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Review

Mouse models of adrenal tumors responsive to gonadotropin-releasing hormone and gonadotropins

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Abstract

In recent years, several mouse models have been established for characterization of the molecular pathways involved in adrenocortical tumorigenesis. Adrenal tumors develop in genetically susceptible mouse strains after prepubertal gonadectomy, in mice transgenic with oncogenes (simian virus 40 T antigen), several gene knockouts (such as *inhibin* or conditional *Gata6*$^{F/F}$), and in mice overexpressing transcription factor GATA binding protein 4. The gonadal rest-type adrenal tumor phenotype is regulated by gonadotropins, mainly luteinizing hormone. Luteinizing hormone/chorionic hormone receptor and gonadotropin-releasing hormone receptor expression has been found in human adrenocortical carcinoma, as well as in several mouse adrenal tumor/adrenocarcinoma models. This mini-review will address recent advancements in this research topic with respect to the molecular basis of adrenocortical tumorigenesis, the clinical relevance of these tumor models, and the potential for future targeted treatment strategies. Furthermore, the ectopic expression of the luteinizing hormone/chorionic hormone receptor or gonadotropin-releasing hormone receptor may open up options for targeted therapy approaches.

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Introduction

Adrenocortical adenomas are rather common in humans, detected in 5% of the population older than 50 years [1]. In contrast, adrenocortical carcinomas are very rare, diagnosed in 1–2 patients per million [2]. The latter are usually found in children (aged <10 years) or in adults (aged 40–50 years), and they are 1.5-fold more common in women than men [2]. Treatment strategies for adrenocortical carcinomas include surgery, chemotherapy, radiation, and/or mitotane with antisteroidogenic and cytostatic properties [3]. Until now, treatment therapies provide 5-year survival in only 10–25% patients [2,3]. Thus, novel therapeutic strategies and better understanding of the molecular pathogenesis of adrenocortical carcinomas are urgently needed.

The role of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), gonadotropin-releasing hormone (GnRH), and their cognate receptors (luteinizing hormone/chorionic hormone receptor [LHCGR], follicle-stimulating hormone receptor [FSHR], or gonadotropin-releasing hormone receptor [GNRHR]) is mainly considered within the reproductive system. Recently, LHCGR, FSHR, and GNRHR expression has been detected in various normal and pathological extragonadal tissues, including those of the breast and prostate [4,5], but also in adrenocortical disorders, such as adrenocorticortophin-independent adrenal hyperplasia, adrenocortical adenoma, and pregnancy-induced Cushing's syndrome [4,6–9]. LHCGR expression has been shown in postmenopausal adrenal glands in connection with elevated circulating LH and dehydroepiandrostenedione despite normal ACTH secretion [10]. The physiological role of the LHCGR in the adult adrenal still remains unknown [6,11], even if highly expressed, they could contribute to the induction of adrenocortical tumors (ACTs). High levels of LH in postmenopausal women or human chorionic

gonadotropin (hCG) during pregnancy have been shown to upregulate the adrenal expression of LHCGR and stimulate cell hypertrophy, proliferation, and steroid hormone production, leading to hypercortisolism or/and hyperandrogenism [6,9,11–14]. LH/hCG stimulation of ACTs induces overproduction of androgens and aldosterone [6] and stimulation of cultured human H295R ACT cells produce dehydroepiandrostenedione-sulphate4 (S) [15].

As in humans, gonadotropins in mice have been implicated in signaling during adrenal pathogenesis. Most of the wild-type or transgenic mice that develop adrenal neoplasia after prepubertal gonadectomy (GDX) present with highly elevated gonadotropin levels relevant to clinical cases with increased LH/hCG [6,9,12,16]. Examples of gonadotropin-induced adrenal neoplasias in genetically modified mice are listed in Table 1.

Mouse models of gonadotropin-dependent tumorigenesis with the gonadal rest phenotype

GDX-induced neoplasia in susceptible mouse strains

The highly differentiated cells of the adrenocortical zones do not proliferate, but are gradually replaced from a subcapsular niche of stem/progenitor cells that migrates toward the medulla, gradually replacing the existing cells [17]. A similar origin for these progenitor cells has been suggested as for the gonadal somatic cells developing in the adrenogonadal primordium in early embryonic life [17]. Some genetically susceptible inbred mouse strains (DBA/2J, CE/J, C3H, NU/J, BALB/c, and B6D2F1) develop adrenocortical neoplasms after neonatal GDX. The arising neoplasms resemble structurally and functionally the gonadal rest ovarian theca and granulosa cells [18] and are considered relevant to human adrenal tumors also induced by elevated LH/hCG levels [6,9,12,16]. In GDX-induced adrenocortical neoplasms, besides increased gonadotropin concentrations, a mechanism inherent to GDX-sensitive strains is believed to be involved [19]. Linkage analysis studies on a susceptible (DBA/2J) crossbreed with nonsusceptible/resistant (C57Bl/6J) mice showed a significant quantitative trait locus on chromosome 8 harboring the dominant *Wnt* inhibitor *Sfrp1*, which was significantly downregulated in GDX-induced tumors [20,21]. This study suggested the involvement of Wnt signaling in the promotion of adrenocortical neoplasia in GDX-induced mice, resembling humans with downregulated *SFRP1* in pediatric ACTs [22].

In mice with adrenal hyperplasia/adenoma/ACT, the histopathological phenotype consists of two types of cells: A and B cells. Basophilic type A cells originate from the adrenal gland capsular cells expressing the GLI-Kruppel family member GLI1 (Gli1) [23]. They travel toward the perimedullary region in a distinct wedge-shaped manner in aging mice of all backgrounds. A cells do not produce steroids or tumors and express transcription factor GATA binding protein 4 (GATA4) [23,24]. The number of A cells is increased after GDX compared with that of intact mice [25], and they express *Lhcgr* [26], which predicts they may respond to LH/LHCGR signaling. ACTs consist of large, lipid-loaded B cells found in gonadectomized inbred (DBA/2J, B6D2F1, NU/J, BALB/c, CE/J, and C3H) [27] or transgenic (21-OH-GATA4) [28] mouse strains. B cells form functional adenomas, produce gonadal steroids, and express gonadal steroidogenic enzymes, Lhcgr and GATA4 [24,28,29].

Genetically modified GDX-induced gonadal rest phenotype ACT models

Inhibin-α knockout model

GDX-induced inhibin-α knockout (KO) mice bred in a mixed C57BL/6 background present with a very early onset (12–17 weeks) of ACTs, which proves inhibin as a strong adrenal tumor suppressor gene [30,31]. LH has been shown to be the key gonadotropin leading to tumorigenesis in this model as crossbreeding with *FSHβ* KO mice did not prevent gonadal tumor formation [32]. No cell lines have been immortalized from any of the tumors of inhibin-α KO mice. Owing to severe cachexia-like syndrome and lethality and the very early onset of tumorigenesis, it may be difficult to use this line as a tumor model for testing treatment strategies.

Inhα/Tag transgenic mouse model

Prepubertally gonadectomized Inhα/Tag mice, expressing the simian virus T antigen (SV40Tag) oncogene under the inhibin alpha (*Inha*) promoter, develop ACTs by the age of 5–8 months [33]. The *Inha* promoter directs oncogene expression to the gonads, adrenals, and pituitary gland, whereas SV40Tag binds to the tumor suppressor p53 (TP53) and retinoblastoma proteins that enhance cell cycle progression [34,35]. Similar to large, lipid-loaded B cells found in GDX-induced inbred mice, ACTs in Inhα/Tag and *Inha*$^{-/-}$mice, the Cα1 cells, immortalized from an Inhα/Tag adrenal tumor, express high levels of gonadal factors such as *Cyp19a1*, *Cyp17a1*, *Lhcgr*, and *Gata4* [26,33,36–38].

As in the *Inha*$^{-/-}$ model, crossbreeding of inhα/Tag with *hpg* mice or pharmacological blockage of gonadotropins prevented the formation of ACT in inhα/Tag mice [39]. Moreover, intercrossing inhα/Tag and *bLHβ*-CTP (having elevated LH levels) mice resulted in simultaneous occurrence of gonadal tumors and ACTs [40]. A recent study with Inhα/Tag intercrossed with LHR KO mice (LuRKO) mice [38] revealed that *Inha*-promoted expression of SV40Tag only induced mild hyperplasia in the adrenal glands of GDX-induced animals at the age of 7 months. This study along with previous observations [39,41] demonstrated that LH/LHCGR signaling is

Table 1

Gonadectomy-induced and gonadotropin-dependent mouse models of adrenocortical neoplasia. Table modified from the study by Basham et al [27].

Model	Gene/promoter	Adrenocortical phenotype after prepubertal GDX/cell line established	Reference
Inha KO	*Inhibin-α* $^{-/-}$ (mouse)	Adrenal tumors (84.8% unilateral, 15.1% bilateral)	[53]
Inha KO; hpg/hpg	*Inhibin-α* $^{-/-}$ (mouse) GnRH (mouse)	Not analyzed	[41]
Inha KO; Fshβ$^{-/-}$	*Inhibin-α* $^{-/-}$ (mouse) *Fshβ* $^{-/-}$ (mouse)	Not analyzed	[32]
Inha KO; LHβ-CTP	*Inhibin-α* $^{-/-}$ (mouse) *LH-β* (bovine) and *hCG-β* (human) chimeria protein	Big unilateral adrenal tumors with early onset, rather similar to Inha KO	[54]
Inha KO; Cyclin D2$^{-/-}$	*Inhibin-α* $^{-/-}$ (mouse) *Ccnd2* $^{-/-}$ (mouse)	Prolonged survival. Adrenal tumors (83% unilateral).	[30]
Inha KO; Madh3$^{+/-}$	*Inhibin-α* $^{-/-}$ (mouse) *Smad3* (mouse)	Prolonged survival with adrenal tumors histologically similar to ovarian tumors	[31]
Inhα/Tag	*SV40* (large T antigen)/6 kb inhibin-α	Progressive and aggressive unilateral adrenocortical tumors by 6–8 months with 100% penetrance. Adrenocortical cell line Cα1 established from the tumor.	[33,39]
Inhα/Tag; LHβ-CTP	*SV40 Tag*/6 kb inhibin-α *LH-β* (bovine) and *hCG-β* (human) chimeric protein	Adrenocortical tumors in intact female mice. GDX: not analyzed	[40]
21-OH-Gata4	*Gata4/Cyp21a1*	Adrenal neoplasia and subcapsular A and B cells	[28]
Gata6 cKO	*Gata6*$^{F/F}$/Sf1-Cre (stochastic)	Subcapsular hyperplasia with A and B cells	[55]
C57BL/6 Gata4$^{+/-}$	*Gata4*$^{F/}$*/Amhr2*-Cre ^{F}	Adrenal neoplasia by 6 months with A and B cells. Impaired adrenal tumorigenesis with *Gata4* haploinsufficiency.	[24]
Inhα/Tag LuRKO	*SV40 Tag*/6 kb inhibin-α LHR$^{-/-}$ *(mouse)*	GDX inhα/Tag/LuRKO only developed SV40Tag-positive hyperplastic cells that were GATA4 negative, cleaved caspase-3 positive, and did not progress into adenoma at the age of 7 months.	[26]

Amhr2, anti-Mllerian hormone receptor type II; Ccnd2, cyclin D2; cKO, conditional knockout; Cyp21a1, cytochrome P450, family 21, subfamily a, polypeptide 1; Fshβ, follicle-stimulating hormone beta subunit; FSH, follicle-stimulating hormone; Gata4, GATA binding protein 4; Gata6, GATA binding protein 6; GDX, gonadectomy; GnRH, gonadotropin-releasing hormone; hCG-β, human chorionic gonadotropin beta subunit; hpg, hypogonadal mutant mouse; Inha, inhibin; KO, knockout; LH, luteinizing hormone; LHβ-CTP, bovine alpha subunit promoter fused to the coding region of a chimeric LHβ subunit; Madh3, mothers against DPP homolog 3; Sf1, steroidogenic factor 1; Smad3, smad family member 3; SV40, simian virus 40; Tag, tumor antigen.

indispensable for the formation of ACTs in GDX models. Besides earlier reported Gata4 and Lhcgr, we recently found upregulated Esr1 and Prlr-rs1 and downregulated Grb10, Mmp24, Sgcd, Rerg, Gnas, Nfatc2, Gnrhr, and Igf2 in inhα/Tag adrenal tumors [38]. A putative normal adrenal remodeling or tumor suppressor role of the downregulated genes (e.g. Nfatc2, Grb10, Gnas, and Rerg) in the ACTs would need further studies.

GATA 4/6 factor–related mouse models of GDX-induced adrenocortical neoplasia

Two GATA family transcription factors GATA4 and GATA6 are known to be crucial for the development of adrenal glands. Normal gonads predominantly express GATA4, whereas adrenals express GATA6 [42]. Interestingly, adrenal tumors with the gonadal-like phenotype have been shown to express high levels of GATA4 [43,44]. A positive and reciprocal feedforward amplification link between LHCGR and GATA4 expression has been shown in conjunction with the SV40Tag expression, leading to the LH-dependent gonadal phenotype ACTs [43]. However, it remains unclear which one of the two genes, GATA4 or LHCGR, is expressed first in the ACTs.

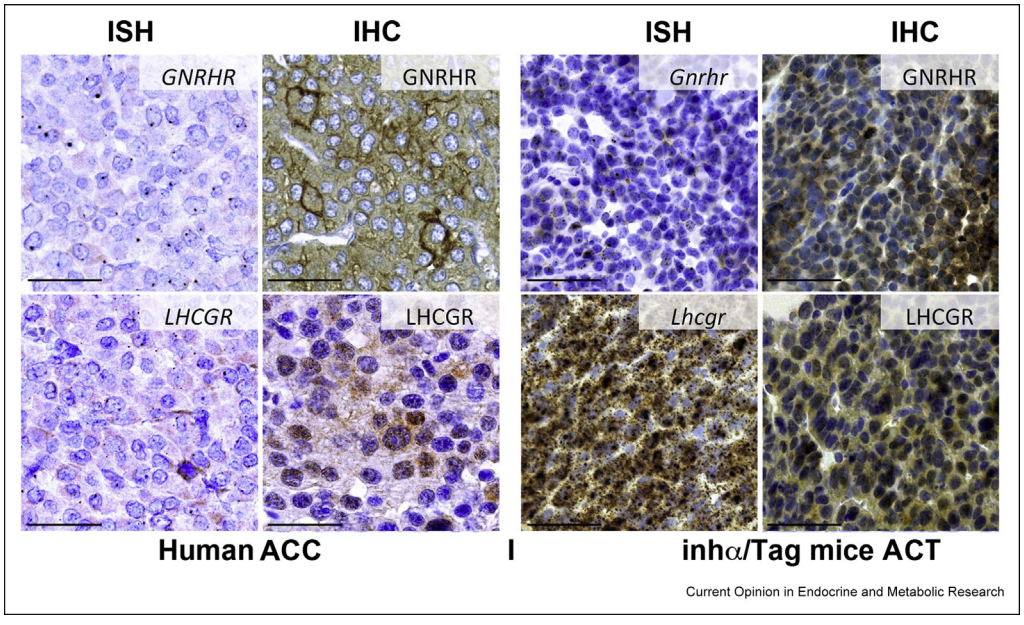
GnRH analog treatment of ACTs: clinical relevance of the GDX-induced mouse models for ACT

The functional LHCGR signaling in the tumorous adrenal glands in humans and GDX-induced mouse models could present a potential for targeted therapy, where gonadotropin ablation could prevent tumor progression. Both agonists and antagonists have been used to treat hormone-dependent tumors [5,45]. Besides suppressing gonadotropin secretion, GnRH analogs could also have direct inhibitory effects on adrenal tumors, which have been shown to express GNRHR [46]. These studies showed that GnRH analog treatment may directly target the cancer cells and decrease their proliferation, metastasis, and angiogenesis [8,45,47,48]. We have recently shown that both human and mouse adrenal tumors express GNRHR and LHCGR (Figure 1), but not FSHR [46]. Moreover, a 21-day GnRH antagonist treatment of mouse ACTs brought about an average of 15-fold decrease in tumor weight [46]. The absence of FSHR expression in our tumors did not support previously published findings on FSHR protein expression in the blood vessels within adrenal tumors [49]. The benefits of GnRH agonist treatment of ACTs could be dual, either by blocking hormonal symptoms by blocking LH release or directly through GNRHR.

Summary and future perspectives

Genetically modified and GDX-induced ACT models have been used successfully to understand the molecular mechanism of adrenal progenitor cell involvements in gonadal rest-type adrenal tumor formation. The molecular and histopathological profiles of the mouse ACTs resemble human gonadal rest tumors and ovarian thecal metaplasia, where subcapsular wedge-shaped clusters

Figure 1



Localization of GNRHR and LHCGR expression in human and mouse adrenocortical tumors/cancer. Representative images of in situ hybridization RNAScope (ISH) and immunolocalization (IHC) of RNA transcripts/protein of GNRHR and LHCGR in human adrenocortical carcinoma (ACC) or adrenocortical tumor (ACT) sections of inhα/Tag mouse (transgenic mouse expressing SV40 T antigen under the inhibin α promoter) (panel B). Bar = 50 μm (This modified figure in this adapted format has been reproduced from the Figure 1 of the study by Doroszko et al [46].) GNRHR, gonadotropin-releasing hormone receptor; LHCGR, luteinizing hormone/chorionic hormone receptor. IHC, ISH.

invade postmenopausal female adrenals [50]. Unlike the aforementioned GDX-induced mouse ACT models, human ACT produces mostly adrenal steroids [51], and sex steroid production is observed only in about 5% of all ACTs [52]. However, a good number of ACTs responds to LH/hCG stimulation [6] by producing cortisol, aldosterone, or androgens. Such situations usually occur in conditions with chronically elevated LH (menopause) or hCG (pregnancy) levels [6,9,12,16]. Thus, studies on the GDX-induced ACT and ACC mouse models are clinically relevant and support the potential of GnRH agonist and antagonist treatments in human ACT/ACC. It would be of great importance to find the pathomechanism of ACT/ACC, other coactivators, and coinhibitors, which could be dependent on the GnRH–gonadotropin–GATA4 pathways and could enhance the treatment strategies.

Conflict of interest statement

Nothing declared.

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* of special interest
** of outstanding interest

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