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**Adrenocortical tumorigenesis, luteinizing hormone receptor and transcription factors
GATA-4 and GATA-6**

Susanna Vuorenoja¹, Adolfo Rivero-Muller¹, Sanne Kiiveri², Malgorzata Bielinska³,
Markku Heikinheimo², David B Wilson³, Ilpo T Huhtaniemi^{1,4} and Nafis A Rahman^{1*}

¹Department of Physiology University of Turku, 20520 Turku, Finland, ²Children's
Hospital and Program for Developmental and Reproductive Biology, University of
Helsinki, 00029 Helsinki, Finland, ³Department of Pediatrics, Washington University, St.
Louis, MO, USA, ⁴Institute of Reproductive and Developmental Biology, Imperial College
London, Faculty of Medicine, London W12 0NN, UK

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Abbreviations: LHR, Luteinizing hormone receptor; hCG, human chorionic gonadotropin,
ACC, adrenocortical carcinoma, ACT, adrenocortical tumor)

**Correspondence:* Nafis Rahman, M.D., Ph.D.
Department of Physiology
University of Turku
Kiinamyllynkatu 10
FIN-20520 Turku, Finland
Fax: +358-2-2502610
E-mail: nafis.rahman@utu.fi

Abstract

Luteinizing hormone (LH/hCG) responsiveness of normal and pathological human adrenal glands as well as the possibility of constitutive expressions of luteinizing hormone receptor (LHR) in adrenal cortex has been reported. Some recent studies showed a correlation between the LHR and abundant GATA-4 expression in both metastasizing and non-metastasizing human adrenocortical tumors, but not in normal adrenals, implicating the putative relevance of LHR and GATA-4 for adrenocortical pathophysiology. However the physio- and pathophysiological significance of LHR and GATA-4 in the mechanism of adrenocortical tumorigenesis remains unclear. The paucity of suitable models for adrenal tumorigenesis makes the establishment of proper animal models highly important. LHR expression in the murine adrenal gland is an exception and not found in wild-type (WT) animal. We have previously shown that ectopic LHR expression in the murine adrenal gland can be induced by chronically elevated LH levels. We have generated a gonadotropin-responsive adrenal tumor model in gonadectomized transgenic (TG) mice expressing the inhibin α promoter/Simian Virus 40 T antigen transgene (*inh α /Tag*). Given the induction of expression and regulation of GATA-4 and GATA-6 zinc finger transcription factors in the gonads by gonadotropins, this review will explore their relationship to LHR expression and their role in adrenocortical tumorigenesis. A functional link between LHR and GATA-4 actions in the adrenal pathophysiology is proposed.

1. Adrenocortical tumors and the luteinizing hormone receptor (LHR)
2. Transcription factors GATA 4 and GATA 6
 - 2.1. GATA-4 and GATA-6 in human adrenocortical tumors
3. Animal models for adrenocortical tumorigenesis
 - 3.1. Transgenic (TG) murine models for adrenocortical tumorigenesis
 - 3.2. Adrenocortical phenotype of the $inh\alpha$ /Tag TG mice
4. Future directions

1. Adrenocortical tumors and the luteinizing hormone receptors (LHR)

Adrenocortical carcinoma (ACC) is a rare type of malignancy. In the United States its incidence is approximately 3 per million children under 16 years of age (Wagner et al. 1994), and 0.5-2 per million adults (Schulick and Brennan 1999). It tends to occur either before the age of five or in the first, fourth or fifth decades of life, and is relatively more common in women (Michalkiewicz et al. 1997, Sandrini et al. 1997, Schulick and Brennan 1999, Wajchenberg et al. 2000). Only a small proportion of adrenocortical tumors (ACT) cause endocrine diseases in humans. It has been shown in 602 cases of ACC, that 62% of the carcinomas were functional and 38% non-functional (Ng and Libertino 2003). The functional tumors presented with Cushing's syndrome (40%) and only rarely with pure virilization in adults (Ng and Libertino 2003). In children the proportion of endocrine disease is even higher (such as primary hyperaldosteronism, hypercortisolism, hyperandrogenism, or hyperestrogenism), and less than 1% are malignant (Bornstein et al. 1999). However, in the group of operated incidentalomas, i.e. incidentally found adrenocortical tumor masses, even 12% can be malignant adrenocortical carcinomas (Arnaldi et al. 2000, Mantero and Arnaldi 2000, Mantero et al. 2000). The tumors are

classified as nonfunctional or functional, depending on their production of corticosteroid, androgen, estrogen, or mineralocorticoid. Most patients present with large tumor masses and with stage IV disease, which normally means the presence of the metastases (Schulick and Brennan 1999). There are still no efficient forms of therapy for adrenal tumors, with the exception of tumor removal. Even in patients undergoing complete resection, recurrent and metastatic disease is quite common (Reincke et al. 1994, Bornstein et al. 1999). The survival rate of ACC according to some studies is 15-35% (Ahlman et al. 2001). In a series of 139 patients from the last 20 decades the 5-year survival rate was shown to be improved than in the older reports to 60% (Vassilopoulou-Sellin and Schultz 2001). In the group of patients with metastases, the five-year survival rate is reported to range between 15-25% (Latronico and Chrousos 1997a, Latronico and Chrousos 1997b).

Human adrenal LH/hCG responsive tumors have been described (Leinonen et al. 1991, Lacroix et al. 1999). LHR expression has also been reported at low levels in normal human adrenal cortex (Pabon et al. 1996) and hCG has been shown to stimulate the dehydroepiandrosterone sulphate synthesis of human fetal adrenals (Jaffe et al. 1981). It would be important to explore further, as suggested in recent studies (Rao Ch et al. 2004, Alevizaki et al. 2006), the possibility whether normal human adrenal glands might contain low levels of LHR and whether they become activated whenever LH levels are elevated as a part of physiological or pathological process. Several cases of LH/hCG dependent and ACTH-independent Cushing's syndrome (CS) have been reported in postmenopausal women (Lacroix et al. 1999, Feelders et al. 2003) and pregnancy associated CS is a known rare condition (Sheeler 1994). In a very recent study, aberrant expression of human LHR by adrenocortical cells was shown to be sufficient to provoke hyperplasia, benign adrenocortical tumorigenesis and Cushing's syndrome features (Mazzuco et al. 2006).

Ectopic expression of LHR in the adrenal gland has been shown not only to be associated with ACTH-independent Cushing's syndrome (CPA), but also with the benign aldosterone-producing adenomas (APA) (Saner-Amigh et al. 2006). The potential role of LH and aberrantly expressed LHR in the regulation of steroidogenesis in both Conn's and Cushing's disease has been shown to have clinical implications (Saner-Amigh et al. 2006). LH has been shown to increase the transcription of aldosterone synthase in an adrenocortical cell line transfected with LHR, which is required for the final reaction in aldosterone synthesis, emphasizing further the potential role of LHR in primary aldosteronism (Saner-Amigh et al. 2006). There are also reports about malignant androgen producing LH-dependent adrenal tumors (Givens et al. 1975, de Lange et al. 1980, Leinonen et al. 1991). Hence, LH/hCG dependent mechanisms do exist among human adrenal pathologies, LH/hCG dependent mechanisms exist and they may be clinically important in certain circumstances, e.g. in connection with polycystic ovarian syndrome (PCOS) and after menopause. It would be interesting to know whether the increased levels of gonadotropins in postmenopausal women or in PCOS patients could affect adrenal function. It is already known that in PCOS patients the elevated levels of LH correlate with the overproduction of androgens (Franks 1995). Only a few animal models exist for studying the initiation, progression, and metastasis of adrenal tumors or for examining new treatment strategies. Thus, there is a great need for novel experimental models for research in adrenocortical cancer.

2. Transcription factors GATA-4 and GATA-6

GATA-4 and GATA-6 belong to the family of zinc finger transcription factors termed the GATA-binding proteins, and they regulate gene expression, cell differentiation

and proliferation in a variety of tissues (Orkin 1992, Heikinheimo et al. 1994, Heikinheimo et al. 1997). However, recent studies on GATA transcription factors showed a role for these factors also in tumor formation, at least in gonads and adrenals (Kero et al. 2000, Ketola et al. 2000, Kiiveri et al. 2002a, Kiiveri et al. 2002b, Barbosa et al. 2004, Kiiveri et al. 2004, Rahman et al. 2004). GATA-4 is expressed in heart, gonads, gastrointestinal tract and pancreas and in limited number of other tissues (Orkin 1992, Arceci et al. 1993, Narita et al. 1996, Heikinheimo et al. 1997, Ketola et al. 1999). The phenotypic characterization of homozygous *GATA-4* deficient mice showed its expression and importance in several tissues during the embryonic period (Arceci et al. 1993, Heikinheimo et al. 1997, Ketola et al. 1999, Ketola et al. 2004). The *GATA-4* deficient mice die *in utero* by *E9.5* mainly due to abnormal cardiac morphogenesis caused by a disturbance in the ventral folding (Kuo et al. 1997, Molkenin et al. 1997). In humans, GATA-4 has been shown to be linked to congenital heart defects and carcinomas of ovary and esophagus (Pehlivan et al. 1999, Lin et al. 2000, Lassus et al. 2001). GATA-6 was primarily recognized as a separate GATA family member from a chicken cDNA library (Laverriere et al. 1994). GATA-6 resembles GATA-4 in many ways and besides the organs mentioned above it is also expressed in the lungs and vascular smooth muscle cells (Narita et al. 1996, Morrisey et al. 1997). GATA-6 is required during embryogenesis and *GATA-6* deficient homozygous mice die at *E7.5* due to a defect in the development of visceral endoderm (Morrisey et al. 1998). Besides being expressed in the male and female gonads and adrenals, GATA-4 and GATA-6 are expressed at the levels of hypothalamus-pituitary-gonadal/adrenal axis (Kiiveri et al. 1999). There are differences in the expression of GATA factors in normal and neoplastic adrenal tissues. GATA-6 is found in fetal and adult adrenocortical tissue (Kiiveri et al. 1999, Kiiveri et al. 2002a, Kiiveri et al. 2002b, Kiiveri

et al. 2004, Kiiveri et al. 2005). GATA-4 expression, instead, is found in fetal but not in wild type murine or human adult adrenal tissue (Kiiveri et al. 2002a). However, while we investigated the adrenal tumors of $inh\alpha$ /Tag mice, we found abundant GATA-4 expression in adrenal tumors, while GATA-6 could not be detected (also see below) (Kiiveri et al. 1999). These results implicate a role for GATA-4 in adrenal tumorigenesis and that GATA-4 and GATA-6 have different roles in the adrenal gland. GATA-4 has been shown to act in synergy with steroidogenic factor 1 (SF-1) in reproduction, steroidogenesis and sexual differentiation (Kiiveri et al. 2005). There is also co-operation between GATA-4 and SF-1 to regulate the expression of AMH (anti-Müllerian hormone) (Kiiveri et al. 2005). The lack of co-operation of these two substances could be the reason for some cases of abnormal sex differentiation in humans (Tremblay and Viger 2003). Recently it has also been found that the human HSD3B2 promoter is activated by GATA transcription factors (Martin et al. 2005), presumably by GATA-6 (Bassett et al. 2005). GATA-6 has been suggested to have a role in androgen synthesis. It is mainly detected in areas, such as zona reticularis, which produce androgens and it appears to co-express with SF-1 and P450c17 which are markers for steroidogenesis (Jimenez et al. 2003, Fluck and Miller 2004, Kiiveri et al. 2005).

2.1. GATA-4 and GATA-6 in human adrenocortical tumors

Recently GATA transcription factors have also been studied in normal human adrenals and adrenocortical tumors. In earlier studies it has been postulated that human adrenals express GATA-6 but not GATA-4, which was in harmony with the findings from mice (Kiiveri et al. 1999, Jimenez et al. 2003). Later studies showed that GATA-4 could also be detected in normal (fetal) human adrenals as well as at high level in tumor tissues

(Pabon et al. 1996, Kiiveri et al. 2002a, Kiiveri et al. 2002b, Barbosa et al. 2004, Kiiveri et al. 2005). However, mRNA expression of GATA-4 was low or hardly detectable in normal adrenal or in adenoma, whereas GATA-6 was significantly increased. These findings support other results that GATA-4 is mainly expressed in adrenal carcinomas, but not in healthy adrenals or adenomas (Barbosa et al. 2004, Kiiveri et al. 2004, Bassett et al. 2005). However, upon comparison at different types of adrenocortical adenomas, some studies showed that GATA-4 expression was mainly associated with cortisol rather than aldosterone production (Kiiveri et al. 2004, Kiiveri et al. 2005), whereas in some cases elevated levels in aldosterone producing adenomas (Bassett et al. 2005). It has also been demonstrated that GATA -4 is expressed both in metastasizing and non-metastasizing tumors, with higher expression in the latter (Barbosa et al. 2004). In contrast to findings in mouse adrenal tumors, expression of GATA-6 has also been found in human adrenal tumors (Kiiveri et al. 2002b, Kiiveri et al. 2004). GATA-6 has been reported to be found highly expressed in both aldosterone producing adenomas and ACTH-independent Cushing's syndrome, compared to the normal human adrenal gland (Bassett et al. 2005). This could implicate that GATA-6 is little affected by the tumorigenic process (Barbosa et al. 2004). GATA-6 is apparently more widely found in cortisol producing adenomas, which supports the finding of the role of GATA-6 in androgen and cortisol production (Kiiveri et al. 2004). The same was found in other studies where human adrenocortical cells lines were investigated (Kiiveri et al. 2004). GATA-6 has been found to be more abundantly present in adenomas than carcinomas except in the virilizing tumor subgroup (Kiiveri et al. 2004). This finding was supported when comparing the Weiss score to GATA-6 expression; if Weiss score was low, i.e. the tumor was more differentiated, there was more GATA-6 expression than in the cases of a high Weiss score (Kiiveri et al. 2005).

These findings could be interpreted to support the role of GATA-6 in the maintenance of benign form of adrenocortical cells and that the absence of GATA-6 could ease the formation of carcinomas. However, GATA-6 has been shown to be a regulator of androgen synthesis together with SF-1 and P450c17 (Fluck and Miller 2004). GATA-6 expression also positively correlates with those of SF-1 and P450c17 (Jimenez et al. 2003, Fluck and Miller 2004, Kiiveri et al. 2005). When non-steroidogenic cells were co-transfected with GATA-6 and SF-1, it was found that these two synergistically activated steroidogenesis (Jimenez et al. 2003). In human adrenocortical cells, GATA-6 was thought to regulate the P450c17, which also ties the production of cortisol and androgens with GATA-6 expression (Jimenez et al. 2003).

3. Animal models for adrenocortical tumorigenesis

There are few early reports from the 1940s about rare “spontaneous” adrenocortical hyperplasia and carcinomas of mouse, showing a strict inbred strain-dependency. The first report on nodular adrenocortical hyperplasia was reported in 1939 for a dilute female brown strain (DBA/2J) of mice after prepubertal gonadectomy (Wooley et al. 1939). A similar phenomenon was reported for gonadectomized DBA male mice in 1941 (Wooley et al. 1941). It has recently been verified that prepubertally gonadectomized DBA/2J mice develop adrenal tumors (Bielinska et al. 2003). Other mouse strains like C3H, BALB/c, after prepubertal gonadectomy have been shown to develop adrenal adenomas and in the case of CE (extreme dilution) strains even adrenal adenocarcinomas, whereas the C57Bl mouse strain failed to form any visible adrenocortical neoplasias (Feteke et al. 1941, Wooley and Little 1945). It has been shown [as early as in the 1940-60s (Feteke et al. 1941, Murthy et al. 1968, Murphy and Rao 1970), and recently revisited and reconfirmed

(Bielinska et al. 2004, Bielinska et al. 2005)] that at first a small groups of densely populated small cells appear below the adrenal capsule (A cells), which further extend as becoming more spindle shaped in between the zona fasciculata cell columns. Later on these A cells presumably further forms the so called B cells, larger round cells which are believed to secrete hormones as estrogens (DBA/2J and CE mice) or androgens (C3H mice) (Rosner et al. 1966, Murphy and Rao 1970). These B cells express several steroidogenic enzymes on the contrary to A cells. This phenomenon has recently been shown in certain inbred mouse strains, like DBA/2J, where pre-pubertal gonadectomy induced sex steroid-producing adrenocortical neoplasm (Bielinska et al. 2003). In this mouse model the adrenal contains first the spindle shaped A which further probably forms B cells, which may implicate that gonadectomy caused undifferentiated stem or progenitor cells in the subcapsular region to transform into steroid producing B cells. Markers for steroidogenesis, for example P450c17, are only found in B cells (Bielinska et al. 2003). The coexpression of GATA-4 and LHR has also been shown in this adrenocortical tumor mouse model (Bielinska et al. 2003).

Adrenocortical neoplasms are found to be a common cause of morbidity in neutered ferrets. A recent study by screening archival specimens of adrenocortical neoplasms from such animals determined that these adrenal tumors expressed nuclear immunoreactivity for GATA-4 in 19/22 (86%) specimens of ferret adrenocortical carcinomas and was prominent in areas exhibiting myxoid differentiation (Peterson et al. 2004), which thus could be used as a tumor marker in this species, as the normal ferret adrenocortical cells lacked GATA-4 expression. Two other markers of adrenocortical tumors in gonadectomized mice (Kiiveri et al. 1999, Rahman et al. 2004), inhibin α and luteinizing hormone receptor (LHR), were coexpressed with GATA-4 in some of the ferret

tumors. Thus neutered ferrets can therefore be used as a model for studying adrenocortical tumorigenesis.

3.1. Transgenic (TG) murine models for adrenocortical tumorigenesis

Until now, among the existing TG murine models the most suitable candidates for adrenal tumors are *inh* α /Tag (mouse bearing Simian Virus 40 T-antigen under the inhibin α promoter) (Kananen et al. 1996, Rilianawati et al. 1998, Rahman et al. 2004), NU/J (Bielinska et al. 2005), *inh*^{-/-} (*inhibin* knockout mice) (Matzuk et al. 1994) and *inh*^{-/-} - LH-CTP (*inhibin* knockout mice crossbred with C-terminal peptide of the human chorionic gonadotropin β subunit TG mice) (Risma et al. 1995) (see below).

One of the models for adrenal tumors, is the female NU/J nude mouse (Bielinska et al. 2004, Bielinska et al. 2005). These mice developed adrenocortical neoplasms in response to either prepubertal gonadectomy or gonadotropin elevation from xenografts of hCG-secreting CHO cells (Bielinska et al. 2004, Bielinska et al. 2005). The CHO cells used stably express a biologically active single chain of hCG variant. Tumors formed in nude mice resemble the ones in DBA/2J strain with spindle shaped A cells expressing GATA-4 and steroid producing B cells expressing GATA-4 and also the steroidogenic factors LHR and P450c17 (Bielinska et al. 2003, Bielinska et al. 2004, Bielinska et al. 2005). Also in this model the chronic elevation of gonadotropins allows the adrenocortical cells in the subcapsular region to differentiate into the gonadal-like stroma. GATA-4, which is expressed in both of the cell types has been hypothesized to be a key factor of the transformation (Bielinska et al. 2005).

Inhibin and activin belong to the TGF β family of growth factors. Inhibin is known as a tumor suppressor in the mouse gonad (Matzuk et al. 1994). It has been shown that

inhibin α knockout mice develop spontaneous gonadal tumors and if gonadectomized prepubertally (<6 weeks of age), they develop adrenal tumors with 99% penetrance (Matzuk et al. 1994). Based on the *inh^{-/-}* observations it was initially suggested that either the overexpression of gonadotropins or the lack of some gonadal factor might be the cause of tumor formation after gonadectomy (Matzuk et al. 1994, Kananen et al. 1996, Kananen et al. 1997). Later studies have indicated that the development of tumors requires a high level of LH (Kero et al. 2000) and a drop in the levels of gonadal activin, which normally inhibits the adrenal tumor growth by apoptosis (Beuschlein et al. 2003). Smad 3, the intracellular signal transducer for TGF β , has shown to be upregulated in *inh^{-/-}*-mice and its expression has also been found in the stem cell compartment indicating that tumors might arise from dys-regulated stem cells. It is also possible that GATA-4 is an important factor inducing adrenal stem cells to transform to tumor cells. This could provide additional proof for the fact that GATA-4 expression is detected in all the tumors developed after gonadectomy (Bielinska et al. 2003). The adrenocortical stem cells may also be unable to downregulate TGF β signalling, which could result in tumor formation (Looyenga et al. 2004). Of interest, GATA-4 has recently been shown to cooperate with Smad3 to mediate TGF β signalling in the regulation of *inhibin α* . (Anttonen et al. 2006). Thus, TGF β , Smad3 and GATA-4 might act in the common pathways to promote adrenal tumor formation. Supporting this connection, TGF β -Smad3 signalling has been linked to GATA-4 upregulation in the adrenals (Looyenga et al. 2004). Recent work on *inh^{-/-}* - LH-CTP mice (Risma et al. 1995, Beuschlein et al. 2003, Beuschlein et al. 2004) showed that when *inh^{-/-}* mice are crossbred with LH-overexpressing LH-CTP mice, they produce large activin-secreting ovarian tumors (Beuschlein et al. 2003). While gonadectomized, they produce large, steroid-producing adrenal tumors putatively arising from the X-zone (Beuschlein et

al. 2003). However, in the presence of activin, apoptosis in the X-zone has been shown to prevent the LH-dependent adrenocortical tumor formation (Beuschlein et al. 2003). Activin is thus considered an important regulator of the X-zone together with inhibin, which again is known to regulate the growth of the adrenal X-zone (Beuschlein et al. 2003, Beuschlein et al. 2004). Hence elevated LH levels alone might not be sufficient to induce adrenal tumor formation in the presence of activin (Beuschlein et al. 2004).

3.2. Adrenocortical phenotype of the $inh\alpha$ /Tag mice

We have studied the molecular mechanisms of adrenal tumorigenesis extensively in the $inh\ \alpha$ /Tag TG mouse model, where the mice bearing the Simian Virus 40 T-antigen (Tag) under a 6-kb fragment of the murine inhibin α -subunit promoter ($inh\alpha$), develop gonadal tumors with 100% penetrance by the age of 5-6 mo (Kananen et al. 1995). When prepubertally gonadectomized, these mice develop adrenocortical tumors by the same age (Kananen et al. 1996, Rilianawati et al. 1998, Rahman et al. 2004). No adrenal tumors could be detected either in any intact TG or in gonadectomized, intact or control non-TG (C57Bl) littermates (Kananen et al. 1996, Rilianawati et al. 1998, Rahman et al. 2004). If functional gonadectomy was induced by administration of a GnRH antagonist or by cross-breeding the transgenic mice into the hypogonadotropic *hpg* genetic background, neither gonadal nor adrenal tumors appeared [10]. This finding led to the hypothesis, that tumor development is related to elevated gonadotropin secretion, which is the distinct difference between the surgical and functional gonadectomy models (Kananen et al. 1997). This high secretion of gonadotropins was thus proposed to stimulate the expression of LHR in tumors upon the malignization process (Rahman et al. 1998, Rilianawati et al. 1998, Rahman et al. 2004).

Recently it has been shown that GATA-4 and LHR have a direct interrelationship during the adrenal tumorigenesis (Rahman et al. 2004). The onset of adrenal mRNA and protein expression of GATA-4 and LHR coincides and colocalizes in the same tumor areas at 4 mo of age after gonadectomy, which preceded the appearance of discernible adrenocortical tumors at 6 mo of age (Rahman et al. 2004). GATA expression might thus be well connected to the LHR expression in adrenocortical tumorigenesis. These results demonstrated an apparent direct and positive feed-forward amplification link between these two factors, LHR action up-regulating the GATA-4 message and perhaps also *vice versa*. These two factors together with Tag expression are thought to cause the formation of LH-dependent adrenocortical tumors in this TG mouse model (Rahman et al. 2004). The adrenal tumor derived cell line (C α 1) from inh α /Tag mice was found to express high levels of functional LHR as assessed by Northern hybridization, immunocytochemistry, ligand binding, and human CG (hCG)-stimulated cAMP and steroid production (Rilianawati et al. 1998). We were unable to find any follicle stimulating hormone receptors (FSHR) in the adrenal tumors or in the C α 1 cells (Rilianawati et al. 1998). While we stimulated the C α 1 cells by hCG, we achieved dose dependent cell proliferation and steroid production (Rilianawati et al. 1998). This helped us additionally to conclude that the expression of the potent oncogene, Tag, in adrenocortical cells allows a tropic hormone (LH) to function as a tumor promoter (Rilianawati et al. 1998). While there are differences between these TG mouse adrenal tumors and human adrenocortical tumors, certain similarities are noteworthy. Even if rare, the human adrenocortical tumors are gonadotropin responsive (Givens et al. 1975, de Lange et al. 1980, Leinonen et al. 1991) (also see above). They may also express some common neuroectodermal and steroidogenic enzymes like in the case of transgenic mice (Kananen et al. 1996, Kananen et al. 1997,

Rilianawati et al. 1998). These conserved features makes the $inh\alpha$ /Tag TG mouse model potentially useful for identifying factors critical for adrenocortical tumorigenesis in humans and a very useful model for human adrenocortical cancer research.

4. Future directions

In the future, it is estimated that somatic gene therapy might be a possible way to treat patients with adrenocortical tumors. A potential probe in gene therapy trials could be the Herpes Simplex Virus Thymidine Kinase (HSV-TK) gene, which alone is non-toxic but induces the killing of dividing cells if exposed to a “prodrug” (Camper et al. 1995). HSV-TK phosphorylates nucleoside analogs such as ganciclovir and acyclovir into monophosphorylated molecules, which are converted into triphosphate substrates by eukaryotic cells’ own enzymes. The cytotoxic monophosphorylated product is incorporated into elongating DNA of proliferating cells, causing chain termination and cell death (Moolten and Wells 1990). HSV-TK has already been used in experimental and clinical gene therapy trials as a conditional suicide gene to ablate tumor cells (Moolten and Wells 1990, Moolten et al. 1990, Mikola et al. 2001). We have produced another TG mouse line, expressing the HSV-TK gene under the $inh\alpha$ promoter and crossbred these mice with the original $inh\alpha$ /Tag tumor mice, to obtain double TG mice, which are susceptible to tumor ablation by treatment with antiherpes drugs (e.g. gancyclovir) (Mikola et al. 2001). We have already successfully treated double TG and single TG mice of both sexes, possessing gonadal tumors, with gancyclovir, and found that the double TG tumors decreased significantly in volume during the antiherpes treatment, whereas controls $inh\alpha$ /Tag tumors continued growing at the same time (Mikola et al. 2001). Thus we hypothesize that targeted expression of the HSV-TK gene would potentially be useful for gene therapy of

adrenocortical tumors in this double TG model. Possibly a novel treatment strategy with siRNA (short hairpin silencing RNA) technology *in vivo* could serve also as a potential and novel therapeutic approach. We would hypothesize that LHR and GATA-4 could be targeted by siRNA *in vivo* strategy simultaneously, which could increase the success rate. Recently it has also been found that the lytic peptide hecate, a fusion protein of a 23-amino acid and a 15-amino acid (81-95) fragment of the human CG β chain could be used as a form of novel anticancer drug that could selectively induce the destruction of cancer cells expressing LHR. The cytotoxic effect strongly correlates with the number of LHR. There are already studies done with Hecate conjugate and its positive treatment effects in prostate (Hansel et al. 2001), and breast (Bodek et al. 2003) cancer, and in gonadal tumors (Bodek et al. 2005). The results have already proven the principle that the Hecate-CG β conjugate may provide a novel specific lead into LHR expressing cancer cell therapy by targeted destruction of LHR expressing tumor cells. Our preliminary data on adrenocortical tumor treatment with Hecate conjugate in inh α /Tag TG mice showed promising results (Vuorenoja et al, unpublished data). Based on the available data (Kiiveri et al. 2002a, Kiiveri et al. 2002b, Barbosa et al. 2004, Kiiveri et al. 2004), we also believe that in the future it will be possible to use the expression levels of GATA-4, as well as GATA-4 associated markers like LHR as a predictor of the prognostic marker for monitoring adrenocortical tumorigenesis.

Table 1 Adrenocortical tumorigenesis animal models

Animal model	Promoter gene	Gonadectomy needed	Age of the tumor formation	Histopathology	LHR	GATA-4	Metastases	Cell line established	Reference
Inha/Tag	Inhibin α	yes	6-8 mo	Tumors from X-zone (innermost layers)	In the tumor area	In the tumor area	Liver occasionally	C α 1	(Kananen et al. 1996), (Rilianawati et al. 1998), (Rahman et al. 2004)
DBA/2J		yes	6 mo	A and B cells in the subcapsular region	In the tumor area	In A and B cells	?	none	(Bielinska et al. 2004, Bielinska et al. 2005)
Inh ^{-/-}		yes	5-6 mo	Tumors derived from zona fasciculata and reticularis and/or X-zone	In the tumor area	?	lung		(Matzuk et al. 1994), (Matzuk et al. 1996)
Inh ^{-/-} -LH-CTP		yes	3 mo	Tumors from X-zone	In the tumor area?	?	?		(Beuschlein et al. 2003)
NU/J nude mice		yes or xenograft	1-2 mo	A and B cells in the subcapsular region (resembles DBA/2J mice)	In B cells	In A and B cells	?		(Bielinska et al. 2004, Bielinska et al. 2005)
ferret		yes		A and B cells in the subcapsular region (resembles DBA/2J mice), myxoid differentiation	In the tumor area	In the tumor area	liver		(Peterson et al. 2004)

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