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Short Communication

Gonadectomy-related adrenocortical tumors in ferrets demonstrate increased expression of androgen and estrogen synthesizing enzymes together with high inhibin expression

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ABSTRACT

The 2 objectives of this study were to (1) measure by quantitative polymerase chain reaction the expression of genes involved in steroid and inhibin synthesis in adrenocortical tumors of gonadectomized ferrets and (2) localize by immunohistochemistry several proteins that are key to adrenal steroidogenesis. Relative to the control adrenals, expression of the *messenger RNAs* encoding StAR (steroidogenic acute regulatory protein; $P = 0.039$), CYP11A ($P = 0.019$), CYP21 ($P = 0.01$), and 3β-HSD ($P = 0.004$), all involved in the synthesis of mineralocorticoids and glucocorticoids, were decreased in the adrenocortical tumors. In contrast, expression of cytochrome B5 (CytB5; $P = 0.0001$) and aromatase ($P = 0.003$), involved in androgen and estrogen synthesis, and both inhibin α-subunit ($P = 0.002$) and β_B-subunit ($P = 0.001$) were upregulated. In tumors, immunostaining of CYP21 was low, whereas staining of Cyp17 and CytB5, necessary for androgen synthesis, was present. It is concluded that ferret adrenocortical tumors express genes for androgen production. In addition, the expression of aromatase and inhibin suggests an even more gonadal differentiation, which is reminiscent to the fact that both gonads and adrenals are derived from a common urogenital primordial cell.

1. Introduction

In neutered ferrets, adrenocortical tumors are very common [1] and are known for their excessive sex steroid production [2]. It is generally believed that the increased secretion of gonadotropins, predominantly luteinizing hormone, which occurs after castration causes adrenocortical hyperplasia and ultimately tumorigenesis. This hypothesis is supported by the evidence of luteinizing hormone receptor (LHR) expression in the adrenal glands of ferrets and a positive response to the gonadotropin releasing hormone (GnRH) stimulation test in neutered ferrets with an adrenocortical tumor [3].

It has been hypothesized that in response to stimulation by increased gonadotropins, undifferentiated gonadal cells in the adrenal gland could differentiate to cells with gonadal characteristics [2]. These steroidogenic cells in the adrenal cortex are thought to stem from a pool of mesodermal progenitors in the urogenital ridge from which the gonads and kidneys are derived as well. During embryogenesis, the urogenital ridge cells associate with neural crest cells to form an adrenal gland [4]. This proposed hypothesis is supported by the finding of many gonadal characteristics in gonadectomy-induced adrenocortical tumors in certain inbred strains of mice which are thought to share the same pathophysiology as ferrets [5].

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In murine adrenocortical tumors, cells with gonadal characteristics demonstrate themselves in a variety of ways. Steroidogenic enzymes more consistent with a gonadal than adrenal phenotype, CYP17, and aromatase have been found in adrenocortical tumors in mice [6]. The enzyme CYP17 possesses a dual action and its 17,20-lyase activity is essential for androgen synthesis. The enzyme is normally expressed in the fetal and postnatal adrenal of human and other higher primates [7] but is not present in the adrenal of postnatal mice. The recurrence of CYP17 expression in adrenocortical tumor cells in susceptible mouse strains suggests gonadal cell differentiation [5].

Another gonadal steroidogenic cell marker expressed in the adrenocortical tumor cells of mice of susceptible strains is the LHR [5]. The functional LHR is thought to serve as a promoter when expressed in the adrenal gland and is stimulated by luteinizing hormone. Inhibin, a member of the transforming growth factor beta family, is another gonadal marker known to be expressed in adrenocortical tumors in mice. Inhibin is a covalently linked heterodimer formed by an inhibin α-subunit with 1 of the 2 β-subunits. The expression of the inhibin α-subunit is limited to steroidogenic tissues, such as the gonads and adrenal cortex, where it regulates growth and differentiation [8]. In inhibin-α knockout mice, there is a high incidence of adrenocortical tumor development after gonadectomy, and inhibin-α expression is increased in adrenocortical tumor cells of certain strains of mice [5].

Some work has been done investigating the development of gonadal cell phenotype in ferret adrenocortical tumors as well. The normal ferret adrenal glands produce the adrenal androgens dehydroepiandrosterone, its sulfate (dehydroepiandrosterone-S), and androstenedione in small amounts [2]. Adrenocortical tumors produce excessive amounts of sex steroids, but so far only cytochrome B5 (CytB5) has been investigated. Cytochrome B5 plays a pivotal role in the positive regulation of androgen production, by stimulating the 17,20-lyase reaction of CYP17 [9]. Wagner et al [10] have shown the presence of CytB5 in most sex steroid–producing adrenocortical tumors in contrast to the absence in normal adrenocortical cells. The expression of LHR is another gonadal characteristic that has been demonstrated on the adrenal cortex of the ferret [3]. Inhibin has been mentioned as a possible key player in the adrenocortical tumorigenesis in ferrets [11] and ferret adrenocortical tumors with myxoid differentiation are known to stain for inhibin-α [12].

To further characterize the gonadal characteristics of the steroid-producing adrenocortical tumor cells, the expression of the *messenger RNA* (*mRNA*) encoding the cholesterol transporting protein StAR, several steroidogenic enzymes (CYP11A, CYP17, CytB5, 3β-HSD, CYP21, and aromatase), and inhibin subunits were investigated by quantitative polymerase chain reaction (qPCR). In addition, the local expressions of various investigated proteins were studied.

2. Materials and methods

2.1. Animals and tissues

The group of normal ferret adrenal glands for the qPCR study consisted of 10 left adrenal glands from healthy male ferrets, all which were euthanized for reasons unrelated to this study. They originated from institutionally approved studies conducted at the Animal Sciences Group, Wageningen University and Research Centre, ID-Lelystad and the Department of Clinical Sciences of Companion Animals of Utrecht University.

The tumor group consists of 9 archival specimens obtained from patients during adrenalectomy at the Department of Clinical Sciences of Companion Animals of Utrecht University. The following selection criteria were used: the ferrets (1) had to be surgically neutered and should not have received an implant containing a depot-GnRH agonist; (2) had to exhibit one or more signs of hyperadrenocorticism (Table 1); and (3) excised adrenal glands were classified histopathologically into adenomas or carcinomas. For some animals, the results from a GnRH stimulation test [13] were available (Table 1). After collection, all tissues were frozen in liquid nitrogen and held at −70°C until RNA extraction.

For immunohistochemistry, adrenal glands of 5-yr-old, surgically castrated male ferrets ($n = 5$) from an unrelated study, approved by the Ethics Committee of the Faculty of Veterinary Medicine, Utrecht University, were used. After a human chorionic gonadotropin stimulation test [13], the ferrets were euthanized and the left adrenal glands were collected and fixed in paraformaldehyde and classified (Table 2).

Table 1
Case details of the ferrets with adrenal tumors.

Number	Sex	Age (yr)	Adrenal pathology	Androstenedione (nmol/L)		Clinical signs			
				$T = 0$	$T = 30$	Symmetrical alopecia	Pruritus	Vulvar swelling	Return of sexual behavior
1	M	8	LA	NT	NT	+	−	−	+
2	M	3	LA	NT	NT	+	+	−	−
3	F	3	LC	NT	NT	+	+	−	−
4	M	4	LA	NT	NT	+	+	−	−
5	M	6	LA	NT	NT	+	+	−	+
6	F	5	LA	NT	NT	+	−	+	−
7	M	5	RC	0.60	NT	+	+	−	−
8	M	6	RA	1.5	NT	+	+	−	−
9	M	3	LC	0.30	2.6	+	−	−	−

Abbreviations: A, adenoma; Age, age at removal adrenal tumor in years; C, carcinoma; F, female; L, left adrenal gland; M, male; NT, not tested; R, right adrenal gland; $T = 0$, androstenedione measurement without stimulation; $T = 30$, androstenedione measurement 30 min after GnRH administration.