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Animal models of adrenocortical tumorigenesis

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Abstract

Over the past decade, research on human adrenocortical neoplasia has been dominated by gene expression profiling of tumor specimens and by analysis of genetic disorders associated with a predisposition to these tumors. Although these studies have identified key genes and associated signaling pathways that are dysregulated in adrenocortical neoplasms, the molecular events accounting for the frequent occurrence of benign tumors and low rate of malignant transformation remain unknown. Moreover, the prognosis for patients with adrenocortical carcinoma remains poor, so new medical treatments are needed. Naturally occurring and genetically engineered animal models afford a means to investigate adrenocortical tumorigenesis and to develop novel therapeutics. This comparative review highlights adrenocortical tumor models useful for either mechanistic studies or preclinical testing. Three model species – mouse, ferret, and dog – are reviewed, and their relevance to adrenocortical tumors in humans is discussed.

Keywords

adrenal cortex; oncology; mouse; ferret; dog

1. Introduction

Animal models provide a considerable range of possibilities for the investigation of adrenocortical tumorigenesis. The high incidence of adrenocortical tumors (ACTs) in certain species can serve as the starting point for genetic and comparative genomic approaches to elucidate the molecular basis for tumorigenesis. Phenotypic evaluation of incidentally discovered ACTs in genetically modified animals can provide clues on pathways involved in adrenal tumor growth that would not have been predicted from structural analysis or *in vitro* exploration. Targeted genetic modification can also be used to verify the functional significance of a given gene or pathway for adrenal growth and steroidogenesis *in vivo*. Finally, well defined tumor models can be used for preclinical intervention trials to screen for novel therapeutic approaches.

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This review provides an overview of ACT models useful for either mechanistic studies or preclinical screening approaches. We focus on three model species: mouse, ferret, and dog. The relevance of each of these models to ACTs in humans is discussed.

2. Comparative anatomy and physiology of the adrenal cortex

The adrenal cortex of humans and most domestic animals, including ferrets and dogs, comprises three principal layers: the zona glomerulosa (ZG), zona fasciculata (ZF), and zona reticularis (ZR) (Table 1) (Bielinska et al., 2006; Else and Hammer, 2005). The ZG secretes mineralocorticoids; ZF and ZR function as a unit to produce glucocorticoids and in some species adrenal androgens. In contrast, the mouse adrenal cortex contains a well-defined ZG and ZF, but there is no discernable ZR. The adrenal cortex of the young mouse contains an additional layer, the X-zone, which is adjacent to the medulla (Keegan and Hammer, 2002). The function of the X-zone, which regresses at puberty in males and during the first pregnancy in females, remains controversial, although evidence suggests that it may be involved in progesterone catabolism (Hershkovitz et al., 2007). Transgenic expression of *LacZ* driven by a specific *Sfl* fetal enhancer element indicates that the X-zone is a remnant of the adrenal primordia that forms before the definitive adrenal cortex (Zubair et al., 2008).

Mice, ferrets, dogs, and humans differ in the repertoire of steroidogenic enzymes and cofactors expressed in the adrenal cortex, and these differences have functional implications. Two key proteins that are differentially expressed among species are CYP17 and its allosteric regulator, cytochrome b₅ (cyt b₅). CYP17 is a bi-functional enzyme that catalyzes both the 17 α -hydroxylation reaction required for the production of cortisol and the 17,20-lyase reaction required for the synthesis of androgens. The 17,20-lyase activity of CYP17 is selectively enhanced by cyt b₅ (Auchus, 2004; Wagner et al., 2008). Cells in the ZF and ZR of ferrets, dogs, and humans possess the 17 α -hydroxylase activity; consequently cortisol is the principal glucocorticoid secreted by the adrenal gland of these species (Bielinska et al., 2006; Fox and Marini, 1998; Meij and Mol, 2008). In humans the adrenal cortex begins to produce dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) at adrenarche (6-8 years of age), and this increase in adrenal androgen production coincides with increased expression of cyt b₅ in the ZR (Nakamura et al., 2009). Under physiological conditions the adrenal glands of ferrets and dogs produce only limited amounts of androgenic steroids (Donovan et al., 1983; Frank et al., 2003; Rosenthal and Peterson, 1996). In the case of ferrets this dearth of adrenal androgen production has been attributed to low cyt b₅ expression in the adrenal cortex (Wagner et al., 2008). The molecular basis for limited adrenal androgen production by the dog adrenal is unknown. Adrenocortical cells in the adult mouse lack CYP17; as a result, corticosterone is the principal glucocorticoid secreted by the mouse adrenal cortex, and under physiological conditions androgenic steroids are not produced in this tissue (Keeney et al., 1995).

The differentiation, growth, function, and survival of steroidogenic cells in the adrenal gland are controlled by a diverse group of hormones, including adrenocorticotrophic hormone (ACTH), angiotensin-II, vasopressin, and insulin-related growth factors (IGFs) (Bielinska et al., 2006). In certain instances, endocrine and paracrine factors traditionally associated with the function of gonadal steroidogenic cells, such as luteinizing hormone (LH) and inhibin, may also influence the differentiation, proliferation, and function of adrenocortical progenitor cells (Bielinska et al., 2006).

3. Mouse models of adrenocortical neoplasia

3.1. Spontaneous development of ACTs in inbred mouse strains

An early case of adrenal tumorigenesis in mice has been reported for the inbred mouse strain CE after surgical gonadectomy (Woolley and Little, 1945) which later was also observed in other inbred strains including DBA/2J, C3H, BALB/c, and NU/J animals (Bielinska et al., 2006). Both surgical gonadectomy and xenografting of hCG producing tumors (Bielinska et al., 2005) were able to induce adrenocortical tumor growth, suggesting that chronic elevation of gonadotropins represents a major determinant of adrenocortical tumorigenesis in these strains. In accordance with early morphological findings, later functional and molecular studies revealed the expression of markers which are otherwise restricted to the gonad including receptors for LH and anti-Müllerian hormone (AMH) as well as steroidogenic enzymes such as CYP17 and CYP19 (Bielinska et al., 2006; Johnsen et al., 2006). Accordingly, an adaption to the gonad's ability to secrete sex steroids was detected (Johnsen et al., 2006). Interestingly, this functional change was also accompanied by a switch in the expression of the transcription factor *Gata6* to that of *Gata4* (Bielinska et al., 2006; Johnsen et al., 2006), which had been implicated in the regulation of tissue-specific gene expression and cellular proliferation in the gonad (Laitinen et al., 2000; Tremblay and Viger, 2001). To provide information on genetic mechanisms associated with gonadectomy-induced adrenal tumorigenesis Bernichtein and colleagues performed a genome-wide association study in non-susceptible C57BL/6J and susceptible DBA/2J animals (Bernichtein et al., 2007). Linkage analysis identified a major locus on chromosome 8 containing, among other genes, *Sfrp1*, a dominant negative regulator of the Wnt/ β -catenin signalling pathway.

Another genetic finding common to mouse strains with high tumor susceptibility is a polymorphism in Steroidogenic Factor-1 (SF1), a transcription factor essential for normal development and function of the adrenal cortex (Luo et al., 1994). A polymorphism which results in a substitution from alanine to serine at residue 172 of the protein appears to influence steroidogenic capacity of adrenocortical cells (Frigeri et al., 2002). Since *Sf1* dosage has been described as a relevant trigger of tumor growth in human ACC (Figueiredo et al., 2005) and in a transgenic mouse model (Doghman et al., 2007), it is possible that the polymorphism could be associated with higher baseline *Sf1* expression levels, thus, predisposing the development of ACT growth.

3.2. Mouse models with transplanted ACTs

Genetic modifications have been applied to primary cultures of normal human and bovine adrenocortical cells to recapitulate molecular alterations found in the course of tumorigenesis *in vivo* and to define those to be necessary and sufficient to induce malignant tumor growth in transplanted animals. Following this approach the role of illicit receptor expression which has been identified as the cause of ACTH-independent hypercortisolism in bilateral adrenal macronodular hyperplasia as well as ACTs (Lacroix et al., 2004) has been investigated. Bovine adrenal cells transduced with either the gastric inhibitory polypeptide receptor (GIPR) (Mazzuco et al., 2006b) or the LH receptor (LHR) (Mazzuco et al., 2006a) developed into hyper-proliferative adenomatous, but not overtly malignant, tissue when transplanted under the kidney capsule and resulted in a mild ACTH-independent hypercortisolism in the host animal. Thus, these experiments show that expression of either *GIPR* or *LHR* is sufficient to induce a phenotype similar to those defining the clinical entity, although these genetic changes are unlikely to be the sole reason for malignant ACT growth.

A commonly used adrenocortical model is that of the human adrenocortical carcinoma (ACC) cell line NCI-H295. Upon subcutaneous injection of 6×10^6 cells in athymic nude

mice, the tumor engraftment rate has been reported in the range of 90% with a median doubling time of 12 days (Logie et al., 2000). This cell line, which originated from a patient with hormonally active ACC, has been shown to retain its ability to produce all major adrenal steroids (Gazdar et al., 1990). Moreover, the tumors were characterized by dysregulation of the IGF system including overexpression of IGF2, similar to what has been observed in primary human tumor specimens. As IGFs have been defined as major contributors of ACT growth (Giordano et al., 2003; Weber et al., 1997), targeting of type I insulin-like growth factor receptor (IGF1R) dependent pathways represents a promising approach to modulate the proliferative phenotype of ACC cells. In accordance with this notion Barlaskar and colleagues could demonstrate that treatment of animals bearing NCI-H295 derived subcutaneous tumors with IGF1R antagonistic compounds resulted in a significant amelioration of tumor growth and increase in survival time (Barlaskar et al., 2009). These experiments among others have paved the way for current clinical trials exploring the efficacy of IGF blockade in patients with ACC. The same NCI-H295 based model was utilized by Luconi and colleagues to evaluate the effects of rosiglitazone on adrenocortical tumorigenesis *in vivo* (Luconi et al., 2010). In these experiments a significant therapeutic effect on tumor growth could be demonstrated in the treatment group concomitant with a decrease in the rate of proliferation and angiogenesis.

While the recent developments provide first examples for successful transition of preclinical findings from mouse tumor models to potential clinical relevant therapeutic approaches, further improvement of existing tumor models is being sought. From a clinical perspective the subcutaneous tumor niche represents an ‘unphysiological’ manifestation of ACC. More relevant metastatic sites would include local lymph nodes, liver, lung and bones. Recently, a technique for an orthotopic model has been described in which transplanted cells are retained within the adrenal gland by formation of a fibrin clot (Cardoso et al., 2010). Using this technique the authors could demonstrate invasion of a tumorigenic bovine adrenocortical cell line into the mouse adrenal cortex. Orthotopic and metastatic models have, however, the disadvantage of hindered follow-up examination. To further improve suitability of such a model, ACC cells stably expressing green fluorescent protein or luciferase could allow *in vivo* imaging approaches in the future. Another aspect to improve ACT models that should be addressed by future research is that of therapeutic testing in the context of personalized medicine. For example tumor material from patients that could be maintained in xenograft models would provide the opportunity to specifically test for the suitability of a specific compound for an individual patient.

3.3. Genetically modified mouse models with ACTs

Mice that have been designed to harbor specific genetic modifications through transgenic techniques or knock-out approaches have been instructive for the identification of molecular mechanisms involved in adrenocortical tumorigenesis. While generation of a genetically modified animal can be hypothesis-driven to specifically explore participation of a given pathway for adrenal tumorigenesis, careful phenotypic characterization of available mouse models in which an adrenal phenotype has been discovered incidentally can also serve as a starting point for further functional analysis.

The Simian Virus 40 (SV40) large T-antigen represents a commonly used oncogene which can be expressed in a tissue specific manner upon transgenic introduction under the control of an appropriate promoter sequence. Promoters that have been used to target the adrenal cortex include 5'-flanking sequences from the human *CYP11A* gene (Compagnone et al., 1997), the aldose reductase-like (*akr1b7*) gene (Sahut-Barnola et al., 2000), and the *inhibin-α* promoter (Kananen et al., 1996). In particular, the *inhibin-α* promoter driven transgenic animal (*inha/Tag*) has been phenotypically characterized in great detail and a large body of literature demonstrates the gonadectomy-dependent induction of adrenocortical

tumorigenesis (Kananen et al., 1997; Rilianawati et al., 2000; Rilianawati et al., 1998). Overall, these findings describe a feed-forward loop of chronic elevated LH levels that induce GATA4 levels which in turn increases expression of the LHR in the adrenal, which provides an important molecular switch in the initiation of adrenal tumorigenesis in this model (Vuorenoja et al., 2007). Gonadectomized *inha/Tag* animals were further utilized as a model of LHR targeted therapy. Using a lytic peptide (hecate) which was conjugated to a fragment of the hCG beta chain (hecate-hCGbeta) treatment of animals with established adrenal tumors induced a significant reduction in adrenal tumor size in male animals (Vuorenoja et al., 2008).

Another example for a transgenic mouse model characterized by adrenal tumor growth has been reported in an animal harboring multiple copies of the *Sf1* genetic locus (Doghman et al., 2007). The gain in *Sf1* copy number which resulted in an increase in adrenal SF1 protein levels was associated with the development of macronodular adrenocortical disease that further progressed into adrenal tumors in an age dependent manner. Together with clinical findings from childhood ACC in which the *Sf1* is amplified and overexpressed (Figueiredo et al., 2005) and from adult patients where *Sf1* expression has been associated with a poor prognosis (Sbiera et al., 2010) this model presents further evidence that gene dosage of *Sf1* can contribute to the phenotype of adrenocortical tumors. As specific SF1 inverse agonists have been developed and proven to be effective in *in vitro* adrenocortical systems (Doghman et al., 2009) the transgenic *in vivo* model might aid in further preclinical testing of these compounds.

Like susceptible inbred mouse strains and the *inha/Tag* transgenic model, mice with a targeted deletion of the inhibin α subunit (*Inha*^{-/-}) develop adrenocortical tumors in response to gonadectomy (Matzuk et al., 1992). Similar to what has been described in *inha/Tag* animals, adrenal tumors in *Inha*^{-/-} mice are characterized by a switch from *Gata6* to *Gata4* (Looyenga and Hammer, 2006) and a further change in expression pattern towards a gonadal phenotype (Beuschlein et al., 2003). LH can also act as an adrenal growth factor in the context of inhibin deficiency, as evidenced by experiments in which introduction of a transgenic background with chronic elevation of LH levels enhanced adrenal tumor growth (Beuschlein et al., 2003). On the contrary, genetic removal of *Smad3* which is required for activin signalling from *Inha*^{-/-} mice could be demonstrated to attenuate adrenal tumor progression by uncoupling extracellular mitogenic signals from the cell cycle machinery including cyclin D2 (Looyenga and Hammer, 2007). Likewise, the loss of *cyclin D2* prolonged the lifespan of double knockouts animals (Burns et al., 2003).

Despite the clear-cut evidence for *TP53* mutations as the underlying cause of childhood ACC (West et al., 2006), only recently has its role as a tumor suppressor gene in murine ACC development has been highlighted in a mouse model of telomere dysfunction in which animals with *p53* haploinsufficiency developed ACC in 5% of cases (Else et al., 2009). While these tumors exhibited locally invasive growth and a malignant histology, no metastatic spread has been reported.

Another well described mechanism in human adrenal tumorigenesis is the activation of β -catenin dependent pathways. The Wnt/ β -catenin pathway is essential for embryonic development and cell renewal including the adrenal cortex (Kim et al., 2008), but its ectopic constitutive activation is associated with cancer development in a number of tissues including adrenocortical tumors. Interestingly and in contrast to other mutations that were either present in benign or in malignant adrenocortical neoplasia, β -catenin mutations were found with similar frequency in adrenocortical adenoma and carcinoma and represented the most frequent genetic event in adrenal tumors in humans (Tissier et al., 2005). Utilizing a transgenic approach Berthoin and colleagues were able to demonstrate that constitutive

activation of β -catenin in the adrenal cortex resulted in progressive adrenocortical cellular hyperplasia and dysplasia. These initial morphological alterations were followed over a long time course into uncontrolled neo-vascularization and loco-regional metastatic invasion. Notably, this particular phenotype was associated with autonomous production of aldosterone leading to primary aldosteronism (Berthon et al., 2010) which had not been reported in other rodent adrenocortical tumor models.

4. Ferrets as a model of adrenocortical neoplasia

4.1. Prevalence and clinical presentation of ACTs in ferrets

Domestic ferrets, like certain strains of mice, develop gonadectomy-induced adrenocortical neoplasia (Bielinska et al., 2006). In the United States and Japan, where most ferrets are gonadectomized at 4-6 weeks of age, the incidence of adrenocortical neoplasia is 15-22% (Li et al., 1998; Miwa et al., 2009; Rosenthal, 1997; Weiss et al., 1999). The average age of diagnosis of adrenocortical neoplasia in ferrets is 4-5 years, and there is no gender predilection (Li et al., 1998; Miwa et al., 2009; Rosenthal, 1997; Weiss et al., 1999). In countries where preadolescent neutering of domestic ferrets is not routine, the incidence of adrenocortical neoplasia is markedly lower (Schoemaker et al., 2000). Other factors besides prepubertal gonadectomy that have been hypothesized to predispose ferrets to adrenocortical neoplasia include inbreeding at commercial facilities and unnatural photoperiodic stimulation (Bielinska et al., 2006).

The neoplastic cells that accumulate in the adrenal glands of gonadectomized ferrets functionally resemble gonadal steroidogenic cells and typically secrete sex steroids rather than corticoids (Rosenthal and Peterson, 1996). The ectopic production of sex steroids by neoplastic adrenocortical tissue causes a syndrome known as adrenal-associated endocrinopathy (AAE) or hyperadrenocorticism, although a more appropriate term for this condition is hyperandrogenism. Clinical signs of this syndrome include bilateral symmetric alopecia (Fig 1A), enlargement of the vulva, resumption of mating behavior, and stranguria (straining to urinate) due to squamous metaplasia of prostatic ductular epithelium (Fox and Marini, 1998). The diagnosis of AAE is confirmed by documenting elevated plasma concentrations of 17α -hydroxyprogesterone, androstenedione, DHEA-S, or estradiol (Fox and Marini, 1998; Rosenthal and Peterson, 1996).

In approximately 85% of ferrets with AAE, only one adrenal gland is enlarged (Rosenthal et al., 1993; Weiss and Scott, 1997). Histological examination of the adrenal cortex may reveal nodular hyperplasia, adenoma, or carcinoma. Adenomas are well-demarcated lesions composed mainly of polyhedral cells (Fig 1B,C) (Rosol et al., 2001). Carcinomas are usually large lesions that invade beyond the adrenal capsule and often contain mixtures of small basophilic ovoid cells, large polyhedral cells, and large clear cells with vacuolated cytoplasm (Rosol et al., 2001). Anaplastic variants of ACC may exhibit myxoid differentiation (Fig 1D,E) (Peterson et al., 2003). In practical terms, however, distinguishing benign and malignant ACTs is often challenging. A separate spindle cell component that expresses smooth muscle actin may be seen in benign or malignant adrenocortical neoplasms in ferrets (Fig 1D), although the prognostic importance of this component is unclear (Gliatto et al., 1995; Newman et al., 2004).

As in mice, the chronic elevation in circulating LH that follows gonadectomy is thought to be a prerequisite for neoplastic transformation of the adrenal cortex (Bielinska et al., 2006). In gonadectomized ferrets harboring adrenocortical tumors, plasma sex steroid levels are higher during the breeding season, when prolonged daylight augments LH secretion by the pituitary (Ryan, 1984). Conversely, signs of AAE can resolve in response to the decline in LH associated with a shortening photoperiod. Inhibition of gonadotropin secretion with

leuprolide acetate (Wagner et al., 2001) or deslorelin acetate (Schoemaker et al., 2008b; Wagner et al., 2005) can decrease sex steroid production by tumors and temporarily ameliorate signs of AAE. LHR immunoreactivity is evident in the both normal and neoplastic adrenocortical cells of the ferret adrenal; however, the receptor appears to be functional only in the neoplastic cells (Schoemaker et al., 2002). Whether other hormonal changes that accompany gonadectomy (e.g., decreased plasma inhibin, elevated plasma FSH, etc.) affect tumorigenesis in the ferret is unknown.

In addition to LHR, gonadectomy-associated tumors in ferrets express other markers characteristic of gonadal steroidogenic cells. Among these markers are inhibin- α (Peterson et al., 2003), AMH (Patterson et al., 2003), GATA4 (Fig 1C,E) (Peterson et al., 2004) and CYP19 (Wagner et al., 2008). Most ferret adrenocortical tumors express cyt b₅, which enhances the 17,20-lyase activity of CYP17 and favors the production of androgens over cortisol in these tumors (Wagner et al., 2008).

Although the vast majority of ferret adrenocortical tumors secrete sex steroids rather than corticoids, not all do. A ferret with LH-dependent Cushing's syndrome has been reported (Schoemaker et al., 2008a), and a case of primary hyperaldosteronism in a domestic ferret with an adrenocortical adenoma has also been described (Desmarchelier et al., 2008).

4.2. Molecular basis of adrenocortical tumorigenesis in ferrets

As in the mouse, the cell of origin for gonadectomy-induced adrenocortical neoplasms in ferrets remains elusive. It might be a committed gonadal progenitor that is ectopically located in the adrenal gland. Rare cells with morphologic features of gonadal steroidogenic cells have been described in the adrenal cortex of mammals (Magalhaes, 1972), and in gonadectomized ferrets sex steroid-producing tumors histologically indistinguishable from adrenocortical tumors have been observed at extra-gonadal sites (Patterson et al., 2003; Smith and Schulman, 2007). On the other hand, the cell of origin could be a multipotential progenitor capable of differentiation into either adrenocortical or gonadal-like steroidogenic cells, depending on the hormonal milieu and other factors (Bielinska et al., 2006).

Why chronic elevation of circulating LH results in only unilateral adrenal growth in the majority of ferrets with AAE is unknown. Adrenocortical tumorigenesis in ferrets, as in other species, is thought to involve both genetic and epigenetic alterations, but the molecular events underlying tumor development in ferrets remain poorly characterized. In human adrenocortical neoplasms, chromosomal changes accumulate during tumor progression, and analyses of heritable and spontaneous types of tumors have documented alterations in either cell surface receptors (e.g., LHR, GIPR, vasopressin receptors) or their downstream effectors that impact tumor development (Bielinska et al., 2009). Whether such genetic changes contribute to adrenocortical neoplasia in gonadectomized ferrets awaits further study. In addition to genetic changes, preexisting epigenetic alterations may impact the phenotypic plasticity of tumor progenitor cells in the adrenal cortex, allowing cells to respond to the hormonal changes associated with gonadectomy or increasing the likelihood of subsequent genetic alterations (Bielinska et al., 2009).

Although the domestic ferret is an established experimental model for studies of reproductive endocrinology and lung pathophysiology, it is not standardized with regard to genotype and therefore not ideal for studies on tumor genetics or epigenetics. However, the field of ferret genetics is advancing rapidly, as evidenced by the recent generation of a knock-in model of cystic fibrosis in this species (Sun et al., 2010). Sequencing of the ferret genome, coupled with the expanding database of genomic DNA sequences and single nucleotide polymorphisms (SNPs) in the mouse and other species, should facilitate the

characterization of alleles and genetic modifiers influencing gonadectomy-induced adrenocortical tumorigenesis.

The phenomenon of gonadotropin-induced adrenocortical neoplasia is not unique to ferrets and mice. Subcapsular tumors have been reported in the adrenal glands of other gonadectomized animals, including guinea pigs, hamsters, goats, and cats (Bielinska et al., 2009; Meler et al., 2011). Often these tumors produce sex steroids. As in ferrets, the human adrenal cortex constitutively expresses low levels of LHR, and this receptor has been shown to be functionally active in the adrenal during pregnancy and other high gonadotropin states (Bernichtein et al., 2008). Thus, it has been proposed that adrenal responsiveness to LH might contribute to adrenocortical tumorigenesis in humans. As in animal models, specific modifier genes in an individual's genetic background might impact the inherent sensitivity of human adrenal gland to chronic gonadotropin elevation (Bernichtein et al., 2008). The overall incidence of gonadotropin-associated adrenocortical neoplasia in humans is difficult to quantify, although autopsy series suggest that incidental adrenocortical tumors ("incidentalomas") are present in approximately 5% of elderly people (Thompson and Young Jr, 2003). There are anecdotal reports of adrenocortical neoplasms with histological features resembling luteinized ovarian stroma in postmenopausal women (Fidler, 1977; Wong and Warner, 1971) and men with acquired testicular atrophy (Romberger and Wong, 1989). While these findings provide indirect evidence that the increase in circulating gonadotropins associated with gonadal failure may contribute to adrenocortical tumor development in humans, the contribution of this phenomenon to the high incidence of adrenal tumors in the elderly remains uncertain.

5. Dogs as a model of adrenocortical neoplasia

5.1. Prevalence and clinical presentation of canine ACTs

The majority of canine ACTs are functional, with cortisol-secreting ACTs being the most common ones (Galac et al., 2010d). They represent about 15% of cases of spontaneous Cushing's syndrome, which in dogs occurs with a frequency of 1-2/1000 dogs/year (Willeberg and Priester, 1982). Most canine ACTs are unilateral solitary lesions, but bilateral tumors occur in about 10% of cases. Adrenal Cushing's syndrome occurs in dogs of middle and old age without sex or breed predilection. Gonadectomy does not impact the incidence of ACTs in dogs.

Histologically, canine ACTs can be divided into adenomas and carcinomas, although as in ferrets this distinction is not always straightforward. Microscopic examination of a seemingly benign canine ACT may reveal its expansion into blood vessels (Labelle et al., 2004). While in-growth into blood vessels is generally considered to be a hallmark of malignancy, canine ACTs may be an exception to this rule (van Sluijs et al., 1995). The probability of malignancy is related to the size of the tumor as lesions < 2 cm are benign and a greater diameter indicates a high risk of malignancy (Labelle et al., 2004).

In dogs the typical clinical signs of a functional ACT are those of glucocorticoid excess, with polyuria and polyphagia being the dominating features. The cardinal physical signs of canine Cushing's syndrome include abdominal obesity, weight gain, fatigue, muscle atrophy and skin changes (Fig 2A vs. B) and are similar to those in humans (de Bruin et al., 2009). The diagnosis of canine adrenal Cushing's syndrome relies on the determination of elevated circulating or urinary cortisol concentrations, which cannot be suppressed by administration of a high dose of dexamethasone, and on suppressed basal circulating ACTH concentrations (Galac et al., 2010d).

After the diagnosis of an ACT has been established, diagnostic imaging is of great assistance to determine the best treatment and to objectively evaluate the prognosis (van der Vlugt-Meijer et al., 2003; van der Vlugt-Meijer et al., 2002; Voorhout et al., 1990).

The preferred treatment for canine ACT is adrenalectomy (Kyles et al., 2003; van Sluijs et al., 1995) with the laparoscopic approach becoming the technique of choice (Jimenez Pelaez et al., 2008). As in humans, the size of the ACT may be a limitation for this procedure. In case of inoperable or metastasized ACTs, medical treatment with o,p'-DDD (mitotane) is used with the aim of complete destruction of all adrenocortical cells including metastases (Kintzer and Peterson, 1994). If neither adrenalectomy nor adrenocortical destruction with o,p'-DDD is an option, trilostane can be considered as a palliative treatment (Benckroun et al., 2008; Eastwood et al., 2003). Trilostane is a competitive inhibitor of 3 β -hydroxysteroid dehydrogenase, an essential enzyme system for the synthesis of cortisol, aldosterone, and androstenedione (Potts et al., 1978). Treatment with trilostane has the potential to significantly decrease basal and ACTH-stimulated circulating cortisol concentrations and therefore can be used successfully to control the clinical signs of glucocorticoid excess (Galac et al., 2010a; Wenger et al., 2004).

5.2 Molecular mechanisms of adrenocortical tumorigenesis in dogs

Canine ACTs share many similarities with those in humans. The major difference, however, is the incidence of cortisol-secreting ACTs, which in dogs is about 1000 fold higher than in humans (Lindholm et al., 2001; Willeberg and Priester, 1982). Therefore, spontaneous canine ACTs can serve as an animal model for studies in humans especially since therapeutic adrenalectomy can generate substantial amounts of primary canine ACT tissue for *in vitro* research purposes. To fully evaluate the feasibility of using canine ACTs as a model for human ACTs, it is necessary to understand the molecular make-up of canine ACTs and to compare this with data in humans.

Recently, the hormonal autonomy of canine ACTs was investigated through expression profiling of steroidogenic enzymes and the ACTH receptor (ACTH-R). The abundances of mRNA encoding steroidogenic enzymes did not differ significantly among adrenocortical adenomas, carcinomas, and normal adrenals. Consequently, hypercortisolemia in canine ACTs cannot be explained by upregulation of the genes encoding for steroidogenic enzymes (Galac et al., 2010c). Moreover, inefficient steroidogenesis, a hallmark of carcinomas, cannot be ascribed to altered expressions of genes encoding for the steroidogenic enzymes. A remarkable feature of canine ACCs is the low expression of the ACTH-R gene compared with that in adrenocortical adenomas (Galac et al., 2010c). This knowledge could be incorporated in the future differentiation between adrenocortical adenomas and carcinomas. In addition to molecular analysis of ACT tissue, the outcome of a preoperative ACTH stimulation test may provide the clinician and the pathologist with extra information about the behavior of a given ACT. In canine cortisol-secreting adenomas the abundances of mRNA encoding ACTH-R did not differ from those in normal adrenals. Therefore in adenomas, a clear rise in circulating cortisol concentration after ACTH administration is expected, while in carcinomas this response should be lacking (Galac et al., 2010c).

In the concept of autonomous steroid production and growth of canine ACTs, the expression of aberrant receptors seems relevant. Hypersecretion of cortisol by canine ACTs is not associated with overexpression of the genes encoding LHR, GIPR, and vasopressin receptors type 1a, 1b and 2 (Galac et al., 2010b). However, immunohistochemistry demonstrated that in the majority of the ACTs there was ectopic expression of GIPR and vasopressin 2 receptor protein and eutopic expression of LHR protein, which suggests a role for these hormone receptors in the pathogenesis of ACTs. Whether these changes in hormone receptor expression are sufficient to induce tumorigenesis, as demonstrated by *in*

vitro studies with bovine adrenocortical cells (Mazzuco et al. 2006a, 2006b), still needs to be answered.

As discussed earlier, the IGF system is one of the most important pathways involved in autonomous growth of human adrenocortical carcinomas. Preliminary results on canine ACT tissue demonstrate that only a minority of canine ACCs overexpress the *IGF2* gene. Ongoing studies are about to elucidate the role of the transcriptional changes of the IGF system in canine ACTs. With the current research we are aiming at a better understanding of the spectrum of molecular differences and similarities between canine and human ACTs and consequently increasing our knowledge of the possibilities and limitations of using the canine ACTs as a model for human ACTs.

Several tools are now available to interrogate the canine genome, including a publically available genome assembly, a SNP map, and SNP arrays (Lindblad-Toh et al., 2005; Rivera and von Euler, 2011). Given the homology between the genome of dogs and humans and the similarities in the biological behavior of ACTs in these two species, the dog may prove to be a useful comparative model for the study of adrenal tumors. Indeed, the Canine Comparative Oncology and Genomics Consortium is already performing comprehensive analysis of other types of tumors in dogs (e.g. lymphoma, osteosarcoma, melanoma, etc.) to gain insight into the pathogenesis and treatment of the corresponding tumors in humans (Breen, 2009).

6. Conclusions

Each of the animal models discussed in this review has inherent strengths and weaknesses, which are highlighted in Table 2. Collectively, these models provide complementary approaches for understanding the factors and signaling pathways involved in adrenocortical tumorigenesis in humans and for developing novel treatments.

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Highlights

> This comparative review highlights adrenocortical tumor models useful for either mechanistic studies or preclinical testing. > Three model species – mouse, ferret, and dog – are reviewed. > The relevance of these models to adrenocortical tumors in humans is discussed.

Figure 1. (A) Ferret with symmetrical alopecia due to adrenocortical neoplasia. (B-E) Histological appearance of ferret adrenocortical neoplasms. Adrenocortical adenoma (A,B) or myxoid adrenocortical carcinoma (C,D) stained with H&E (A,C) or GATA4 antibody (avidin-biotin complex peroxidase method) (B,D). The arrowhead highlights the spindle cell component of the tumor. Bars = 100 μ m.

Figure 2.
(A) Dog with typical clinical signs of Cushing's syndrome: abdominal obesity, muscle atrophy, thin skin and fatigue. (B) The same dog after treatment.

Table 1

Properties of the adrenal cortex in 4 species

	Mouse	Ferret	Dog	Human
Morphologic zones	ZG ZF X-zone	ZG ZF ZR	ZG ZF ZR	ZG ZF ZR
CYP17 expressed	No	Yes	Yes	Yes
Major glucocorticoid	Corticosterone	Cortisol	Cortisol	Cortisol
Production of androgens	No	Minimal	Minimal	Yes

Abbreviations: ZG, zona glomerulosa; ZF, zona fasciculata; ZR, zona reticularis.

Table 2

Advantages and shortcomings of the 3 experimental models vis-à-vis human ACTs

	Mouse	Ferret	Dog
Advantages	1.Genetically & epigenetically tractable, which facilitates mechanistic studies. 2.Strain dependent, allowing modifier gene screens. 3.Very high tumor incidence in certain strains (near 100%). 4.Short tumor latency (weeks to months). 5.Suitable for xenograft & other transplantation experiments.	1.High tumor incidence in gonadectomized individuals (~ 20%). 2.Overall physiology of the ferret resembles that of humans, making this a useful organism for the study of reproductive endocrinology & airway diseases (e.g., cystic fibrosis).	1.Adrenal gland is anatomically & functionally similar to that of humans. 2.Tumors usually produce cortisol as in humans. 3.High incidence of tumors compared to humans (1000x). 4.Genomic tools available. 5.Some treatments mirror those used in humans (e.g., o,p'-DDD).
Shortcomings	1.Adrenal gland is anatomically & functionally different from that of humans. 2.Tumors are often “gonadal-like” & produce sex steroids rather than gluco- or mineralocorticoids.	1.Species is not standardized with regard to genotype & therefore not ideal for studies of tumor genetics. 2.Long tumor latency (years). 3.Tumors are typically “gonadal-like” and produce sex steroids rather than gluco- or mineralocorticoids.	1. Tumors do not show a clear breed predilection.