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Human Adrenocortical Carcinoma Cell Lines

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Summary

The human adrenal cortex secretes mineralocorticoids, glucocorticoids and adrenal androgens. These steroids are produced from unique cell types located within the three distinct zones of the adrenal cortex. Disruption of adrenal steroid production results in a variety of diseases that can lead to hypertension, metabolic syndrome, infertility and androgen excess. The adrenal cortex is also a common site for the development of adenomas, and rarely the site for the development of carcinomas. The adenomas can lead to diseases associated with adrenal steroid excess, while the carcinomas are particularly aggressive and have a poor prognosis. In vitro cell culture models provide an important tool to examine molecular and cellular mechanisms controlling both the normal and pathologic function of the adrenal cortex. Herein we discuss the human adrenocortical cell lines and their use as model systems for adrenal studies.

Keywords

Adrenocortical cell line; Steroidogenesis; Cancer therapy; Adrenal cortex; model systems; cell lines

1. Introduction

The adrenal cortex is composed of three functionally distinct regions, the zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR). The ZG synthesizes mineralocorticoids; the ZF produces cortisol and the ZR secretes the so called adrenal androgens, DHEA and DHEA-sulfate. Each zone is preferentially regulated by different circulating factors that include angiotensin II (Ang II) and potassium (K^+) for the ZG, adrenocorticotropic hormone (ACTH) for the ZF, and ACTH plus other yet to be determined factors for the ZR (Parker and Rainey, 2004)(Figure 1). It has been established that the reason each zone secretes a unique set of steroids is related to the selective expression of steroid-metabolizing enzymes within each zone (Rainey, 1999; Rainey et al., 2002; Vinson, 2003; Nguyen and Conley, 2008) (Figure 2). However, the molecular mechanisms that cause zone-specific expression patterns of enzymes are yet to be resolved.

Adrenal steroid production remains an area of active research, which supports the need to develop appropriate cell models that can mimic adrenal physiology or pathology. Primary

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cultures of adrenocortical cells have proven to be useful for examining the mechanisms controlling many aspects of adrenal physiology (Chen and Hornsby, 2006; Kuulasmaa et al., 2008; Cardoso et al., 2009; Xing et al., 2010; Xing et al., 2011). However, several issues have limited the use of primary adrenal cells as *in vitro* models. The most common limitations are the constant requirement for fresh tissue and the difficulties associated with the isolation of adequate cortical cells. In addition, cells from different human donors are subject to considerable variability; whereas cells from rodents do not produce cortisol or adrenal androgens due to the lack of steroid 17α-hydroxylase (CYP17) expression. To overcome the problems with tissue accessibility and quality, many groups have attempted to establish cell lines from adrenocortical carcinomas. This approach has been somewhat successful leading to adrenal cell lines from several species and we have previously reviewed the overall development of these models (Rainey et al., 1994; Rainey et al., 2004). Herein, we focus only on the human adrenocortical cell lines and provide details with regard to their development and utility.

2. Human adrenocortical carcinoma cell lines

2.1 The NCI-H295 derived adrenocortical carcinoma cell lines

The NCI-H295 cell line was established from a female patient diagnosed with an adrenocortical carcinoma(Gazdar et al., 1990). A large invasive adrenocortical tumor was detected in this patient and was later reported to have metastasized to the lungs and liver. Following tumor extraction, the tissue was finely minced, defragmented and maintained in various serum-containing and serum-free culture media for a one year period. The most vigorous growing cells were selected and designated as the NCI-H295. Radioimmunoassay (RIA) and gas chromatography/mass spectroscopy (GCMS) analysis demonstrated that the selected cells could produce a variety of steroids (Gazdar et al., 1990).

Because the original NCI-H295 cells grow very slowly as loosely attached cell clusters, alternative growth conditions were sought to segregate a population of cells with better monolayer attachment and more rapid growth. To achieve this goal, cells were continuously flushed with growth medium to remove the floating suspended cells and retain the attached subtype. Based on the serum supplement used for growth, three strains were developed and have been termed H295R-S1, H295R-S2 and H295R-S3 (Rainey et al., 2004). All three strains grow as adherent monolayer cultures (Table 1). However, the responses of these strains and their growth characteristics vary significantly, which appears related to the different growth medium. As a result the functional aspects of the H295R cells vary in individual laboratory where tissue culture conditions are different that those used for their isolation.

H295R-S1 grows in a commercially available Nu-Serum type I (5%, Collaborative Biomedical Products, Bedford, MA) supplemented medium. This strain is available from the American Type Culture Collection as ATCC CRL-2128. Strain 2 (H295R-S2) grows in the medium with the serum substitute called Ultroser-G (2.5%, Pall Corporation, Port Washington, NY). Previous assessments reported increased adrenal cell growth and steroidogenic function with this media supplement (McAllister and Hornsby, 1987; Hornsby and McAllister, 1991; McAllister et al., 1994). Indeed these cells remain highly differentiated and respond to Ang II and K^+ treatment by increased steroid production. Strain 3 (H295R-S3) grows in a serum called Cosmic Calf (10%, Invitrogen, Grand Island, NY).

Walter Miller and colleagues used the parental NCI-H295 cell line to select another monolayer strain called H295A (Rodriguez et al., 1997). The method for isolation of this strain was similar to that described for H295R, relying on the removal of non-attached cells

with medium changes, to select a population of cells that grew as a monolayer. Interestingly, H295A cells have limited response to Ang II (Table 1).

In an attempt to develop a new human adrenocortical carcinoma (HAC) cell line with ACTH responsiveness, Parmer et al. isolated clonal populations of cells from what was thought to be a "novel" adrenal tumor (Parmar et al., 2008). However, subsequent single-nucleotide polymorphism (SNP) array analysis indicated that the clones were isolated from contaminated H295R cells. Two of the clones (HAC13 and HAC15) responded well to Ang II and K^+ treatment, with increased aldosterone production. HAC15 cells also exhibited modest response to ACTH through significant increases in cortisol production and steroidogenic enzyme expression (Table 1). Compared to the NCI-H295 cell strains, isolated from a mixed population of tumor cells, the HAC cell clones are monoclonal which may provide a more stable steroidogenic phenotypes with time in culture.

2.2 SW13 human adrenal carcinoma derived cell line

SW13 cells were derived from a small cell carcinoma in the adrenal cortex of a 55-year-old female (Table 1). Although the cell line was taken from a surgically removed adrenal, SW13 cells produce no steroids and it is unclear whether the cell line was derived from a primary cancer arising from the adrenal cortex or from metastasis to the adrenal cortex (Leibovitz et al., 1973). SW13 cells are available from the American Type Culture Collection (CCL-105). These cells have a mosaic pattern of vimentin expression and are deficient in the mammalian homologues of Brahma genes, Brm and BRG1 (Hedberg and Chen, 1986; Butler et al., 2000; Yamamichi-Nishina et al., 2003). Due to the lack of steroidogenic phenotype, the usefulness of these cells as an adrenocortical model system is limited.

2.3 PRKAR1A inactivated adrenocortical cells

This cell line was derived from the adrenal glands of a patient diagnosed with primary pigmented nodular adrenocortical disease (PPNAD) and Carney complex (Table 1). The PRKAR1A gene that encodes the regulatory subunit type 1A (RIα) of cAMP-dependent protein kinase (PKA) was inactivated via gene mutation after the introduction of this cell line. PRKAR1A cells originally produced cortisol in the early passages, but lost this capacity while in culture (Nesterova et al., 2008). Other than steroid production, this cell line was mainly used for cAMP/PKA signal pathway studies, which involved adrenal tumorogenesis (Almeida and Stratakis, 2011).

2.4 ACT-1 human adrenal carcinoma derived cell line

ACT-1 cells were isolated from a 62 year old male patient who was initially diagnosed with a left adrenocortical carcinoma. ACT-1 cells were only shown to express HSD3B2 enzymes, and were devoid of any adrenocortical steroid production (Ueno et al., 2001). Thus, ACT-1 cells have limited adrenocortical function and therefore serve in a restricted role for adrenal steroidogenic studies (Table 1). However, these cells may prove useful to screen for adrenocortical carcinoma therapies (Kiiveri et al., 2004).

2.5 RL-251 human adrenal carcinoma derived cell line

A right adrenal mass was removed and placed in cell suspension from a 75 year old male who presented with high blood pressure and fever. As with H295R cell lines, the isolated RL-251 cultures exhibited an abnormal karyotypic profile, consisting of numerous deletions and translocations (Table 1). Assessments of adrenocortical function showed atypical steroidogenesis, and lack of response upon ACTH stimulation (Schteingart et al., 2001). To date, steroid production has not been observed in the RL-251 cell line. Intriguingly, in the initial report, RL-251 cells expressed ample amounts of interleukin-8, epithelial cell-derived

neutrophil-activating peptide 78, growth regulated oncogene-α and growth regulated oncogene-*y*. These molecules are CXC chemokine family cytokines that have potent angiogenic activity essential for tumor growth. It is speculated that these chemokines may play a role in the enhancement of carcinoma growth and proliferation in an autocrine or paracrine manner (Schteingart et al., 2010). While a lack of reported steroidogenesis and hormonal responses make these cells an inappropriate model to study steroidogenesis, these cells may prove useful in identifying the role of chemokines in adrenocortical carcinomas.

2.7 Pediatric adrenocortical adenoma derived cell line

Primary adrenocortical adenoma cells were isolated from a 1 year old female that presented with virilization and Cushing's syndrome (Almeida et al., 2008). The cell line initially grew at a slow rate with a spindle-like morphology. Melan-A, a melanocytic differentiation marker, which is expressed in steroid hormone producing adrenal adenomas and carcinomas, was detected in the cells (Ghorab et al., 2003). Biosynthesis of cortisol, aldosterone, androstenedione, and 17-hydroxyprogesterone was observed along with the expression of steroidogenic enzymes that included HSD3B2, CYP11B1 and CYP21 (Table 1). Taking into consideration that this newly developed pediatric cell line was last reported to have only reached eight passages, the likelihood of the cell line's wide-spread use remains uncertain.

2.8 SV40 transformed adrenal cell lines

Simian virus 40 (SV40) T-antigen is a viral oncogene which is capable of transforming many cell types (Hornsby et al., 1989). The use of a SV40 T-antigen transformation strategy resulted in the production of human fetal adrenal cell clones that responded to cAMP with an increase in both CYP17 and CYP11A1, but no change in CYP21 and CYP11B1 (Table 1). These transformed cells were maintained in culture for 30 to 40 population doublings after isolation, but then entered a "crisis" stage and stopped dividing (Cheng et al., 1992). The inability to maintain these cell cultures for extended periods limits their widespread use as a steroidogenic or carcinoma model.

3. Steroidogenic enzyme expression and steroid production

SW13, PRKAR1A, ACT-1, and RL-251 cells appear to lack steroidogenic potential. The previously described pediatric adrenocortical adenoma derived cell line and the SV40 transformed adrenal cell lines showed some steroidogenic capacity, but both stopped growing after several passages. In this section, we will limit our discussion to the continuous growing NCI-H295 cell strains and clonal HAC15 cells.

After establishment of NCI-H295 cells, primary assessment of steroidogenic capacity was performed using GCMS and radioimmunoassay. Of a total of 30 different steroids reportedly synthesized and secreted by parental NCI-H295, approximately 20 were formally identified as known steroid hormones. The production of these steroids suggested the original NCI-H295 cells expressed all of the enzymes participating in normal human adrenal steroidogenesis (Gazdar et al., 1990). Along with the parental NCI-H295, the H295R and H295A cell strains have also been used as models for studying steroidogenic enzyme gene expression. Transcripts encoding StAR, HSD3B2, as well as the five forms of cytochrome P450 known to be involved in normal adrenal steroidogenesis (CYP11A1, CYP17, CYP21, CYP11B2 and CYP11B1), are detectable in the H295 cell strains, and HAC15 clonal cell line (Bird et al., 1993b; Bird et al., 1993a; Bird et al., 1995a; Bird et al., 1995b; Bird et al., 1996; Denner et al., 1996; Samandari et al., 2007; Parmar et al., 2008).

The ability to produce steroids that span the multiple zones of the adrenal cortex, suggests that the NCI-H295 derived cell lines remain pluripotent with regard to adrenocortical differentiation. These cells produce an array of steroids even under basal conditions (Rainey

et al., 1994; Xing et al., 2011). It is noteworthy that treatment with agonists appears to selectively promote the synthesis of certain zone-specific steroid hormone groups in H295R. For instance, Ang II and K^+ stimulation drastically increased aldosterone production (Bird et al., 1993b; Bird et al., 1993a; Rainey et al., 1994; Clark et al., 1995), while treatment with forskolin induced cortisol, 11β-hydroxyandrostenedione, DHEA, DHEA-sulfate, corticosterone, 11-deoxycortisol, and androstenedione production (Rainey et al., 1993; Xing et al., 2011). As stated above, with a wide variety of effective agonists, a mosaic of unique steroid expression profiles are possible in H295A, H295R and HAC15 cells, thus making these cell lines potential models for steroid hormone biosynthesis in all adrenocortical zones.

4. Receptors and responsiveness to agonist

Although Ang II, K^+ and ACTH are the primary regulators of adrenal steroid hormone production, there was no indication of hormonal responsiveness in the original description of the NCI-H295 cells (Gazdar et al., 1990). However, subsequent to original report, the responses to Ang II, K^+, and ACTH treatment, as well as expression of corresponding receptors, were characterized for the H295A, H295R, and HAC15 cell models. Ang II is the primary hormonal regulator within the renin-angiotensin-aldosterone system (RAAS), and it acts on the adrenal glomerulosa by binding to type 1 Ang II (AT1) receptors to increase the production of aldosterone. The H295R cell has proven to be a useful model to study Ang II regulated aldosterone production(Nogueira et al., 2007; Otani et al., 2008; Nogueira et al., 2009). Only AT1 receptor antagonists have a significant inhibitory effect on these cells, while type 2 AngII (AT2) receptor antagonists have little impact on Ang II stimulation, demonstrating that H295R cells respond almost exclusively to the AT1 receptors(Bird et al., 1993a; Bird et al., 1994). Subsequent studies also revealed AT1 receptor coupled with the expression of phosphoinositidase C increased inositol phosphates in H295R cells(Bird et al., 1993b). The HAC15 cell line also resulted in an increase of aldosterone production when stimulated by Ang II(Parmar et al., 2008). In contrast, the H295A cell strain did not show a significant increase of steroid production when stimulated by Ang II, which is consistent with the known low level of AT1 receptor expression in this cell strain(Samandari et al., 2007).

The other major physiologic regulator of adrenal aldosterone production is extracellular K^+. An increase in intracellular calcium levels, in response to elevated K^+, mediates an increase in aldosterone biosynthesis. In addition, there is evidence of an intra-adrenal renin/angiotensin system, in which K^+ stimulation increases the production of both Ang I and Ang II(Hilbers et al., 1999). The H295R cell line is used by many laboratories as a model to study the mechanisms of K^+ regulation of adrenal steroid production(Romero et al., 2006; Bandulik et al., 2010; Nogueira et al., 2010). HAC15 cells also show a significant increase of aldosterone production in response to the K^+ treatment. However, response of the H295A cell strain to K^+ is currently unknown.

ACTH, along with its receptor (melanocortin 2 receptor, MC2R), is the primary hormonal regulator of adrenal cortisol production. Interestingly, the H295R cell line is only mildly responsive to ACTH, while most other adrenocortical cell lines are completely unresponsive(Parmar et al., 2008). However, ACTH treatment of H295R cells results in an acute increase in aldosterone biosynthesis, though the cell line lacks long term responsiveness to ACTH(Staels et al., 1993; Janes et al., 2008; Parmar et al., 2008). The H295A strain exhibited similar ACTH receptor expression to H295R according to a comparison study between these two cell strains(Samandari et al., 2007). Since ACTH primarily regulates cortisol production through cAMP signaling, the addition of either forskolin (to activate adenylyl cyclase) or cAMP analogues is often used to overcome this lack of effect of ACTH in H295A and H295R strains. (Rainey et al., 1993; Rainey et al.,

1994; Bird et al., 1996; Samandari et al., 2007). Another alternate strategy could involve the use of transgenic technology to reinstate MC2R expression in the cell lines. However, attempts to do so have resulted in an increase in receptor expression, but not in a detectable response to ligand in H295R cells (unpublished observation). The MC2R gene is expressed in HAC15 cells, with mRNA levels that are higher than those found in the H295R cell line(Parmar et al., 2008). However, the expression of the MC2R gene and the response to ACTH of the HAC15 clones is lower compared to primary culture of adrenal cells. At present, investigations of ACTH responsiveness rely heavily on the primary culture of human adrenal cells and mouse Y1 adrenal cell line (Schimmer et al., 2006; Xing et al., 2011).

5. Cell lines as adrenal cancer therapy tools

Adrenocortical carcinoma (ACC) is a rare malignant neoplasm with a poor prognosis. Previous studies showed that several genes are associated with ACC. Fernandez-Ranvier et al. compared the gene profiles between 11 malignant adrenocortical carcinomas and 78 benign adrenocortical adenoma, and showed that the overexpression of *CCNB2* (cyclin B2) and *IL13RA2* (interleukin 13 receptor, alpha 2); and decreased expression of *SLC16A9* (solute carrier family 16, member 9), *HTR2B* (5-hydroxytryptamine receptor 2B) and *RARRES2* (retinoic acid receptor responder) are related to the risk of malignancy of adrenocortical tumors(Fernandez-Ranvier et al., 2008). Interestingly, H295 cell strains had higher *CCNB2, IL13A2* and lower *SLC16A9, HTR2B* and *RARRES2* mRNA levels compared with normal adrenal tissue (Figure 3). de Reynies et al. found that *DLG7* (Discs large homolog 7), *PINK1* (PTEN induced putative kinase 1) and *BUB1B* (budding uninhibited by benzimidazoles 1 homolog beta) were related to the outcome prognosis of adrenocortical carcinoma(de Reynies et al., 2009). These genes were also differentially expressed between H295 cell strains and normal adrenal tissues in a manner similar to that seen for ACC versus normal adrenal tissue (Figure 4). Giordano *et al* compared ACC with adrenocortical adenoma and normal adrenal tissue by using cDNA microarray, and showed that more than 20 genes significantly highly expressed in ACC(Giordano et al., 2009). Most of the genes in their analysis list are also expressed higher in SW13, PRKAR1A, and H295 cell strains when compared to normal adrenal tissue according to our microarray analysis (Figure 5). These data suggest that H295 derived cell lines are likely appropriate models for certain aspects of ACC study.

In vitro cancer therapy screening represents an important preclinical assessment of anti-cancer compounds before any clinical evaluation (Weiss et al., 1980; Fisher et al., 1981; Bodrogi, 1989; Shoemaker, 2006). SW13 and NCI-H295 cell strains have been widely used as tools for screening anti-cancer drugs. Suramin, one of the first anti-cancer drugs tested in the NCI-H295 cells, reduced the production of glucocorticoids, mineralocorticoids and adrenal androgens(La Rocca et al., 1990). Another proposed anti-cancer drug, mitotane, strongly suppressed cell growth of NCI-H295 cells(Schteingart et al., 1993), and later adopted as an anti-adrenocortical carcinoma drug(Veytsman et al., 2009; Bertagna, 2010; Fassnacht et al.). Recently, a study showed that mitotane increased the effect of radiotherapy on both H295R and SW13 cell models(Cerquetti et al., 2010). Fallo et al. tested the cytotoxic/anti-proliferation drugs, taxol and paclitaxel, which effectively exhibited dose-dependent inhibition of cellular growth and steroidogenesis in NCI-H295 cells (Fallo et al., 1996; Fallo et al., 1998). The adrenostatic compounds aminoglutethimide (AG), metyrapone (MTP) and etomidate (ETO) were also tested in NCI-H295 cells for their anti-proliferative properties. AG and ETO inhibited cell proliferation and ETO was much more potent than AG(Fassnacht et al., 2000). Thiazolidinediones (TZDs) are specific peroxisome proliferator-activated receptor (PPAR)-gamma ligands. Examination in NCI-H295 cells suggested that TZDs might have favorable effects in the treatment of a variety of tumors and appear to act

as differentiation-inducing agents(Betz et al., 2005). Rosiglitazone, a member of TZDs, showed autophagy in H295R cells and cell cycle deregulation in SW13 cells, which suggested a potential therapy role for rosiglitazone in ACC therapy(Cerquetti et al., 2011) Interestingly, TZD also showed suppression in CYP11B2 expression and aldosterone production when tested in H295R cells(Uruno et al., 2011).

Human adrenocortical carcinoma cell lines have also been utilized to assess the role of certain interferons, chemokines and growth factors in adrenocortical cancers.(Schteingart et al., 2001; van Koetsveld et al., 2006; Almeida et al., 2008; Schteingart et al., 2010) T-cell factor/β-catenin antagonists, PKF115-584 and type I insulin-like growth factor receptor inhibitors have been recently tested in the NCI-H295 cells(Doghman et al., 2008; Barlaskar et al., 2009). These biochemicals are targeted to a limited number of known genetic mutations in adrenocortical carcinomas which are also present in the NCI-H295 cells. Finally, NCI-H295 cells were also tumorigenic when inoculated subcutaneously into nude mice lacking a thymus, a species model frequently used for preclinical anti-carcinoma drug screening(Gazdar et al., 1990).

6. Conclusion

Human adrenocortical cell lines represent a crucial tool for molecular and cellular studies that cannot practically be done in animal models. Currently available human cell lines can produce mineralocorticoids, glucocorticoids and adrenal androgens, respond to AngII, ACTH and K^+, and act as a screening tool for cancer therapies. The reliance on one adrenal carcinoma (the NCI-H295) as a source for most of the models is a concern and a limitation that supports the need for the development of additional human adrenal cell lines.

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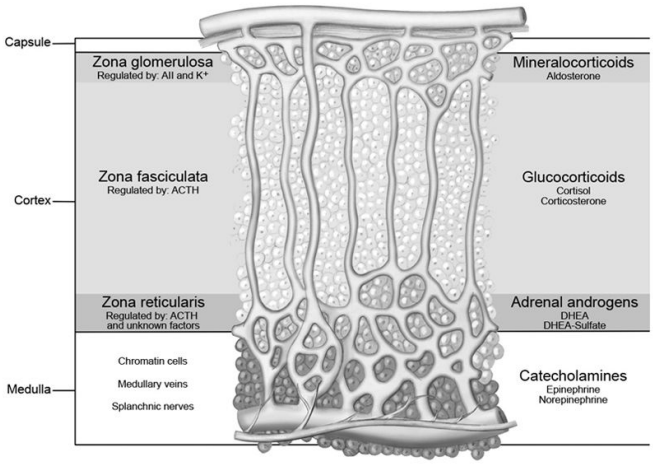

Figure 1.

The adrenal cortex is divided into three histological and functionally distinct zones: the zona glomerulosa synthesizes mineralocorticoids; zona fasciculata produces cortisol and zona reticularis secrets the so called adrenal androgens, DHEA and DHEA-sulfate.

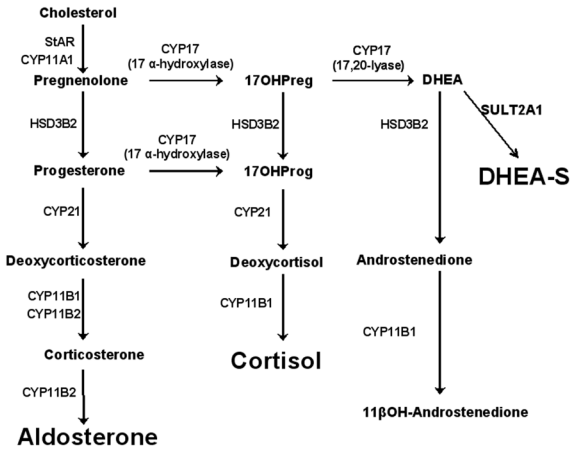

Figure 2.

Human adrenal steroid biosynthetic pathways illustrating the three main products of the human adrenal cortex: aldosterone, cortisol, and adrenal androgens (DHEA, DHEA-S) as well as the enzymes that synthesize these steroids. StAR = steroidogenic acute regulatory protein; CYP11A1 = cholesterol side-chain cleavage enzyme; HSD3B2 = 3 beta-hydroxysteroid dehydrogenase type II; CYP21 = 21-hydroxylase; CYP11B1 = 11-beta-hydroxylase; CYP11B2 = aldosterone synthase; CYP17 = 17-alpha-hydroxylase/17, 20 lyase; SULT2A1 =Steroid-sulfotransferase.

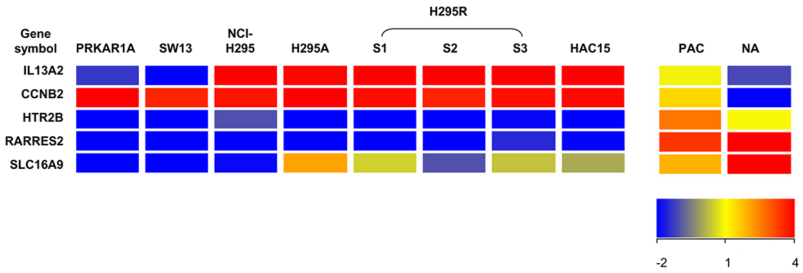

Figure 3.

Heatmap of *CCNB2*, *IL13A2*, *SLC16A9*, *HTR2B* and *RARRES2* genes in human PRKAR1A, SW13, H295 cell strains, HAC15 cells, primary adrenal cells and normal adrenal tissue based on microarray analysis. *CCNB2*, *IL13A2* were expressed at higher level while *SLC16A9*, *HTR2B* and *RARRES2* were expressed at lower level in most of the cell lines compared with the human normal adrenal tissue or primary culture of adrenal cells. The expression of these genes in cell lines showed a consistency with its expression in malignant adrenocortical tumor according to Fernandez-Ranvier et al. study(Fernandez-Ranvier et al., 2008). The color of heatmap indicates the expression value of each gene in the corresponding sample, and is based on the bar in the lower right-hand corner. PAC=primary adrenal cells; NA= normal adrenal tissue.

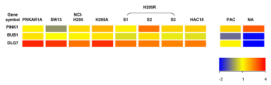

Figure 4.

Heatmap of *DLG7*, *PINK1* and *BUB1B* genes, which were able to predict adrenocortical tumor prognosis(de Reynies et al., 2009), in human PRKAR1A, SW13, H295 cell strains, HAC15 cells, primary adrenal cells and normal adrenal tissue based on microarray analysis. Expression in these cancer prognostic genes varied between the cell lines and normal adrenal tissue. The color of heatmap indicates the expression value based on the bar in the lower right-hand corner. PAC=primary adrenal cells; NA= normal adrenal tissue.

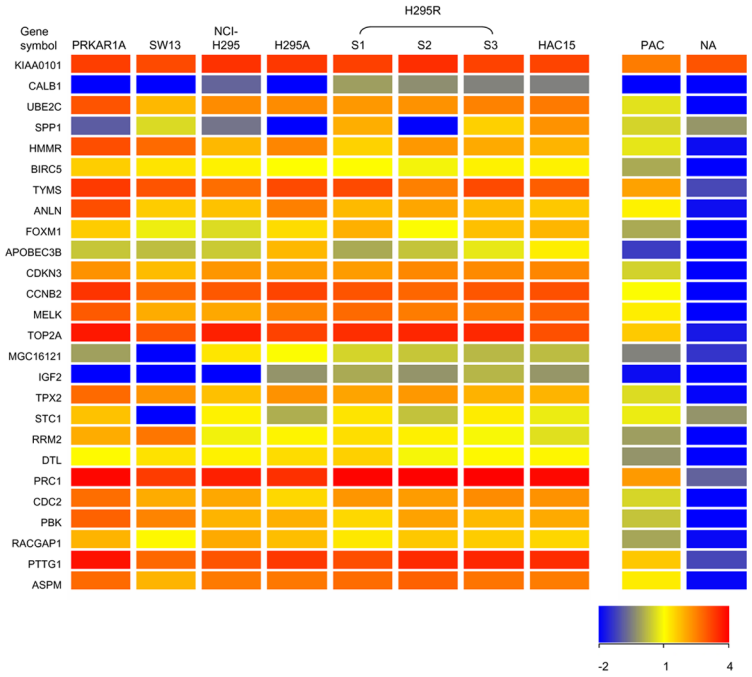

Figure 5.

Heatmap of genes found to be elevated in ACC by Giordano et al. study(Giordano et al., 2009). These genes also showed a higher expression level in most cell lines compared with normal adrenal tissue. Primary cultures of adrenal cells had a phenotype between normal adrenal tissue and the cell lines. The expression level of each gene was indicated by bar in the lower right-hand corner. PAC=primary adrenal cells; NA= normal adrenal tissue.

Table 1

Summary of Human Adrenocortical Cell Lines

Cell Line Name	Ang II	K^+	ACTH	cAMP	Steroids produced	Reference
NCI-H295	ND	ND	−	+	Mineralocorticoids Glucocorticoids Adrenal Androgens	(Gazdar et al., 1990; Staels et al., 1993; Rainey et al., 1994)
NCI-H295A	−	ND	−	+	Mineralocorticoid Glucocorticoids Adrenal Androgens	(Rodriguez et al., 1997; Huang et al., 2005)
NCI-H295R Strains	+	+	−/+	+	Mineralocorticoids Glucocorticoids Adrenal Androgens	(Bird et al., 1993;Rainey et al., 1994; Bird et al., 1995; Clark et al., 1995; Denner et al., 1996)
L-251	ND	ND	−	ND	None reported	(Schteingart et al., 2001)
ACT-1	ND	ND	ND	ND	None reported	(Ueno et al., 2001)
SW13	ND	ND	ND	ND	None reported	(Leibovitz et al., 1973)
Pediatric adrenocortical adenoma derived cell line	ND	ND	ND	ND	Mineralocorticoids Glucocorticoids	(Almeida et al., 2008)
HAC15	+	+	+	+	Mineralocorticoids Glucocorticoids Adrenal Androgens	(Parmar et al., 2008)
PRKAR1A inactivated cell line	ND	ND	−	−	None reported	(Nesterova et al., 2008)

Abbreviation: ND, Not determined