm.

Regulation of adrenocortical stem cells

Transcription factors

SF-1—As mentioned above, SF-1 is an essential regulator of adrenal development and steroidogenic function. Two laboratories independently identified SF-1/Ad4BP as the nuclear receptor that activates transcription of the steroidogenic enzymes responsible for catalyzing steroid biosynthesis.[46,47] Shortly thereafter, the importance of SF-1 in specification of adrenal cell identity and in adrenal growth became evident from studies of mice in which the *Nr5a1* gene was knocked out. SF-1 expression starts at E9.0 in the urogenital ridge, where it specifies precursors of the steroidogenic lineage and leads to the formation of the AGP.[48] Mice globally deficient for SF-1 exhibit degeneration of the AGP due to apoptosis between E11.5 and E12.0, which results in agenesis of the adrenal glands and gonads.[21] Unlike *Nr5a1* knockout animals, mice heterozygous for *Nr5a1* survive to adulthood yet possess smaller adrenal glands that have reduced capacity for corticosterone production under conditions of stress.[49] Of note, *Nr5a1* heterozygotes are unable to mount a compensatory growth response following unilateral adrenalectomy owing to lack of peripheral adrenal cell proliferation.[50] Although the incidence of *NR5A1* mutations in humans is low, patients with mutations in the DNA-binding domain of SF-1 exhibit primary adrenal failure and gonadal dysgenesis (Table 1).[51,52]

In a genetic mouse model in which SF-1 overexpression is mediated by the *FAdE* enhancer (*FAdE-Ad4BP-[Ad4BP/SF-1]* transgenic mice), the animals exhibit ectopic formation of

adrenal tissue throughout the thoracic cavity.[53] Similarly, forced expression of SF-1 in embryonic[54] and mesenchymal stem cells[55] is sufficient to promote steroidogenic cell differentiation *in vitro*. Other studies have demonstrated that increased subcapsular proliferation occurs in mice with transgenic overexpression of SF-1.[56] Amplification and overexpression of SF-1 are associated with paediatric adrenocortical adenomas and carcinomas,[57–59] and are correlated with poor clinical outcomes in patients with adrenocortical carcinomas (Table 1).[60]

Altogether, these data highlight a critical role for SF-1 in the regulation of adrenocortical cell specification, differentiation and proliferation. How does SF-1 mediate the seemingly opposing tasks of proliferation and differentiation? Regulation of the transcriptional activity of SF-1 by post-translational modifications and/or stimulatory and inhibitory ligands has been posited to dictate what genetic programs are enacted by SF-1; this topic has been reviewed elsewhere.[61,62] The role of these modifications on the transcriptional activity of SF-1 and its effects on homeostatic maintenance is an area of active investigation.

DAX-1—The *Nr0b1* gene encodes DAX-1 (nuclear receptor subfamily 0 group B member 1), an atypical nuclear receptor that lacks a classic nuclear receptor DNA-binding domain, instead containing three-and-a-half repeated amino-acid stretches rich in glycine and alanine.[63] Initial reports showed that DAX-1 functions as a negative regulator of SF-1-mediated transcription[64] and these findings were subsequently extended to other nuclear receptors.[65] Whereas DAX-1 recruits the corepressors NCoR and ALIEN for transcriptional inhibition,[66,67] data suggest DAX-1 also has a dose-dependent coactivator capacity.[68] DAX-1 inhibits SF-1-mediated steroidogenesis *in vitro* and *in vivo*.[69] Additionally, DAX-1 directly represses *Star*, which encodes steroidogenic acute regulatory protein, by binding to hairpin structures present in the promoter region of this gene.[70] Whether DAX-1 exerts its inhibitory actions on steroidogenesis in differentiated cells or in progenitor cells in which steroidogenesis is uniquely down-regulated was unclear. More recent studies have shown that DAX-1 is essential to maintain embryonic stem cell pluripotency.[71–74] Data from our laboratory show that in the adult murine adrenal cortex, DAX-1 is critical for maintenance of adrenocortical progenitor cells. When compared with wild-type littermates, DAX-1 knockout mice exhibit enhanced steroidogenesis and proliferation at early ages but display progressive loss of proliferating cells, which is associated with adrenal gland dysplasia and hypofunction, as they age.[75]

Mutations or deletion of *NR0B1* in humans are the underlying basis of X-linked adrenal hypoplasia congenita (AHC; Table 1).[63,76] Classically, these patients present with primary adrenal insufficiency and hypogonadotropic hypogonadism, although a subset of patients exhibit signs of adrenal hyperfunction prior to adrenal failure.[77,78] These human data together with the DAX-1 knockout mouse study indicate that DAX-1 deficiency leads to precocious differentiation of adrenocortical progenitor cells, which ultimately leads to progenitor depletion and adrenal insufficiency.

Paracrine signalling pathways

Wnt signalling—The mammalian wingless-type MMTV integration site (Wnt) pathway is a paracrine signalling pathway critically involved in development and stem-cell maintenance in multiple organ systems.[79] Secreted Wnt molecules bind to a variety of receptors (including the frizzled family, and the receptor tyrosine kinases RYK and ROR) to elicit distinct downstream signalling through the canonical Wnt–β-catenin pathway and/or the noncanonical Wnt (planar cell polarity) and calcium signalling pathways.[80] For activation of the canonical pathway, Wnt ligands bind to frizzled receptors resulting in the inactivation of the multiprotein complex that promotes the destruction of the transcription factor β-catenin. Once freed from the destruction complex, β-catenin enters the nucleus and transactivates Wnt-responsive genes through interaction with DNA-bound TCF/LEF transcriptional regulators. In the developing adrenal glands of mice, β-catenin expression occurs as early as E12.5.[81] In transgenic mice expressing reporter genes, active canonical Wnt–β-catenin signalling is evident in a few scattered cells in the periphery of the adrenal gland at this time. By E18.5, Wnt–β-catenin signalling is restricted to the outer subcapsular region of the adrenal cortex.[81] To ablate β-catenin expression in adrenocortical cells, *Ctnnb1*^{tm2kem} mice were crossed with *Sf1/Cre*^{high} mice. *Sf1/Cre*^{high} mice have five copies of the *Sf1/Cre* transgene, which results in high Cre expression in SF-1^{+} cells, and *Sf1/Cre*^{high}:*Ctnnb1*^{tm2kem} mice have little or no β-catenin in SF-1 expressing cells.[81] Adrenal gland development is initiated in the absence of β-catenin; however, the proliferation of adrenocortical cells is dramatically decreased between E12.5 and E14.5, and the entire gland disappears by E18.5.[81] *Sf1/Cre*^{low} mice contain only one copy of the *Sf1/Cre* transgene and exhibit inefficient Cre recombination. In *Sf1/Cre*^{low}*:Ctnnb1*^{tm2kem} mice, a subset of cells escapes recombination leading to an overall ~50% decrease in β-catenin-mediated Wnt signalling.[81] The adrenal glands of *Sf1/Cre*^{low}*:Ctnnb1*^{tm2kem} mice develop fairly normally until 15 weeks of age, after which the adrenal cortex undergoes progressive thinning, marked by loss of SF-1^{+} cells, which results in adrenal failure by 45 weeks of age.[81] Of note, *Nr0b1*, which encodes DAX-1, is a target gene of β-catenin; DAX-1-deficient adrenal glands in part phenocopy *Sf1/Cre*^{low}*:Ctnnb1*^{tm2kem} adrenal glands.[75,82] Conversely, mice in which adrenocortical Wnt–β-catenin signalling is upregulated, either through genetic deletion of *Apc* (a factor critical for adequate function of the β-catenin destruction complex) or through gain of a constitutively active *Ctnnb1* allele, display increased subcapsular proliferation[83,84] and exhibit progenitor cell expansion.[84] In addition, observations in mice suggest β-catenin facilitates the differentiation of zona glomerulosa cells while suppressing differentiation of the zona fasciculata through indirect regulation of aldosterone production,[83,85] as well as through inhibition of *Cyp11b1* expression and corticosterone production.[86] Alterations resulting in Wnt pathway activation, such as genetic loss of *APC*[87] or gain-of-function mutations in *CTNNB1*,[88] are frequent perturbations in human adrenocortical carcinomas and are correlated with poor prognosis (Table 1).[84,88,89] These collective data support a role for Wnt–β-catenin signalling in the maintenance and commitment of the adrenocortical progenitor pool.

The WNT4 ligand is expressed in the developing adrenal glands of mice as early as E11.5, with restricted expression in the periphery of the cortex observable by E14.5.[5] Whether *Wnt4* expression is a cause or a consequence of the activated canonical Wnt pathway

remains unclear, as *Wnt4* is genetically downstream of β-catenin in other tissues.[90,91] Mice globally deficient for *Wnt4* have fairly normal adrenal gland size and morphology during development, but exhibit reduced *Cyp11b2* expression at birth resulting in decreased aldosterone production.[5] *Wnt4*-knockout mice also contain ectopic adrenocortical cells at the anterior tips of the gonads, the gonadal region that is closest to the forming adrenal glands. Given the shared lineage between the adrenal glands and the gonads, these data suggest that WNT4 regulates adrenal cell homing or specification during separation of the AGP in early adrenal development.[5,92] In humans, *WNT4* loss-of-function mutations result in SERKAL syndrome, an autosomal-recessive disorder whose manifestations include female sex reversal and dysgenesis of the kidneys, lungs and adrenal glands (Table 1).[93] In contrast to mice, human fetuses with SERKAL syndrome have reduced adrenal gland size but fairly normal adrenal histology.[93]

SHH signalling—SHH signalling is implicated in developmental patterning and stem cell biology in many tissues in species from flies to humans.[35] Evidence for the importance of SHH signalling in adrenal gland development arose from the discovery that frameshift mutations that result in truncation of GLI3 into a constitutive repressor are the underlying cause of Pallister–Hall syndrome (PHS).[94] PHS is characterized by a constellation of congenital anomalies including polydactyly, hypothalamic hamartoma, kidney abnormalities and adrenal insufficiency.[95] Studies of a genetic mouse model of PHS with a truncated GLI3 protein (GLI3Δ699) have led to discrepant findings; one research group reported these mice had adrenal aplasia,[96] whereas another group found no adrenal malformations in these animals.[97] Despite the findings of the second of these studies, several reports have highlighted a critical role for SHH in adrenocortical progenitor biology, as previously discussed. Mice with global *Shh* ablation ($Shh^{-/-}$ mice[36]), as well as mice with conditional ablation of *Shh* in steroidogenic cells ($Sf1/Cre{:}Shh^{fl/fl}$ and $Sf1/Cre{:}Shh^{fl/-}$ mice[36–38]), exhibit hypoplastic adrenal glands during embryonic development, owing to reduced cortical and capsular cell proliferation (Table 1).[37,38] Adult $Sf1/Cre{:}Shh^{fl/-}$ adrenal glands contain fewer cells that are hypertrophic yet retain the capacity of steroid secretion.[38] Despite having smaller adrenal glands, *Shh*-deficient mice have adrenal histology and organization comparable to those in their wild-type counterparts, indicating that adrenocortical encapsulation and zonation, as well as migration of medullary cells, do not rely on SHH signalling. However, $Sf1/Cre{:}Shh^{fl/-}$ embryonic and adult adrenal glands possess markedly thinner capsules compared with wild-type glands.[36,38] Coupled with the observation that *Shh*-deficient mice have reduced capsular proliferation, these data indicate SHH might be a regulator of mitogenic signalling for capsular stem and progenitor cells.

FGF signalling—The complexity of the fibroblast growth factor (FGF) signalling pathway is due to the diversity of ligands, receptors and receptor isoforms, as well as to the diversity of the intracellular signalling cascades that participate in FGF signal transduction. Binding of FGFs to FGF receptor (FGFR) tyrosine kinases is facilitated by transmembrane heparan sulfate proteoglycans. Ligand binding promotes FGFR dimerization and autophosphorylation, which can stimulate multiple downstream signalling pathways, including the JAK–STAT (Janus kinase and signal transducer and activator of transcription), phos-pholipase C (PLC) γ, PI3K (phosphatidylinositol-4,5- bisphosphate 3-kinase) and

MAPK (mitogen-activated protein kinase) pathways.[98] Both the adrenal capsule and the adrenal cortex express components of the FGF pathway (Figure 3). FGF-2 and FGF-9 expressed in the capsule signal to FGFR-1 IIIc and FGFR-2 IIIc, which are expressed in both the cortex and the capsule.[99,100] FGF-1 secreted from the cortex is thought to elicit signalling from FGFR-1 IIIc, FGFR-2 IIIb and FGFR-2 IIIc, which are expressed in both compartments.[99,100] Ablation of *Fgfr2* in steroidogenic cells results in adrenal hypoplasia observable by E15.5.[101] The effects of FGFR-2 deletion are predicted to be solely due to loss of the *IIIb* isoform, as global deletion of *Fgfr2 IIIb* results in embryonic adrenal hypoplasia,[99,102] whereas the adrenal glands of *Fgfr2 IIIc* knockout mice have no discernible phenotypic differences from wild-type glands at postnatal day 2.[103] The adrenal hypoplasia evident in *Fgfr2-IIIb*-deficient mice results from reduced cortical proliferation during embryogenesis (Table 1),[99] which demonstrates a requirement for mitogenic FGF signalling for proper adrenal gland development. FGFs also exert mitogenic effects important for adrenal gland maintenance in the postnatal period. FGF-2 has been reported to stimulate proliferation in primary cultures of bovine adrenocortical cells and the Y1 mouse tumour cell line.[104,105] Adrenal re-growth under the paradigm of reconstitution by injecting primary adrenocortical cells in mice or following unilateral adrenalectomy in mice is critically dependent on the pro-proliferative and angiogenic properties of FGFs.[11,106,107] In humans, over-expression of *FGFR1* and *FGFR4* has been found in adrenocortical adenomas and adrenocortical carcinomas (Table 1).[108–110] *FGFR4* overexpression was detected in up to 65% of adrenocortical tumours and is a predictor of poor outcome, and, therefore, a new potential target for the treatment of adrenocortical carcinoma.[110]

TGF-β and inhibin signalling—The transforming growth factor β (TGF-β) signalling pathway affects a wide variety of cellular processes and is involved in maintenance and differentiation of stem cells.[111] The TGF-β superfamily consists of a diverse array of ligands, including (but not limited to) bone morphogenetic proteins, growth and differentiation factors, activins and inhibins. Ligand binding to TGF-β receptor type-2 (TGFR-2) promotes phosphorylation of TGF-β receptor type-1 (TGFR-1), which in turn results in phosphorylation and nuclear translocation of mothers against decapentaplegic (SMAD) proteins that modulate transcription of target genes. Activins and inhibins are present in the fetal and adult adrenal cortex[112] and profoundly influence adrenocortical differentiation. Activins augment steroidogenesis induced by adrenocorticotropic hormone (ACTH) in fetal adrenal cells and stimulate aldosterone production induced by angiotensin II (Ang II) and ACTH in adult adrenal cells.[112,113] Whether inhibins directly affect adrenal steroidogenesis is unclear;[114] however, an important role for the gene encoding inhibin-α (*Inha*) in the biology of adrenocortical progenitor cells has been demonstrated. Following gonadectomy, *Inha*-deficient mice develop estrogen-secreting steroidogenic tissue in the adrenal cortex,[115] a process that is dependent on high levels of circulating luteinizing hormone (LH).[116] LH induces a transcriptional program switch from adrenal-specific *Gata6* expression to gonadal-restricted *Gata4* expression.[117] The loss of *Inha* results in constitutive TGF-β2 and SMAD3 activation in adrenocortical progenitor cells, with subsequent expansion of GATA4-expressing cells. The resultant ectopic ovarian tissue includes differentiated theca and granulosa cell lineages and partially matured follicles.[117] These data establish a role for inhibin-α in the determination of the adrenal (versus gonadal) fate of

adrenocortical progenitor cells. Whether the ovarian theca metaplasia observed in the adrenal cortex of postmenopausal women reflects constitutive TGF-β2 activation remains an intriguing hypothesis.[118]

IGF signalling—The insulin-like growth factor (IGF) signalling pathway has been implicated in growth and differentiation of the adrenal cortex. The IGF family consists of two ligands structurally similar to proinsulin, IGF-1 and IGF-2. These secreted proteins interact with IGF-1 receptor (IGF-1R), a receptor tyrosine kinase structurally similar to the insulin receptor, to promote cell growth and survival, signalling through MAPK and/or PI3K–AKT (also known as protein kinase B) pathways.[119] IGF-2 receptor (IGF-2R), unlike IGF-1R, does not contain a tyrosine-kinase domain and acts as a molecular sink to restrict the bioavailability of IGF-2.[119] Six IGF-binding proteins (IGFBPs) bind to and exert stimulatory or inhibitory effects on IGFs; however, their actions in the adrenal gland are poorly understood. All components of the IGF pathway are expressed in the adrenal gland across mammalian species.[4] IGFs have strong mitogenic effects on fetal and adult human[120] and bovine[121] adrenal cells. *IGF2*, the gene that encodes IGF-2, is highly expressed in human fetal adrenal glands. However, *IGF2* expression is decreased in the adult adrenal gland to a level equivalent to the expression level of *IGF1*.[119] IGFRs are expressed throughout all zones of the human adrenal cortex, with enrichment of IGF-1R in the subcapsular region.[122]

Mice deficient in both the insulin receptor gene (*Insr*) and *Igf1r* exhibit gonadal dysgenesis, male-to-female sex reversal and adrenal aplasia.[123] Adrenal agenesis is evident by E16.5 and is likely to be due to global loss of cell proliferation throughout the genital ridge and the AGP beginning as early as E10.5.[123] Mice with an *Insr* and *Igf1r* double knockout have an AGP containing almost half the number of SF-1^+ cells found in wild-type mice at E11.5, as well as having reduced *Nr5a1* expression. These data indicate that IGF signalling is a potent adrenal mitogen and is critical for adrenal cell specification early in development. Additionally, IGFs augment basal and ACTH-induced steroidogenesis in both fetal and adult adrenal cells *in vitro*,[124] suggesting a role for IGF signalling in adrenocortical cell differentiation in addition to adrenal cell proliferation.

IGF2 is located on chromosome 11p15.5 within an imprinted locus that also includes *CDKN1C*, which encodes the cyclin-dependent kinase inhibitor 1C (also known as p57Kip2), and *H19*, which is transcribed into a nontranslated RNA.[125] Genetic and epigenetic aberrations in this locus result in diseases with clinically important adrenal gland abnormalities, such as Beckwith–Wiedemann syndrome (BWS) and IMAGe (intrauterine growth retardation, metaphyseal dysplasia, AHC and genital anomalies) (Table 1). BWS, a heterogeneous paediatric overgrowth syndrome characterized by embryonic tumours, macrosomia, macroglossia and other developmental defects, results from loss of imprinting of 11p15.5 leading to upregulation of *IGF2* and down-regulation of *CKDN1C* and *H19*.[125] Whereas the common adrenal phenotype in patients with BWS is adrenal cytomegaly with adrenocortical cysts,[126,127] adrenocortical carcinoma occurs in 7% of BWS cases.[127] Of note, *IGF2* was found to be markedly upregulated in most genomic studies of sporadic adrenocortical carcinoma, with loss of methylation marks observed at the *IGF2* locus,[89,128,129] as reviewed previously.[130] Interestingly, loss of the major imprinting control

region of the *Igf2* locus in the mouse adrenal gland increases *Igf2* expression but does not by itself increase the incidence of adrenal tumours. However, in combination with genetically increased β-catenin levels, elevated IGF-2 expression contributes to an earlier formation and increased severity of adrenocortical tumours in mice.[84,131] In contrast to BWS, IMAGe is characterized by adrenal insufficiency. The constellation of congenital anomalies that characterize IMAGe result from gain-of-function mutations in *CDKN1C* that increase the stability of the p57Kip2 protein.[132,133]

Endocrine signalling pathways

The peptide hormones Ang II and ACTH mediate different aspects of endocrine homeostasis through regulation of adrenocortical steroidogenesis and cell differentiation (Figure 3). Under the control of the RAAS, Ang II promotes aldosterone production through activation of PLC, calcium and protein kinase C signalling (reviewed in depth previously[134]). By contrast, pro-opiomelanocortin (POMC)-derived ACTH, under regulation of the HPA axis, induces glucocorticoid synthesis by stimulating cAMP–protein kinase A (PKA) and MAPK signal transduction pathways (reviewed in depth previously[135]). Mice deficient for both of the type-1 Ang II receptors (*Agtr1a*$^{-/-}$:*Agtr1b*$^{-/-}$); the ACTH receptor (*Mc2r)*; or *Pomc* exhibit normal adrenal histology at birth,[136–138] indicating that Ang II and ACTH are dispensable for normal fetal adrenal gland development. However, these endocrine signals profoundly influence adrenocortical remodelling and maintenance of the adrenal cortex during adult life. The effects of activation of the RAAS and the HPA axis on adrenal gland architecture in mice are similar; initial differentiation and hypertrophy of steroidogenic cells occurs acutely, with subsequent chronic stimulation resulting in increased cell proliferation through mechanisms that are not well established.[139] Rats consuming a low-salt diet exhibited hypertrophic cells that had increased *Cyp11b2* expression in the zona glomerulosa over the initial 3 days of the diet, which was followed by adrenal gland hyperplasia peaking 3–5 days after initiation of diet.[140] The increase in the number of CYP11B2^{+} cells that occurred prior to proliferation is thought to be the direct result of differentiation of *Shh*-expressing cells into zona glomerulosa cells (see above), followed by proliferation to replace the population of progenitors. In accord with this notion, rats subsisting on a low-salt diet for 3 days gained *Cyp11b2*-expressing cells without induction of proliferation, which was concomitant with a ~50% reduction in *Shh* expression.[141] The bulk of replicating cells under chronic RAAS or ACTH stimulation initially reside within the undifferentiated zone before extending to the other zones.[140,142] Conflicting data exist on whether ACTH is mitogenic in itself;[143] ACTH has been suggested to stimulate expression of growth factors, such as IGF-2, rather than directly promoting cell proliferation.[144,145]

The integration of endocrine and local paracrine pathways toggles adrenocortical gene expression. *Nr0b1*, which encodes DAX1, is one of the genes regulated in this fashion. *Nr0b1* is activated by local paracrine Wnt signalling in presumptive adrenocortical progenitors, which is thought to contribute to the maintenance of their undifferentiated state.[61,75,82] DAX1 inhibits SF-1-mediated steroidogenesis (see above)[64] and DAX1 expression is also activated by excess glucocorticoid levels, leading to negative feedback regulation of steroid production.[146] By contrast, ACTH clears transcriptional coactivators

from the proximal promoter of *Nr0b1*, presumably relieving DAX1-mediated repression of SF-1-dependent steroidogenesis and enabling adrenocortical cell differentiation.[146]

cAMP–PKA signalling pathway

The cAMP–PKA pathway, the intracellular signalling mediator of the effects of various hormones, is crucial for adrenal steroidogenesis and cell proliferation. Under normal conditions, activation of G-protein coupled receptors by hormones results in the activation of G proteins with subsequent adenylate cyclase stimulation and generation of cAMP. cAMP binds to the regulatory subunits of PKA, which frees the catalytic subunits of PKA to phosphorylate downstream substrates that activate transcription of cAMP-responsive genes.

Genetic mutations of the cAMP–PKA pathway are associated with an array of hyperproliferative adrenocortical diseases in humans and in mouse models (Table 1). Activating mutations in the *GNAS* gene, which encodes adenylate cyclase-stimulating Gα protein (also known as G_s protein), have been identified in patients with cortisol-producing adrenal adenomas[147–149] and ACTH-independent macronodular adrenal hyperplasia (AIMAH),[150] and are the genetic basis of McCune–Albright syndrome.[151] Gain-of-function mutations in *PRKACA*, one of the three genes encoding the catalytic subunits of PKA, are the genetic basis of many cortisol-producing adrenal adenomas.[149,152–154] Loss-of-function mutations in *PRKAR1A*, which codes for one of the regulatory subunits of PKA, are responsible for primary pigmented nodular adrenal dysplasia[155] and Carney complex.[155,156] In different mouse models, genetic ablation or reduction of expression of *Prkar1a* variably recapitulates aspects of Carney complex.[157–160] Adrenal-specific knockout of *Prkar1a* in mice results in autonomous adrenal hyperplasia and glucocorticoid production reminiscent of Cushing syndrome.[160] Additionally, *Prkar1a*-deficient mouse adrenal glands exhibit reduced numbers of normal adult adrenal cells concomitant with centrifugal expansion of apoptosis-insensitive fetal adrenal-like cells, suggesting that normal adrenocortical cell differentiation and proliferation require proper regulation of PKA activity.[160] Interestingly, aberrant upregulation of PKA activity in the adrenal cortex has been associated with dysregulated Wnt signalling in both mice and humans.[147,159] Finally, inactivating mutations in *PDE11A* and *PDE8B*, which encode phosphodiesterases that dampen cAMP signalling, have been associated with adrenal hyperplasia,[161,162] AIMAH[163] and adrenocortical adenomas.[164]

Conclusions

Historical studies have provided evidence that the adrenocortical stem cells and progenitor cells that regulate homeostasis of the adrenal cortex reside within the capsule and subcapsular region of the gland. More recent transgenic mouse studies have shown that SHH-receiving, $Gli1^+$;SF-1^- cells in the adrenal capsule, some of which derive from SF-1^+ fetal cells of the adrenal primordium, serve as adrenocortical stem cells during embryonic development.[36,39] These cells give rise to subcapsular SHH-producing cells that are undifferentiated progenitors of steroidogenic cell lineages important for homeostasis during the postnatal period.[36,39] Despite the knowledge gained by these lineage-tracing experiments, the signalling cues that trigger delamination of a $Gli1^+$ capsular cell to form an

adrenocortical cell remain elusive. Additional questions remain, such as what the relative contributions of capsular stem cells and long-lived subcapsular progenitor cells are to normal homeostasis, and whether these populations engage differently under conditions of normal tissue maintenance and injury repair.

The studies detailed above highlight the involvement of paracrine and endocrine signalling in the establishment and maintenance of the adrenocortical stem and progenitor cell niche. The Wnt pathway is critical for both development and maintenance of the adrenocortical tissue, in part through activation of the Wnt target gene *Nr0b1*.[81,82] DAX-1 maintains the adrenocortical progenitor pool postnatally, by acting as a negative regulator of SF-1-mediated steroidogenesis and cell differentiation.[64,75] The adrenal (as opposed to gonadal) fate of adrenocortical progenitors is sustained by the TGF-β family member inhibin-α.[117] The proliferative capacity of adrenocortical cells seems to be under the control of IGF-2, FGFs, cAMP–PKA signalling and SF-1,[50,56,99,104,105,121,122] although the rules dictating which molecules regulate proliferation temporally and spatially remain to be elucidated. A key issue to understand is how these different paracrine signals coordinate to maintain the adrenocortical stem and progenitor cell unit in terms of promoting proliferation and/or self-renewal, quiescence, or terminal differentiation. Differentiation of adrenocortical cells is in part under control of the hormones Ang II and ACTH, which activate steroidogenesis in conjunction with cAMP–PKA signalling.

Future studies should aim to elucidate how the integration of paracrine and endocrine signals regulates proliferation and differentiation of adrenocortical stem cells and progenitor cells in time and space. Although potential markers of adrenocortical stem cells and progenitor cells have been identified, the inability to isolate and culture these cells *in vitro* has been an impediment in this area of research. Given that the population of adrenocortical stem cells and progenitor cells is very small, methodologies that require ample starting material would necessitate expansion of these cells *in vitro* following their isolation, yet protocols for culturing adrenocortical stem and progenitor cells are lacking. This challenge is likely to reflect the complexity of the integration of multiple signalling pathways, an incomplete understanding of essential factors influencing the stem and progenitor cell niche and the inability to recapitulate the *in vivo* environment in a culture dish. Establishment of methods for the culturing of adrenocortical stem cells and progenitor cells will be critical for future research efforts.

Diseases resulting in adrenal insufficiency (AHC, SERKAL, PHS and IMAGe) are consequences of loss-of-function mutations in the specific transcription factors (SF-1, DAX-1) and paracrine signalling pathways (Wnt, Hedgehog, IGF) that are critical for adrenocortical stem and progenitor cells. Conversely, activating mutations in these pathways result in adrenal tissue ‘overgrowth’ that contributes to the formation of adrenocortical hyperplasias, adenomas and carcinomas. These diseases might therefore reflect the consequences of adrenocortical stem and progenitor cell failure or overactivation. We present a comprehensive list of genetic mutations underlying a spectrum of adrenocortical diseases in Table 1 (primary disorders of steroidogenesis due to loss-of-function mutations in *MC2R*, *MRAP* and in genes encoding steroidogenic enzymes that result in primary hypoplasias or compensatory hyperplasias [congenital adrenal hyperplasias and familial

glucocorticoid deficiency] are not included but have been discussed extensively elsewhere[135]). Although some of the genes listed in Table 1 have been shown to be important in the biology of adrenal disease, their involvement in normal adrenal gland development and homeostasis remains unclear. Conversely, a number of genes discussed in this Review have been shown to be involved in adrenocortical development and homeostasis in mice, yet their role in human adrenal biology and disease remains unclear. Many genes that participate in stem and progenitor cell biology and contribute to disease states have been determined, but most likely many remain unknown. The powerful tool of next-generation high-throughput sequencing enables assessment of genetic perturbations on a genomic level. These experimental approaches will be likely to uncover new genes and pathways that are required for the maintenance of adrenocortical stem and progenitor cells and contribute to our understanding of the aetiology of adrenocortical diseases across the spectrum of adrenal failure to neoplasia.

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Key points

- Fetal adrenal cells are critical for the establishment of the capsular–cortical unit of the adrenal gland; these cells populate the mesenchymal capsule to become stem cells for the underlying adult (definitive) cortex
- The adrenal capsular and subcapsular cell populations have a crucial role in gland maintenance; paracrine signals between the capsule and the cortex coordinate normal homeostasis by regulating capsular and subcapsular cellular lineages
- Genetic defects in signalling pathways that regulate adrenocortical stem and progenitor cells contribute to diseases across the spectrum of adrenal failure and neoplasia

Review criteria

PubMed and Google Scholar were used to find original full-length research articles and reviews published between 1866 and 2014. Search terms included “adrenocortical stem AND/OR progenitor”, “adrenocortical shh”, “adrenocortical wnt AND/OR beta-catenin”, “adrenocortical FGF”, “adrenocortical IGF”, “adrenocortical TGF-beta AND inhibin”, “adrenocortical PKA”, “adrenocortical hypoplasia”, “adrenocortical development”. The references contained within articles found through this search also served as a resource to identify additional pertinent articles for discussion.

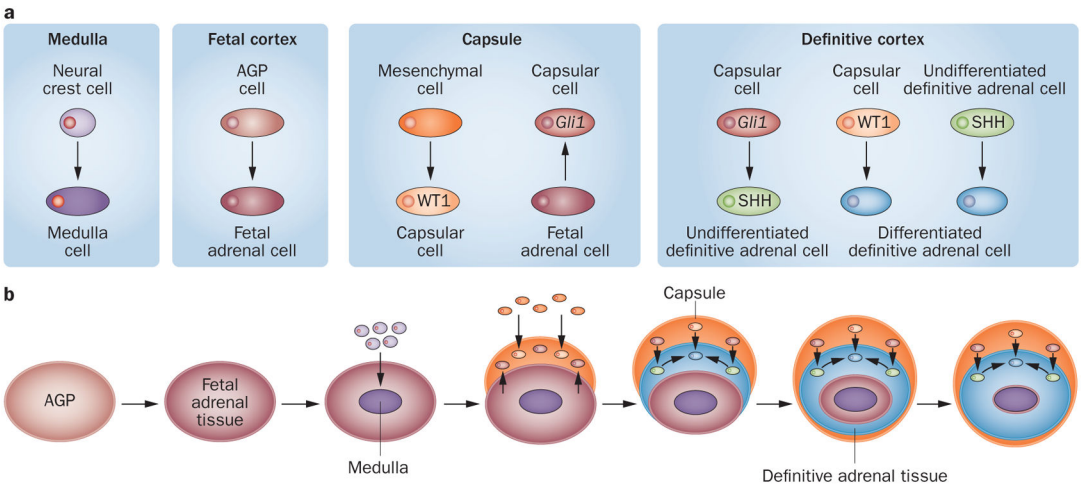

Figure 1.

Cell lineages in adrenal gland development and homeostasis. **a** | Cell lineages contributing to the adrenal gland. The catecholamine-producing cells of the adrenal medulla (purple) are derived from neural crest precursors. WT1-expressing mesenchymal cells (orange) contribute to the adrenal capsule. Fetal adrenal cells (dark pink) give rise to a subpopulation of *Gli1*-expressing capsular cells. *Gli1*$^{+}$ capsular cells give rise to undifferentiated SHH-secreting cells (green) and differentiated steroidogenic adrenocortical cells (blue) of the adult gland. WT1-expressing cells give rise to differentiated cells of the adult cortex that no longer express WT1. **b** | Adrenal gland development begins when the AGP forms and separates into a fetal gonad (not shown) and a fetal adrenal gland. Cells from the neural crest infiltrate the fetal adrenal gland to form the medulla, while mesenchymal cells participate in encapsulation. Fetal adrenal cells give rise to a portion of the adrenal capsule. As the definitive cortex grows and the fetal cortex regresses, *Gli1*$^{+}$ capsular cells give rise to SHH-secreting progenitors and steroidogenic adrenocortical cells. WT1^{+} cells also contribute to the cortex, albeit infrequently. Postnatally, the capsular and subcapsular progenitor cells are retained throughout adulthood, during which time they maintain homeostasis of the definitive adrenal cortex. Abbreviations: AGP, adrenogonadal primordium; SHH, sonic hedgehog; WT1, Wilms tumour protein homolog. Adapted with permission from Wood, M. A. *et al. Development* **140** (22), 4522–4532 (2013) http://dx.doi.org/10.1242/dev.092775.[39]

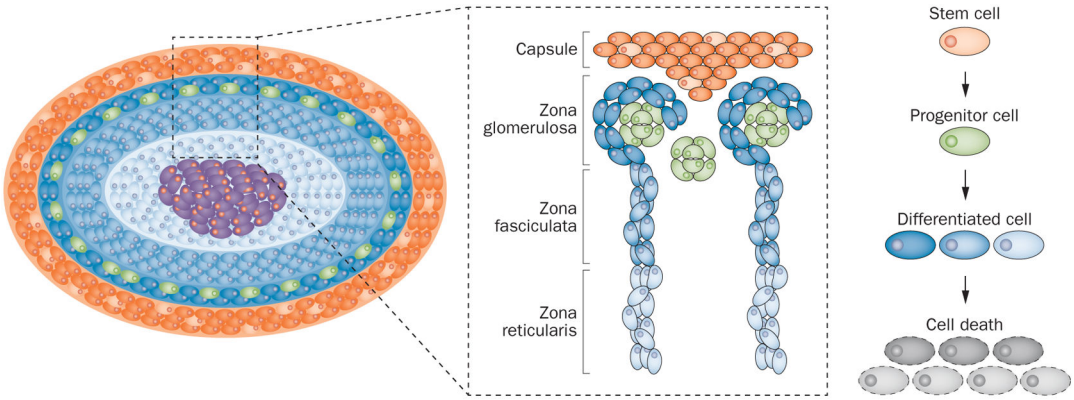

Figure 2.

Simplified view of the adrenocortical homeostatic unit. The adrenal gland consists of the adrenal medulla (purple cells) and the adrenal cortex (blue concentric layers) that is encased within the adrenal capsule (orange circle). The adrenocortical capsule contains mesenchymal cells (dark orange) and stem cells (light orange). Adrenocortical stem cells give rise to undifferentiated, nonsteroidogenic adrenocortical progenitor cells (green cells) and differentiated, steroidogenic cells of the adrenal cortex (blue cells of various hues). Cells stream through the cortex until they reach the cortical–medullary boundary where they undergo apoptosis (grey cells).

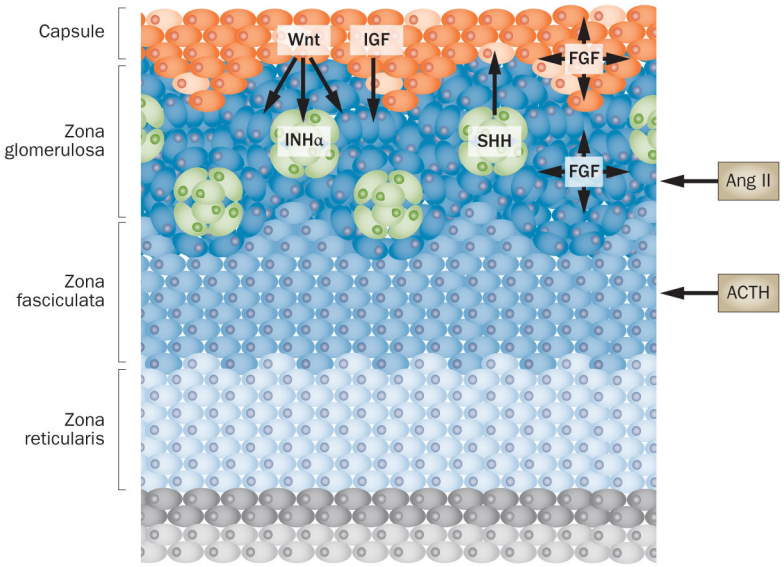

Figure 3.

Paracrine and endocrine signals that act on adrenocortical cells. Ang II and ACTH are endocrine signals that stimulate steroidogenesis in differentiated cells of the zona glomerulosa (dark blue cells, Ang II), the zona fasciculata (medium blue cells, ACTH) and the zona reticularis (light blue cells, ACTH), respectively. Undifferentiated progenitor cells (green cells) secrete SHH ligands that act on stem cells within the adrenal capsule (light orange cells). Wnt ligands are received by cells of the zona glomerulosa, are critical for maintenance of adrenocortical progenitors, and indirectly regulate aldosterone production (not shown). INHα maintains the adrenal (as opposed to gonadal) fate of progenitor cells. FGFs are secreted from and received by both the capsule and the cortex and might be involved in regulation of cell proliferation. During adulthood, IGFs are expressed in the capsule, act on the cortex to promote proliferation, and can participate in steroidogenesis. Abbreviations: ACTH, adrenocorticotropic hormone; Ang II, angiotensin II; FGF, fibroblast growth factor; IGF, insulin-like growth factor; INHα, inhibin-α; SHH, sonic hedgehog; Wnt, Wingless-type MMTV integration site.

Table 1

Genes implicated in adrenocortical disease

Gene(s)	Genetic defect	Syndrome and/or phenotype	References
Aplasia, hypoplasia, dysplasia			
AAAS	Germline LOF	Triple A syndrome	165
Acd	Germline LOF	Adrenocortical dysplasia	166
CDKN1C	Germline LOF	IMAGe syndrome	132,133
Cited2	Gene knockout	Adrenocortical agenesis	29
Ctnnb1	Gene knockout	Adrenocortical aplasia, postnatal homeostatic defects	81
Fgfr2	Gene knockout	Adrenocortical hypoplasia	101
Foxd2	Gene knockout	Adrenocortical hypoplasia	167
GLI3, Gli3	Germline LOF	Pallister–Hall syndrome (humans and mice)	94,96
Insr, Igf1r	Gene knockout	Adrenocortical aplasia	123
NR5A1, Nr5a1	Germline LOF	AHC (humans), aplasia (mice)	51,52
NR0B1, Nr0b1	Germline LOF	AHC (humans), postnatal dysplasia (mice)	63,76
Shh	Gene knockout	Adrenocortical hypoplasia	36–38
WNT4	Germline LOF	SERKAL syndrome	93
Wt1	Gene knockout	Adrenocortical agenesis	27,28
Hyperplasia, neoplasia			
APC	Germline and somatic LOF	Gardner syndrome	87
ARMC5	Germline LOF	Adrenocortical hyperplasia: AIMAH	168
ATP1A1, ATP2B3	Somatic GOF	Adrenocortical adenoma: primary aldosteronism	169
CACNA1D	Germline and somatic GOF	Adrenocortical adenoma: primary aldosteronism	170
CTNNB1	Somatic GOF	Adrenocortical adenoma	88,89
DAXX	Somatic LOF	Adrenocortical carcinoma	89
FGFR1	Overexpression	Adrenocortical adenoma and adrenocortical carcinoma	108,109
FGFR4	Overexpression and amplification	Adrenocortical adenoma and adrenocortical carcinoma	108–110
GNAS	Germline GOF	Adrenocortical hyperplasia: AIMAH Adrenocortical adenoma: McCune–Albright syndrome	147,148,150
IGF2, CDKN1C, H19	Imprinting defects in the 11p15.5 locus	Adrenocortical carcinoma: Beckwith–Wiedemann syndrome	125
KCNJ5	Somatic GOF	Adrenocortical adenoma: primary aldosteronism	171
MED12	Somatic LOF	Adrenocortical carcinoma	89
MEN1	Germline and somatic LOF	Adrenocortical carcinoma: multiple endocrine neoplasia (germline LOF mutations); somatic LOF mutations	172,173
MSH2, MSH6, MLH1, PMS2	Germline LOF	Adrenocortical carcinoma: Lynch syndrome	174
NR5A1	Somatic amplification, overexpression	Adrenocortical adenoma Adrenocortical carcinoma	57–59
PDE11A, PDE8B	Germline LOF	Adrenocortical hyperplasia: AIMAH	161–164

Gene(s)	Genetic defect	Syndrome and/or phenotype	References
		Adrenocortical adenoma	
PRKACA	Somatic GOF	Adrenocortical adenoma: Cushing syndrome	149,152–154
PRKAR1A	Germline LOF	Adrenocortical hyperplasia and adrenocortical carcinoma: Carney complex	156
RB1	Somatic LOF	Adrenocortical carcinoma	89,175
TERT	Somatic amplification	Adrenocortical carcinoma	89
TP53	Germline and somatic LOF	Adrenocortical adenoma and adrenocortical carcinoma: Li–Fraumeni syndrome	176–178
ZNRF3	Somatic LOF	Adrenocortical carcinoma	89

Abbreviations: AHC, adrenal hypoplasia congenita; AIMAH, adrenocorticotropic-hormone-independent macronodular adrenal hyperplasia; GOF, gain of function; IMAGe, intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita, genital abnormalities; LOF, loss of function; SERKAL, sex reversion, kidneys, adrenal and lung dysgenesis.