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New strategies for applying targeted therapies to adrenocortical carcinoma

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Abstract

Adrenocortical carcinoma (ACC) is a rare, aggressive, and frequently deadly cancer. Up to 75% of all patients will eventually develop metastatic disease, and our current medical therapies for ACC provide limited – if any – survival benefit. These statistics highlight a crucial need for novel approaches. Recent studies performing comprehensive molecular profiling on ACC have illuminated that ACC is comprised of three clinically distinct molecular subtypes, bearing differential regulation of cell cycle, epigenetics, Wnt/β-catenin signaling, PKA signaling, steroidogenesis and immune cell biology. Furthermore, these studies have spurred the development of molecular subtype-based biomarkers, contextualized outcomes of recent clinical trials, and advanced our understanding of the underlying biology of adrenocortical homeostasis and cancer. In this review, we describe these findings and their implications for new strategies to apply targeted therapies to ACC.

Keywords

adrenocortical carcinoma; ACC; genomics; targeted therapy; biomarkers

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Conflict of Interest

D.R. Mohan, A.M. Lerario, and G.D. Hammer are co-inventors on a provisional patent application describing compositions and methods for characterizing cancer, owned by The Regents of the University of Michigan. G.D. Hammer is founder and advisor for Millendo Therapeutics. G. D. Hammer is also founder of Vasaragen.

1. Introduction

Adrenocortical carcinoma (ACC) is a rare cancer of the adrenal cortex with a global annual incidence of 0.5 to 2 individuals per million (1,2). Despite its rarity, outcomes for patients diagnosed with ACC remain dismal, with 5-year overall survival of ~35% (3). Furthermore, while 50% of patients are diagnosed with surgically resectable locoregional disease, ~75% of all patients will ultimately develop metastases (4). Treatment options for patients with metastatic disease are limited and often ineffective: patients receive the DDT-derived adrenolytic agent mitotane +/− cytotoxic chemotherapy, sometimes paired with palliative surgery for resectable lesions, but <10% of patients with metastatic disease survive 5 years on these agents (5,6). Taken together, these statistics highlight a critical need for novel therapeutic strategies to fight ACC, contingent on a deeper understanding of the molecular basis of this disease.

Recent advances in comprehensive molecular profiling, biomarker identification, clinical trials, and *in vivo* and *in vitro* modeling have now illuminated a spectrum of pharmacologically-targetable molecular programs essential for adrenocortical development and homeostasis and uniquely derailed in cancer, including: cell cycle, DNA damage response, epigenetics, Wnt signaling, PKA signaling, and steroidogenesis. In this review, we summarize these developments and describe their implications for the next generation of targeted therapies for ACC. Note that the scope of this review is largely restricted to adult ACC.

2. Methods

We selected literature for inclusion in this review by querying the NIH/NCBI PubMed database on or before June 23, 2019 for at least any of the following search terms: “adrenocortical carcinoma” (3490 items as of June 23, 2019); “adrenocortical carcinoma preclinical” (58 items); “adrenocortical carcinoma clinical trial” (103 items); “adrenocortical carcinoma trial” (133 items). We curated search results manually using the following criteria: relevance, impact, and recency

3. Results and Discussion

3.1. Multiplatform genomics reveal ACC is comprised of 3 distinct subtypes and provide pan-cancer contextualization

Our current understanding of the molecular basis of ACC is informed by two major studies utilizing multiplatform omics approaches to profile primary tumors – Assie, Letouze *et al.* (7) and The Cancer Genome Atlas Study on ACC (ACC-TCGA; (8)). These studies confirmed that 90% of ACC exhibit loss of heterozygosity of the *IGF2* locus leading to upregulation of IGF2/IGF1R signaling (7,8). ACC also bear recurrent somatic alterations facilitating rapid cell cycling (*TP53, CDKN2A, RB1, CDK4, CCNE1*), telomere maintenance (*TERT, TERF2*), constitutive Wnt/β-catenin signaling (*ZNRF3, CTNNB1*), and constitutive PKA signaling (*PRKAR1A*); and involved in chromatin remodeling (*MEN1, DAXX*), transcription (*MED12*), and translation (*RPL22*) (7,8). While Assie, Letouze *et al.* observed ACC exhibit frequent copy number changes (7), ACC-TCGA identified three

recurrent somatic copy number alteration (SCNA) signatures: “quiet” (rare; tumors possess a diploid genome), “chromosomal” (~2/3 of tumors possess loss of heterozygosity of entire chromosomes +/− hypodiploidy or whole genome doubling), and “noisy” (~1/3 of tumors possess frequent arm-level gains and losses throughout the genome +/− whole genome doubling) (8).

ACC-TCGA provided a novel molecular classification of ACC, identifying three comparably frequent and distinct molecular subtypes via a cluster of cluster (COC) analysis – COC1, COC2, and COC3 (Fig 1) (8). Good prognosis COC1 ACC have fewer somatic alterations, a quiet or chromosomal SCNA profile, a transcriptional signature characterized by immune infiltration and low expression of steroidogenic machinery (overlapping with a signature identified by Assie, Letouze *et al.* as “C1B”) (8). Intermediate prognosis COC2 and dismal prognosis COC3 ACC bear frequent Wnt/β-catenin pathway alterations (8). COC2 ACC bear a quiet or chromosomal SCNA profile and high expression of steroidogenic machinery (overlapping with Assie, Letouze *et al.* C1A). COC3 ACC bear frequent cell cycle alterations, a noisy SCNA profile, and high expression of steroidogenic and proliferative machinery (also overlapping with C1A) (8). Finally, on an epigenetic level, COC1 tumors bear low levels of genome-wide CpG island methylation (CIMP-low), COC2 bear intermediate levels (CIMP-intermediate), and COC3 bear high levels (CIMP-high) (8).

The completion of ACC-TCGA enabled the incorporation of ACC into pan-cancer analyses. These studies reveal that ACC has the lowest degree of immune infiltration of nearly all TCGA cancers (9,10) and a subset of ACC also exhibit a genomic signature suggestive of homologous recombination deficiency (11) . While primary ACC are notable for bearing a lower mutational burden than most TCGA cancers (10), metastatic ACC bear a mutational burden nearly 3-fold higher than that of primary tumors (12). A recent landmark study using ATAC-seq on TCGA samples revealed that the chromatin accessibility landscape of ACC is largely driven by critical transcription factor for adrenal organogenesis and steroidogenesis, SF1 (encoded by *NR5A1*), consistent with the hallmark steroidogenic transcriptional program active in most ACC (13).

3.2. Novel biomarkers stratify ACC into homogeneous classes

Currently, proliferation-based grade measured by Ki67 or mitotic counts on histologic sections of primary tumor samples is used to prognosticate ACC (14–16). However, the availability of high-throughput and multiplatform genomics data profiling ACC has enabled the discovery of several novel biomarkers that stratify ACC into molecular subtypes; such stratification is essential for the application of targeted therapies to specific subgroups of patients. The first of these molecular markers was developed by de Reynies, Assie, and colleagues, who demonstrated that cell cycle avid C1A and adenoma-like C1B tumors can be distinguished using a score derived from the mRNA expression of genes *BUB1B* and *PINK1* (*BUB1B-PINK1* score) (17). More recently, in line with ACC-TCGA, investigators have also shown that high mRNA expression of E2F target genes like *EZH2* (18) or novel SF1 transcriptional targets like *VAV2* are also associated with worse clinical outcomes (19,20).

Recent biomarkers take advantage of orthogonal approaches to capture the DNA hypermethylation signature characteristic of aggressive ACC (21,22). Our group recently demonstrated that uniform hypermethylation and silencing of the gene *G0S2* accurately captures a subgroup of patients with homogeneously dismal disease course akin to patients with COC3/CIMP-high tumors in ACC-TCGA (22). Indeed, this signature can be combined with *BUB1B-PINK1* to approximate the three molecular subtypes described by ACC-TCGA ((22); Fig 1).

While promising, most molecular biomarker studies use frozen primary tumor tissues, which are not available at all clinical centers. In an integrated study performing targeted assessment of somatic alterations, gene expression, and methylation in formalin-fixed paraffin-embedded tissues, Lippert *et al.* demonstrated that it is possible to molecularly prognosticate ACC using routinely available clinical samples (23). Other investigators have assessed less invasive approaches, demonstrating that benign and malignant lesions of the cortex can be distinguished by circulating steroids (24) or circulating microRNAs (25), and that it is possible to measure circulating tumor DNA from patients with ACC (26,27); such approaches may ultimately enable molecular subtype-directed neoadjuvant therapy and radiation-free tracking of ACC burden.

3.3. Clinical trials expose weaknesses of single pathway, “one size fits all” therapy

Molecular biomarkers are not currently used to direct therapies in ACC; patients with advanced and/or high-risk disease are uniformly directed to cytotoxic chemotherapy with or without mitotane (5,28,29). However, ongoing trials are evaluating the efficacy of prognostic grade in predicting therapeutic response to adjuvant mitotane alone or with combination cytotoxic chemotherapy (ADIUVO – NCT00777244, ADIUVO-2 – NCT03583710). While grade is certainly effective in pinpointing patients who are less likely to respond to standard of care, such an approach still falls short of rationally directing patients to therapy based on oncogenic pathways driving their specific type of ACC. Moreover, the therapeutic potential of mitotane is poorly understood in the context of ACC subtypes as recent studies suggest this drug may drive cytotoxic ER stress in responsive cells through SOAT-1 (30,31), the target of the investigational adrenolytic agent nevanimibe (ATR-101) (32,33). The molecular biomarker-directed application of novel or existing targeted agents to specific subtypes will be essential for advances in the care of this disease. However, previous evaluation of targeted therapies for ACC weaves a cautionary tale.

Overexpression of *IGF2* in 90% of ACC (confirmed in (7,8)) and early observations that inhibition of IGF2/IGF1R signaling was efficacious in subcutaneous xenograft models (34) fueled phase I-III clinical trials evaluating IGF2/IGF1R inhibition by figitumumab, cixutumumab, or linsitinib in patients with advanced ACC (35–37). Shockingly, these studies revealed that only 3-5% of patients with refractory metastatic ACC responded to IGF2/IGF1R inhibition with longterm regression (37), suggesting downstream genetic events may confer resistance to IGF2/IGF1R monotherapy. ACC-TCGA suggests that patients with COC2-3 tumors will likely require additional therapies targeting the Wnt pathway and cell cycle ((8); Fig 1). More recently, the US Food and Drug Administration’s accelerated approval of PD-1/PD-L1 checkpoint therapy for patients with mismatch repair-

deficient solid tumors has fueled studies demonstrating such therapies may be effective for some patients with ACC (38,39). However, the immunosuppressive effects of glucocorticoids (40) and the anti-correlation between steroidogenesis and immune infiltration in ACC-TCGA (8) suggests combination inhibition of steroidogenesis may be additionally required in mismatch repair-deficient, functional COC2-3 ACC. Taken together, these studies suggest that the application of targeted therapies to ACC likely requires multiple agents and a deeper understanding of the collaborative oncogenic pathways turned on in each tumor subtype.

3.4. Revisiting ACC at the bench

Advances in *in vivo* modeling of adrenocortical homeostasis and cancer have refined the understanding of oncogenic pathways derailed in ACC. Among the earliest of such studies was one by Heaton *et al.,* who demonstrated that *IGF2* overexpression likely requires collaboration with additional pathways (e.g. Wnt/β-catenin signaling) to promote adrenocortical tumorigenesis (41). More recent *in vivo* models point to one putative cell of origin of a subset of ACC, perhaps lying in the boundary between the mineralocorticoid-producing zona glomerulosa (zG) and glucocorticoid-producing zona fasciculata (zF) (Fig 2).

It is well known that proliferation and differentiation of the adrenocortical zF is reliant on ACTH-dependent PKA signaling (42). We recently demonstrated that ACTH-dependent proliferation during zF regeneration also relies on intact Wnt/β-catenin signaling (43). This, combined with identification of recurrent mutations leading to constitutive activation of both pathways in ACC (7,8) and strong association between cortisol production and Wnt/β-catenin pathway alterations (8), suggests that components of the ACTH signaling pathway may contribute to Wnt/β-catenin-dependent adrenocortical carcinogenesis. This is strongly supported by a recent study from our group demonstrating that adrenocortical deletion of a negative regulator of ligand-dependent Wnt signaling, ZNRF3, leads to functional zF hyperplasia (44). Additionally, a transgenic mouse model of ACC driven by adrenocortical expression of the SV40 large T-antigen (which simultaneously inactivates pRb and p53) also demonstrated that glucocorticoid-producing, metastatic ACC emerge from dysplastic lesions lying in the zG/zF boundary that upregulate Wnt/β-catenin signaling (45). Intriguingly, these lesions also exhibited E2F-dependent upregulation of histone methyltransferase EZH2 (45), akin to cell cycle-avid ACC (18). The recent demonstration that adrenocortical deletion of EZH2 leads to zF aplasia and glucocorticoid insufficiency suggests that the integrity of ACTH signaling in the adrenal cortex is exquisitely reliant on this pharmacologically targetable epigenetic modifier (46).

New developments in xenograft and *in vitro* modeling of ACC also hold promise for enabling a deeper understanding of ACC biology. The steroidogenic NCI-H295R cell line has long been the classical, most established model of ACC (47), possessing high expression of SF1, constitutively active β-catenin (48), and inactivation of pRb (49,50) and p53 (51). Recently, several new adult ACC cell lines and xenograft models have emerged. Pinto *et al.* characterized the first pediatric xenograft model of ACC, SJ-ACC3, enabling the preclinical identification of topotecan as an efficacious medical therapy for a child with recurrent ACC

(52). Hantel, Beuschlein and colleagues developed the adult ACC-derived, steroidogenic, and Wnt/β-catenin-active MUC-1 xenograft model and cell line which exhibited resistance to IGF-targeting therapy and recapitulated resistance to cytotoxic chemotherapy observed in the original patient (53–55). Kiseljak-Vassiliades and colleagues also developed two new adult ACC-derived cell lines and xenograft models, CU-ACC1 and CU-ACC2; CU-ACC1 is a cortisol-producing cell line bearing constitutively active β-catenin, whereas CU-ACC2 is a mismatch repair-deficient cell line bearing a mutation in *TP53* (56). Comprehensive molecular profiling of the NCI-H295R cell line as well as these novel models will be essential for preclinical evaluation of ACC subtype-specific therapeutic approaches.

3.5. Implications for novel strategies to direct targeted therapies

The new molecular classification of ACC paired with biomarkers capturing molecular subtypes (Fig 1) and recent clinical and translational studies have clarified our understanding of ACC's molecular basis and illuminated several novel therapeutic strategies. Notably, these studies have suggested that COC1, COC2, and COC3 ACC may be differentially responsive to therapies targeting the IGF2/IGF1R pathway, Wnt/β-catenin pathway, cell cycle, and immune system. The apparent reliance of most ACC on multiple oncogenic pathways may explain the observed broad resistance to IGF2/IGF1R monotherapy (37), suggesting that biomarker-based strategies to improve patient selection and new strategies incorporating combination therapy are paramount. Indeed, recently developed biomarkers that approximate ACC-TCGA molecular subtypes hold promise for enabling both prospective classification of ACC (Fig 1) and application of efficacious adjuvant therapies to patients likely to recur on standard of care (22). Advances in biomarker detection in archival material (23) and blood (24–27) will undoubtedly expand the patient population for which biomarker assessment is feasible.

The outlook for targeted therapies in ACC is promising. Currently, only a rare population of individuals with ACC respond to immunotherapy as a single agent (39); however, it is possible that combination therapy with inhibitors of steroidogenesis and/or cytotoxic agents (57) may enhance neoantigen presentation and immune clearance. The enrichment for Wnt/β-catenin pathway alterations in COC2-COC3 ACC suggests that individuals with these ACC types may be responsive to therapies targeting this pathway. Those tumors with ZNRF3 deficiency are likely reliant on Porcupine-dependent Wnt ligand secretion (44) and may be responsive to Porcupine inhibitors currently in phase I trials (e.g. NCT01351103). Tumors with mutations in *CTNNB1* leading to constitutive stabilization of β-catenin may instead be responsive to therapies targeting the oncogenic β-catenin/CBP transcriptional program, which have recently completed phase I trials for solid tumors (e.g. NCT01302405, NCT01764477). Patients with COC3 ACC likely require additional therapies targeting the cell cycle, perhaps in combination with DNA demethylating agents (58,59). Well characterized *in vitro* and *in vivo* models of ACC will undoubtedly facilitate preclinical assessment of these approaches (47,52–56).

Finally, recent developments in murine modeling of adrenocortical homeostasis and cancer have implicated a role for collaboration between ACTH/PKA and Wnt/β-catenin signaling in enabling cell cycle activation in a proliferating population of cells residing in the zG/zF

boundary (Fig 2). While it is yet unknown how targeting these cells will influence established ACC, the susceptibility of this population to hyperplasia and malignant transformation (44,45) suggests that targeting interplay between paracrine and endocrine signaling may ultimately be required to extinguish at least one type of ACC cell of origin.

4. Conclusions

ACC is a rare and often fatal cancer. In the last few years, our field has made numerous advances in the molecular understanding of ACC and adrenocortical biology. Moreover, the public availability of multiplatform data profiling ACC (particularly through ACC-TCGA (8)) has enabled the development of novel biomarkers and a deeper understanding of ACC from a pan-cancer perspective. The recent development of new scientific models of ACC and agents targeting pathways differentially upregulated in tumor subtypes supplies researchers and clinicians with a new set of therapeutic tools and renewed hope to fight this devastating disease.

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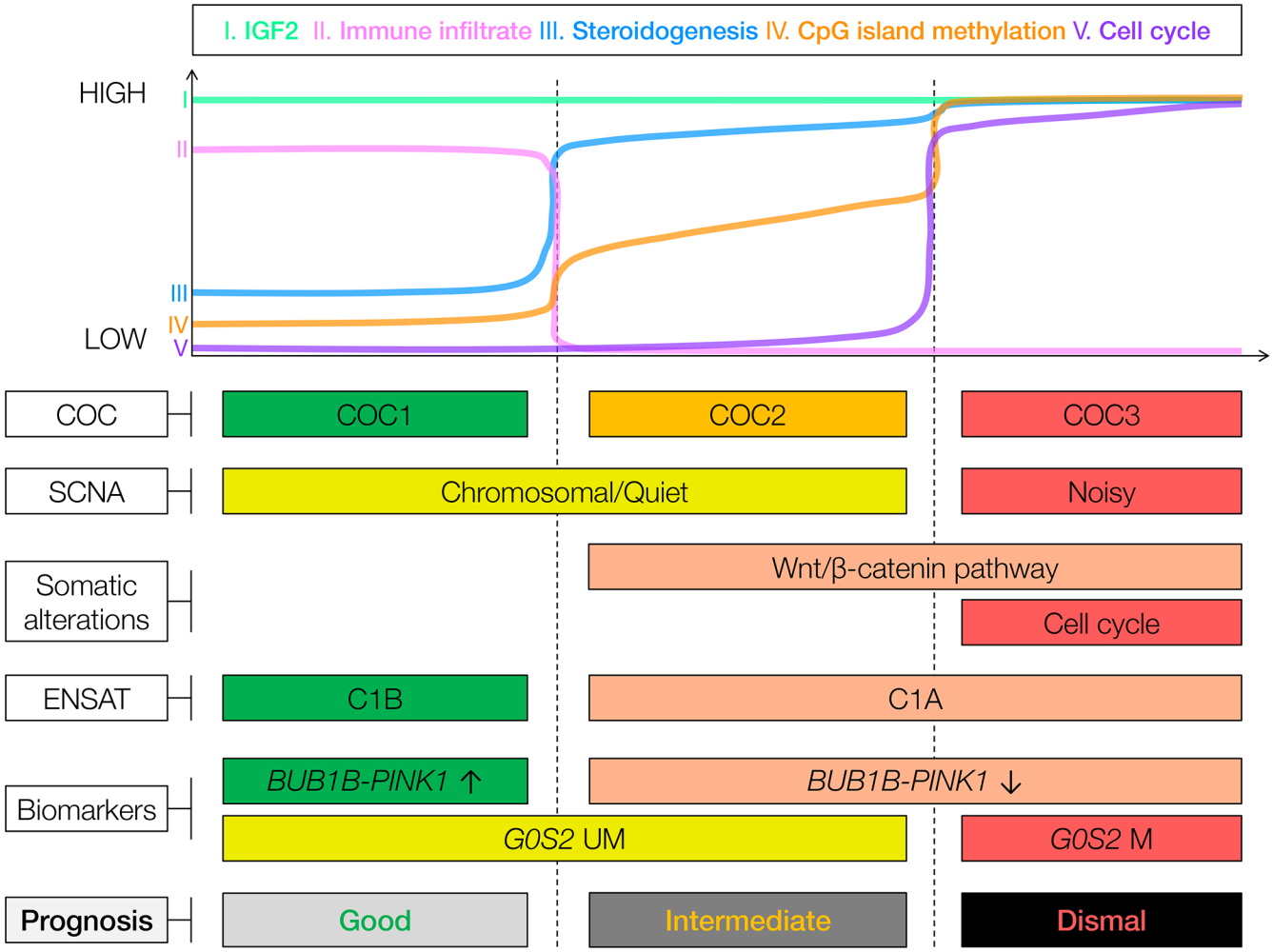

Figure 1. ACC is comprised of three distinct molecular subtypes amenable to targeted assessment.

As described in this review, ACC-TCGA (8) identified that ACC is comprised of three distinct molecular subtypes, COC1, COC2 and COC3. COC1 tumors have the best prognosis (longest progression-free survival), while COC2 tumors have intermediate prognosis and COC3 tumors have dismal prognosis. All subtypes have high expression of *IGF2* (I). COC1 tumors bear the highest degree of immune infiltration (II), while COC2-3 tumors bear higher expression of steroidogenic machinery (III). COC3 tumors bear CpG island hypermethylation (IV) and high expression of cell cycling machinery (V). COC1-COC2 tumors bear a somatic copy number alteration (SCNA) profile termed chromosomal or quiet, while COC3 tumors bear an SCNA profile termed noisy. COC2-3 tumors bear a higher burden of somatic alterations leading to constitutive activation of the Wnt pathway, while COC3 tumors bear a higher burden of somatic alterations leading to constitutive cell cycling. ACC-TCGA also showed that COC1 tumors possess a transcriptional program identified by de Reynies, Assie and colleagues as C1B (17), while COC2-COC3 tumors possess a transcriptional program akin to C1A (17). Notably, these molecular subtypes can

be captured using biomarkers, namely *BUB1B-PINK1* score (17) and *G0S2* methylation (22).

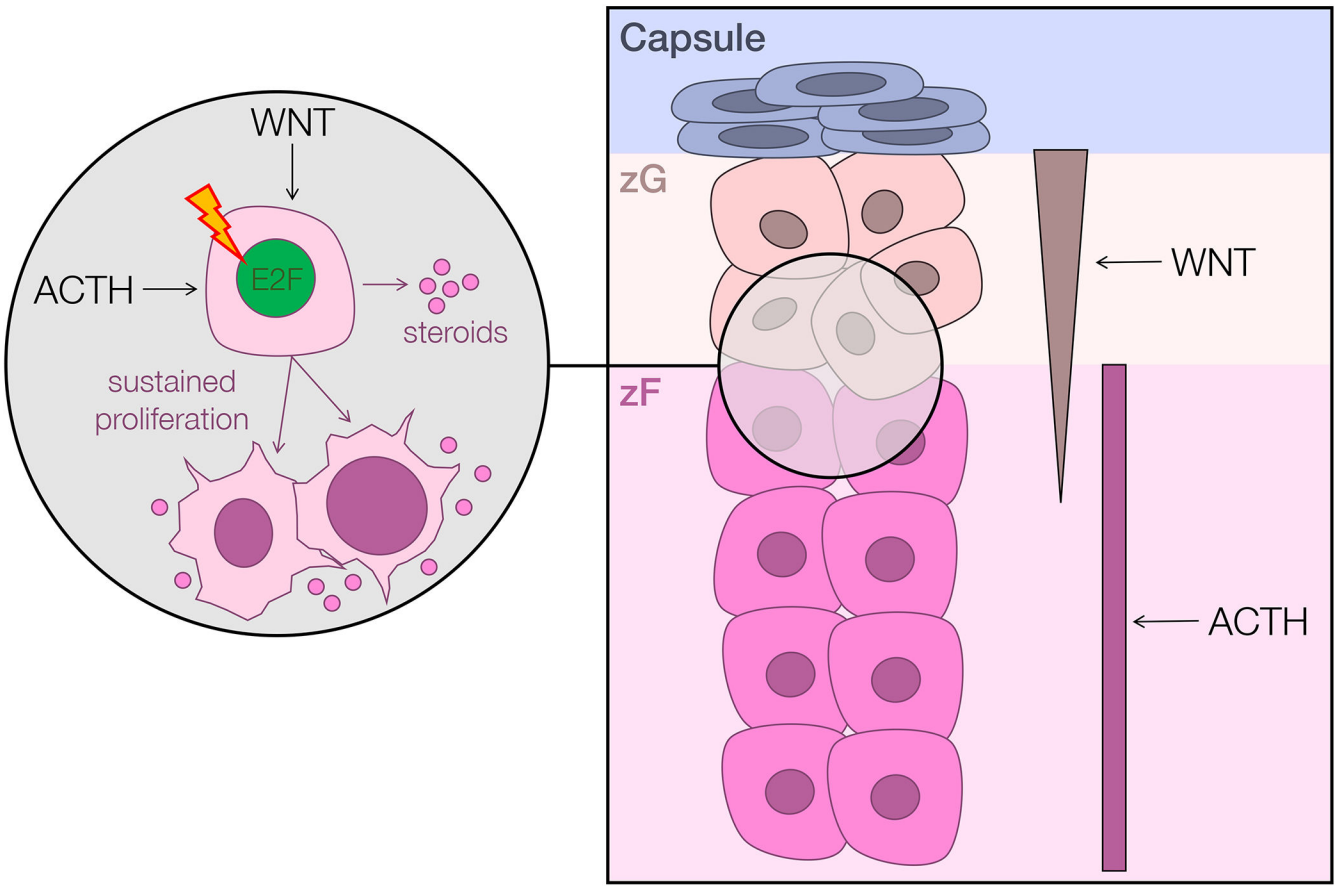

Figure 2. Murine models of adrenocortical homeostasis and cancer point to a putative cell of origin for Wnt-active ACC.

Schematized right is a portion of the capsular/cortical unit of the adrenal cortex. The upper layer of the adrenal cortex, the zona glomerulosa (zG), bears a gradient of active Wnt signaling and produces mineralocorticoids. The second layer of the adrenal cortex, the zona fasciculata (zF), proliferates and produces glucocorticoids in response to ACTH/PKA. Comprehensive molecular profiling studies identified recurrent mutations leading to constitutive activation of both ACTH/PKA and Wnt signaling ACC (7,8), and demonstrated that Wnt/β-catenin pathway alterations are significantly associated with clinical cortisol production (8). Recent mouse models of ACTH-driven zF regeneration (43), augmented Wnt/β-catenin signaling supported by ZNRF3 deficiency (44), and sustained proliferation triggered by adrenocortical expression of the SV40 large T-antigen (45) also demonstrate a unique interplay between Wnt/β-catenin and ACTH/PKA signaling in enabling proliferation of cells residing in the zG/zF boundary. Taken together, these studies support the existence of a small population of zG/zF boundary cells that are capable of rapidly proliferating in response to sustained Wnt/β-catenin and/or ACTH signaling. Prolonged cell cycle activation (schematized here by E2F) may render these cells susceptible to malignant transformation and ligand-independent growth.