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Review

Multiple sites of tumorigenesis in transgenic mice overproducing hCG

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Abstract

We have produced transgenic (TG) mice expressing under the ubiquitin C promoter either the glycoprotein hormone common α-subunit (Cα) or human chorionic gonadotropin (hCG) β-subunit. Cα overexpression alone had no phenotypic effect, but the hCGβ expressing females, presenting with moderately elevated levels of bioactive LH/hCG, due to dimerization of the TG hCGβ with endogenous Cα, developed multiple gonadal and extragonadal neoplasias. Crosses of the Cα and hCGβ mice (hCGαβ) had >1000-fold elevated hCG levels, due to ubiquitous transgene expression, and presented with more aggressive tumour formation. The ovaries displayed initially strong luteinisation of all somatic cell types, leading to formation of luteomas, and subsequently to germ cell tumours (teratomas). The pituitary glands of TG females were massively enlarged, up to >100 mg, developing macroprolactinomas with very high prolactin (PRL) production. This endocrine response probably induced breast cancers in the mice. In contrast to the females, similar high levels of hCG in male mice had only marginal effects in adulthood, with slight Leydig cell hyperplasia and atrophy in the seminiferous epithelium. However, clear Leydig cell adenomas were observed in postnatal mice, apparently originating from fetal Leydig cells. In conclusion, these studies demonstrate marked tumorigenic effects of supraphysiological hCG levels in female mice, but clear resistance to similar changes in males. The extragonadal tumours were induced by hCG stimulated aberrant ovarian endocrine function, rather than by direct hCG action, because gonadectomy prevented all extragonadal phenotypes despite persistent hCG elevation. The phenotypes of the TG mice apparently represent exaggerated responses to hCG/LH and/or gonadal steroids. It remains to be explored to what extent they simulate respective responses in humans to pathophysiological elevation of the same hormones.

Keywords: Choriongonadotropin; Transgenic mice; Ovarian tumours; Pituitary tumours; Mammary tumours; Teratoma; Prolactinoma; Luteoma; Leydig cell adenoma

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1. Introduction

The two gonadotropins, follicle-stimulating hormone (FSH) and luteinising hormone (LH), are the key trophic regulators of ovarian and testicular development and mature function. Both clinical conditions with insufficient gonadotropin secretion (e.g. Kallmann syndrome) and animal experiments with suppressed gonadotropin secretion (e.g. hypophysectomy) provide compelling evidence that normal gonadotropin secretion and action are needed for the maintenance of sufficient gonadal sex hormone production and fertility. Recent findings of inactivating gonadotropin and gonadotropin receptor mutations in humans and respective knockout models in mice have largely confirmed the earlier developed concepts of the hypothalamic–pituitary–gonadal function (Themmen and Huhtaniemi, 2000; Burns and Matzuk, 2002). However, many interesting novel details about gonadotropin action have emerged from both the human and mouse mutations, and in some situations the need of the two gonadotropins may not be as absolute as previously assumed. For instance, men and male mice without FSH action appear to be fertile (Tapanainen et al., 1997; Kumar et al., 1997) and mice can develop qualitatively, though not quantitatively normal spermatogenesis in the absence of LH-driven testosterone production (Zhang et al., 2003).

In addition to gonadotropin deficiency there are also situations with physiologically or pathologically elevated gonadotropin action. The clearest example are the postmenopausal high gonadotropin levels to which elderly woman are exposed for decades. Pregnancy represents another situation with temporary elevation of gonadotropins through placental production of choriongonadotropin (hCG). There are pituitary gonadotroph adenomas, but they rarely produce high gonadotropin levels (Young et al., 1996). Finally, activating mutations of gonadotropin receptors, especially of those of LH, represent a situation of chronic elevation of gonadotropin action. Whether such conditions have other effects than providing elevation of the physiological gonadal stimulation remains unclear. Epidemiological data are in favour of the role of lifetime exposure to gonadotropins in the occurrence of ovarian cancer (Konishi et al., 1999). There is one particular type of activating LH receptor mutation (Asp578His) that seems to be associated with Leydig cell tumours (Liu et al., 1999). In transgenic (TG) mice developing SV40 T-antigen induced tumours of gonadal somatic cells, and in inhibin-α knockout mice, gonadotropins have a clear tumour promoting function (Kananen et al., 1997; Kumar et al., 1999). Hence, although the information is scattered, there is evidence that gonadotropins may have a role as tumour promoters in certain conditions, which calls for detailed studies to explore this possibility. We therefore developed a TG mouse model expressing bioactive hCG at high level. In this way we can amplify subtle aberrant regulatory mechanisms that otherwise would remain unnoticed. Indeed, multiple tumorigenesis was observed in these mice in support to our original hypothesis. The purpose of this review is to summarize the main findings of these studies.

2. Generation of hCG overexpressing transgenic mice

To generate the TG mouse models with elevated LH/hCG bioactivity we used the human ubiquitin C promoter that displays ubiquitous low level expression from the late fetal age onwards. Two different TG mouse models were generated using the promoter: mice expressing the hCGβ-subunit (Rulli et al., 2002) and mice expressing the common-α subunit (Rulli et al., 2003). For the former model, a 579-bp cDNA fragment of the β-subunit of hCG was cloned under the ubiquitin C promoter, followed by the bovine growth hormone polyadenylation signal. For expressing the common α-subunit (Cα) as a transgene, a 2.4-kb long genomic fragment coding for the α-subunit was cloned under the same promoter and used for TG mouse generation. The TG mice were produced using conventional pronuclear microinjection technique.

Two of the four ubiquitin C-hCGβ male founders were fertile, and consequently, two independent mouse lines expressing the transgene ubiquitously (hCGβ mice), with similar phenotype, were established by breeding the fertile males with wild-type (wt) FVB/N females. The hCGβ mice showed transgene expression also in the pituitary, and therefore the TG β-subunit was able to dimerize with the mouse Cα and form high levels of biologically active dimeric hCG (Fig. 1). The dimerization process is expected to occur mainly in gonadotrophs and thyrotrophs (Kendall et al., 1994) but possibly also in some extra-pituitary tissues, such as the ovary, where Cα expression has been detected (Markkula et al., 1995). This resulted in a 40-fold increase in circulating hCG/LH bioactivity in the hCGβ females, while in males only 3–4-fold elevation of hCG/LH bioactivity was detected. This difference is likely to result from sexually dimorphic regulation of the Cα gene in the pituitary gland (Risma et al., 1995). The high increase in bioactive hCG/LH in females is not due to such high excess of free Cα subunit in the pituitary gland, but rather to the much longer circulatory half-life of hCG in comparison to mouse LH.

TG mice expressing Cα were indistinguishable from their wt littermates, indicating that free α-subunit is devoid of

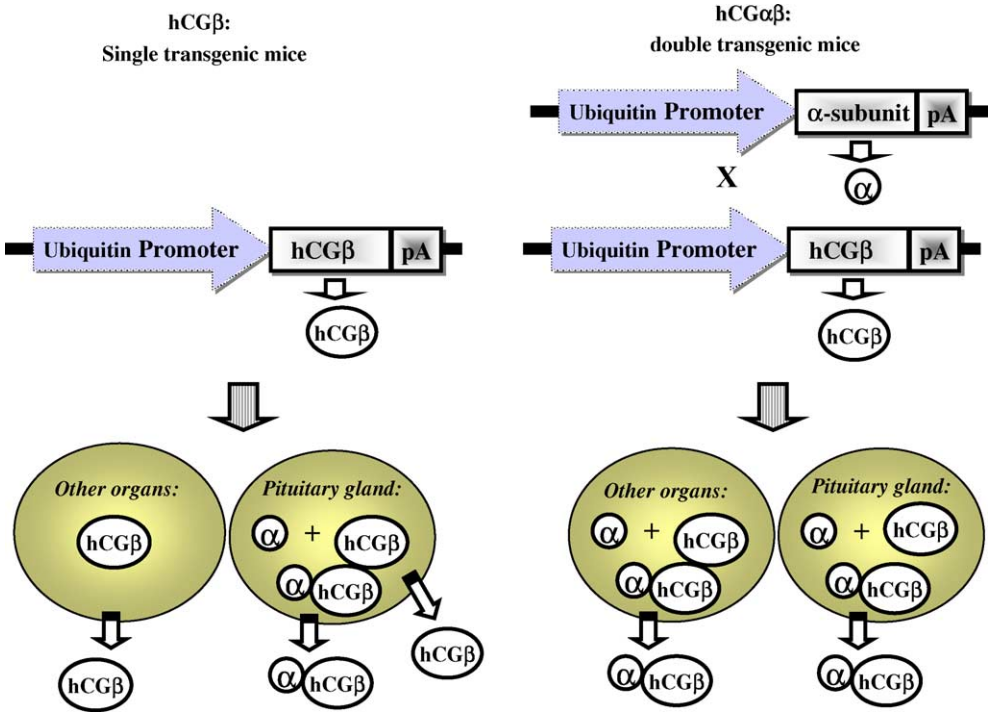

Fig. 1. Schematic presentation of bioactive hCG production of the single TG hCGβ and double TG hCGαβ mice. In the former mice, the TG hCGβ formed in pituitary gonadotroph and thyrotroph cells dimerizes with endogenous common α-subunit giving rise to bioactive hCG. In the latter model, most organs, in addition to the pituitary gland, produce TG Cα and hCGβ, giving rise to massively elevated hCG levels.

detectable phenotypic effects. In order to produce double-TG mice, the combination of four different Cα lines with one of the hCGβ TG lines was attempted. Pregnancy and delivery of double TG offspring were successful only with one of the Cα TG lines, coincident with the lowest levels of transgene expression. This suggested that a high level of hCG production by the fetuses resulted in termination of pregnancy. Since the double TG males and females were infertile, constant crossbreeding of hCGβ mice with the Cα mouse line was necessary to obtain hCGαβ mice. In these double-TG mice, the bioactive LH/hCG exceeded 1000-fold the respective levels measured in wt mice (Fig. 1).

In summary, two mouse models were generated: those expressing only the hCGβ subunit resulting in moderate increase in LH/hCG bioactivity (four-fold in males, 40-fold in females), and those expressing both hCG subunits and giving rise to pharmacological levels of LH/hCG (>1000-fold increase in both sexes). A TG model with mildly elevated LH was previously described by Risma et al. (1995), in which the bovine α-subunit promoter was fused to the coding region of a chimeric bovine (b) LHβ-hCGβ C-terminal peptide (CTP) gene. This model displays certain similarities with our hCGβ mice. Both present with severe disturbances in the female reproductive system, such as precocious puberty, associated with increased oestrogen and androgen levels (Risma et al., 1997), infertility, and anovulation (Mann et al., 1999), while the males are not affected. Remarkable differences also exist between the two models. Firstly, because of different promoters, the human ubiquitin C promoter used in our study is universally and constitutively activated (Schorpp et al., 1996), whereas the α-subunit promoter used in the bLHβ-CTP mice is specifically directed to gonadotrophs and responds to negative feedback regulation of gonadal steroids (Abbud et al., 1999). This apparently explains the different levels of circulating LH/hCG in the two models, 5–10-fold in the bLHβ-CTP females (Risma et al., 1995), and around 40-fold in the hCGβ females (Rulli et al., 2002).

3. Ovarian tumorigenesis in TG females

In the female hCGβ mice, reproductive function was severely disrupted, and the mice showed precocious puberty as manifested by vaginal opening 5–6 days before wt littermates, followed by disrupted oestrous cycles and infertility. As a consequence of hyperstimulation of the immature ovary by hCG, abnormal ovarian morphology was observed, characterized by fluid- and blood-filled cysts and massive somatic cell luteinisation resembling luteomas (Fig. 2). Enhanced steroidogenesis accompanied these changes throughout life, resulting in high levels of oestradiol, testosterone and progesterone during early stages of sexual maturation. In adulthood, the most conspicuous change was the 40–100-fold elevation of progesterone, while oestradiol remained in the physiological range, and a 3–8-fold increase was found in testosterone.

Observations in other TG mouse models support the contention that gonadotropins are implicated in ovarian

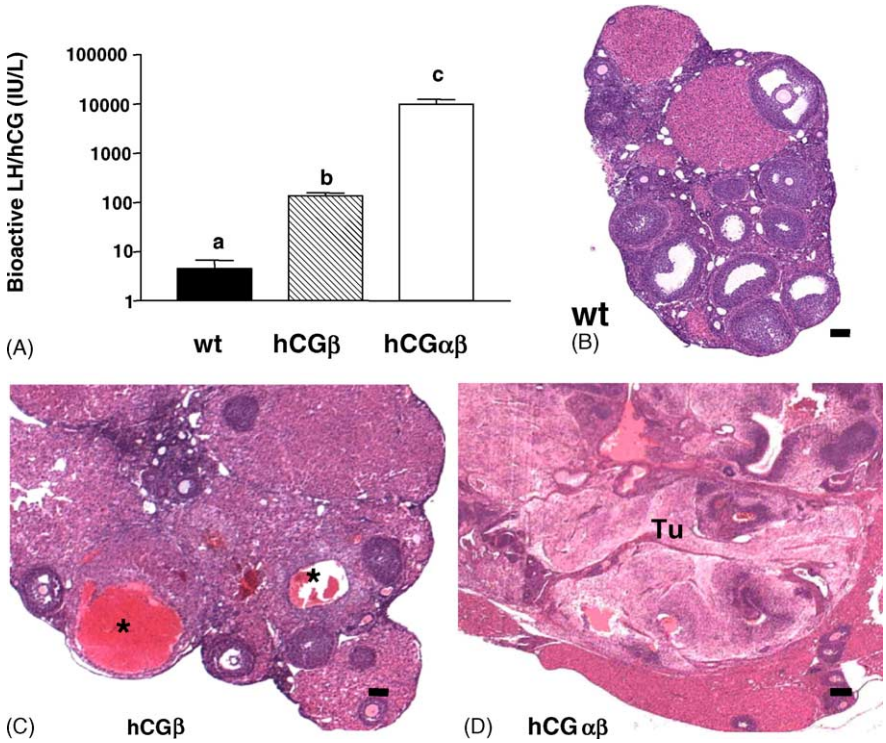

Fig. 2. (A) Serum levels of bioactive hCG/LH in adult wt, hCGβ, and hCGαβ female mice (n = 5–10). Different letters denote statistically significant differences ($P < 0.05$); (B) histology of the adult ovary from wt mice showing follicles at different stages and corpora lutea; (C) ovary of an hCGβ mouse showing massive luteinisation and hemorrhagic cysts (asterisks); (D) ovary of a hCGαβ mouse showing a solid tumour (Tu) with different tissues resembling a teratoma. Hematoxylin and eosin; bar = 50 μm (modified from Rulli et al., 2002, 2003, unpublished data).

tumorigenesis. As was previously shown, chronic elevation of LH/hCG could lead to polycystic ovaries, luteomas and development of granulosa cell tumours, depending on the genetic background of the mouse strain (Keri et al., 2000; Owens et al., 2002). In TG mice expressing the mouse inhibin α-subunit promoter/Simian virus 40 T-antigen fusion gene elevated LH levels likewise act as tumour promoter and induce gonadal and adrenal tumorigenesis (Mikola et al., 2003). In addition, mice deficient of the inhibin α-subunit gene develop mixed granulosa/Sertoli cell tumours in the presence of LH and FSH (Kumar et al., 1996).

In the double TG mouse model overexpressing both Cα- and hCGβ-subunits, and producing over 1000-fold excess of bioactive hCG in circulation (Rulli et al., 2003), females also showed precocious puberty, infertility, and enhanced steroidogenesis from early sexual development. In young adulthood, these mice developed ovarian germ cell tumours that were phenocopies of human teratomas (Rulli et al., unpublished observation). The tumours comprise a wide variety of tissue types including ectodermal, mesodermal and endodermal derivatives, like keratinizing squamous epithelium, hair follicles, sebaceous glands, neural tissue, cartilage, digestive or respiratory epithelia (Fig. 2). These tumours apparently originate through parthenogenetic activation of oocytes located within the ovary (Mutter, 1987, 1997; Ulbright, 2004). These germ cell-derived tumours are one of the most frequent types of ovarian tumours in women, representing around 20% of all ovarian neoplasm. They are benign, however displaying malignant transformation with an incidence of approximately 1.4–2% (Comerci et al., 1994). The molecular pathogenesis of this neoplasm in humans has not been fully clarified. Consequently, our TG hCGαβ mouse model provides a useful resource to reveal the molecular mechanisms involved in the formation of ovarian tumours and to allow a better understanding of their treatment and prevention.

4. Pituitary macroprolactinomas in TG females

Pituitary glands of the hCGβ overexpressing female mice were enlarged from the age of 2 months and progressed to clear adenomas in older ages (Rulli et al., 2002). The penetrance of the adenomas was 100% at 10–12 months of age, and the tumours weighed up to 100 mg. In parallel with the persistent pituitary growth throughout life, serum prolactin (PRL) levels increased up to 600-fold in connection with the largest adenomas (Fig. 3). Macroscopically the prolactinomas presented with suprasellar expansion and hemorrhagia, and histologically the tumours were nodular and of irregular shape, and showed distorted architecture with dilated blood-filled spaces and multifocal nodules compressing the adjacent tissue. The neoplastic foci showed breakdown of the reticulin fiber network indicating the diagnosis of adenoma. Densely packed, small rounded cells were present in some

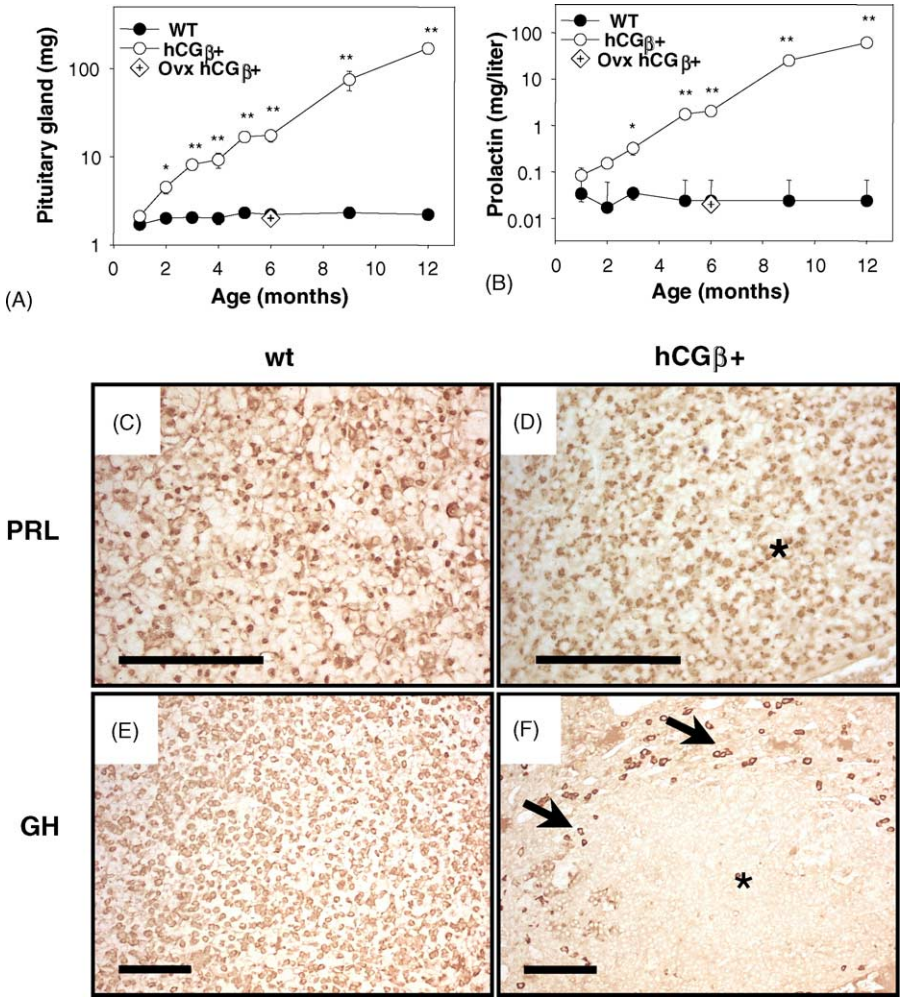

Fig. 3. Age-related changes in weights of pituitary glands (A) and concentration of serum prolactin (B) in wt (filled circles) and hCGβ females (open circles). The effects of ovariectomy on hCGβ females are indicated by a diamond-shaped symbol at 6 months of age in both figures ($n = 6$; $^{*}p < 0.05$; $^{**}p < 0.01$ vs. wt). Immunohistochemistry of PRL (C and D) and growth hormone (GH, E and F) in pituitaries of 11-month-old wt (C and E), and hCGβ females (D and F). In (D), strong immunoreactivity for PRL is seen in the focal adenomatous nodule (asterisk) and its surroundings. In (F), the arrows indicate cells positive for GH, whereas the asterisk indicate the lack of immunoreaction for GH in the same focal nodule; bars = 100 μm (modified from Rulli et al., 2002).

areas of the tumour, whereas enlarged cells with abundant cytoplasm populated other areas. The adenomas showed strong immunocytochemical reaction for PRL in the different tumour areas, whereas immunoreactions for ACTH, GH, FSHβ and TSHβ were scattered in areas surrounding the nodules, probably representing remnants of the normal pituitary gland (Fig. 3). The specific immunostaining for PRL in the neoplastic foci of the pituitary, along with severe hyperprolactinemia, confirmed the diagnosis of prolactinoma.

Even though the same tendency to severe hyperprolactinemia associated with pituitary enlargement existed in the double TG hCGαβ females, we were unable to find pituitary adenomas in this model, since the animals had to be sacrificed before the neoplastic growth appeared, due to excessive growth of their ovarian tumours.

All the effects on the pituitary gland observed in the hCGβ TG mice were attributed to the chronic ovarian hyperstimulation by hCG, since pituitary hyperplasia and subsequent development of lactotroph adenomas were prevented by ovariectomy despite the persistently high levels of hCG, suggesting the involvement of gonadal steroids or other gonadal hormones. In this respect, oestrogens have been recognised in pituitary lactotroph proliferation and PRL expression (Freeman et al., 2000). It has been known for long time that certain inbred strains of rats are sensitive to oestrogens, developing PRL producing pituitary tumours after oestrogen treatment (Spady et al., 1998; Heaney et al., 1999). Ovary-dependent functional adenomas from cells of the Pit-1 lineage were described in a mouse model with targeted overexpression of LH (Mohammad et al., 2003). In addition, the increased lactotroph proliferation and PRL production during pregnancy in women are attributed to high oestrogens (Scheithauer et al., 1990). In our model, the aberrant exposure to an early oestrogen peak during peripuberty was followed by persistently elevated levels of androgens as a source of locally produced oestrogens (Carretero et al., 1999), which may

explain the occurrence of PRL producing pituitary adenomas at older ages. Due to its complex and multistep hormonal dysregulation, the hCGβ mouse is a good model to understand the hormone-dependent pathogenesis of pituitary adenomas.

5. Mammary gland tumours in TG females

One of the most remarkable features of the hCGβ females was the development of alveolar hyperplasia in their mammary glands at 3–6 months of age, with structures resembling prelactating mammary gland epithelium (Fig. 4). At the age of 9–12 months, the hyperplastic mammary glands developed into palpable tumours, and by 12 months, more than 90% of the females had developed multiple palpable mammary gland tumours. The mammary tumours presented with characteristics of adenocarcinoma and were highly malignant, sending metastases at high frequency to several tissues. As described above, the hCGβ females developed pituitary prolactinomas. The ensuing hyperprolactinaemia is likely to play a central role in development of the mammary gland tumours. The physiologic role of oestrogens in inducing lactotroph proliferation and PRL gene expression has been well characterized (Heaney et al., 1999; Spady et al., 1998). Also in humans, pregnancy induces a coordinated increase in lactotroph proliferation and PRL production, an effect attributed to oestrogens (Asa et al., 1982; Scheithauer et al., 1990), and few lactotroph adenomas may grow during gestation. Hence, the fact that the hCGβ mice were exposed to elevated oestrogens at an early age would, at least partially, explains the occurrence of pituitary lactotroph adenomas in the females at adulthood, and the subsequent mammary gland tumours.

Our results demonstrate that the abnormal lobuloalveolar development and tumorigenesis of mammary glands of the hCGβ female mice was dependent on ovarian hyperfunction. It is likely that the high levels of hCG increased oestrogen, progesterone and PRL production, which then stimulated the proliferation and differentiation of the mammary gland, resulting the metastatic adenocarcinomas. Sex hormones, especially oestrogens, are well characterized priming factors for breast cancer, and the role of PRL in the initiation and progression of mammary carcinomas is also increasingly appreciated (Clevenger et al., 2003). Hence, this TG model provides the opportunity to study the sequence of hormonal effects putatively involved in mammary gland hyperstimulation and breast cancer progression from the initial tumour formation to the final metastatic stage.

6. Mild phenotype in adult TG males with no tumorigenesis

We observed that the hCGβ TG males presented only with very mild phenotypes, which was explained by the fact that their bioactive hCG levels were only slightly elevated, and their testicular and serum testosterone levels remained normal. In contrast to the hCGβ males, the double TG hCGαβ males presented with 1000-fold increase in serum hCG/LH bioactivity, leading to highly elevated serum and testicular testosterone levels. Because of properly functioning negative feedback system, the high testosterone levels induced a clear decrease of FSH secretion. This apparently resulted in decreased postnatal proliferation of Sertoli cells and subsequently in decreased total length of seminiferous tubules,

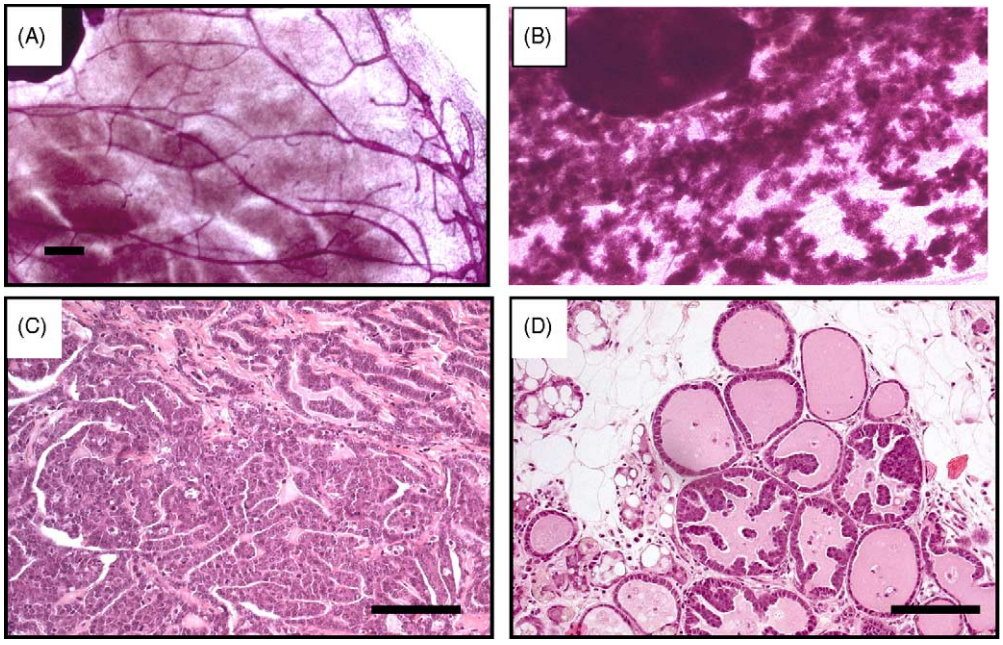

Fig. 4. Lobuloalveolar development and tumorigenesis in the hCGβ female mice: (A) wt mouse; (B) hCGβ female at the age of 6 months; C and D: papillomatous tumour structures in aged hCGβ females; bars = 100 μm (modified from Rulli et al., 2002).

as was reflected by a 50% decrease in testis weights of the hCGαβ males in comparison to wt littermates (Rulli et al., 2003).

Upon histological evaluation, the structure of the seminiferous tubules was normal with full spermatogenesis between 2 and 4 months of age, but thereafter, severe tubular degeneration with large vacuoles in the basal compartment of the seminiferous epithelium was observed. Only slight Leydig cell hypertrophy/hyperplasia was observed in the double TG testes (Fig. 5). Furthermore, no signs of testicular tumorigenesis were found.

We saw signs of infravesical urinary tract obstruction in the hCGαβ males (Rulli et al., 2003). The kidneys of the animals were enlarged, the urinary bladder was dilated, and the distal parts of vasa deferentia were distended and filled with sperm. However, the urethral lumen was visible indicating functional, not anatomical, obstruction. Also the seminal vesicles and ventral prostate were greatly enlarged in the adult double TG males, but no signs of neoplastic transformation were seen in the prostate. Histology of the epididymides was normal in young males, but revealed some sperm granulomas, increased amount of connective tissue, and abnormal sperm morphology after the age of 8–9 months.

The hCGαβ males were infertile already as young adults, before any changes in the epididymis occurred, and we found no vaginal plugs after mating were mated with PMSG-treated females. In addition, the males behaved very aggressively during matings, and some of the females were even killed by the TG males, indicating that their infertility is most likely explained by their abnormal behaviour rather than anatomical or physiological defects. Similar findings were reported by Matzuk et al. (2003) with their hCG overexpressing males. In conclusion, we can conclude that adult males are well protected against adverse effects of LH/hCG hyperstimulation, and enhanced LH/hCG action is an unlikely inducer of tumours in adult male mice.

7. Leydig cell adenomas in prepubertal TG males

The development of precocious puberty and formation of Leydig cell adenomas with a particular mutation (Liu et al., 1999), have been described in association with activating LHR mutations (Themmen and Huhtaniemi, 2000). Also, the administration of hCG/LH by injection has shown to lead to formation of Leydig cell hyperplasia (Christensen and Peacock, 1980).

Testicular histology of the hCGαβ mice at the ages of 5, 10, 21 and 60 day showed adenomatous growth of Leydig cells, and we found some mitotic figures in the Leydig cells of 10-day-old TG males indicating their active proliferation (Ahtiainen et al., submitted for publication). At the same age, the diameters of the largest Leydig cell islets found in the TG testes exceeded those of seminiferous tubules, thus fulfilling the criterion of Leydig cell adenomas (Clegg et al., 1997). The volume density of Leydig cells was highly increased in prepubertal TG males, but not in adults. Also, the largest Leydig cell islets were observed in the 10-day-old TG males. Conspicuously, the index of total Leydig cell volume was increased in prepubertal but not in adult TG males (Ahtiainen et al., submitted for publication).

There exist two growth phases of Leydig cells in the testes of most mammalian species, the fetal and the adult type of Leydig cell populations (Habert et al., 2001). We analyzed the expression pattern of marker genes for fetal and adult type Leydig cells to determine the origin of Leydig cell adenomas in prepubertal TG males. The data demonstrated that the expression of thrombospondin 2, a fetal Leydig cell

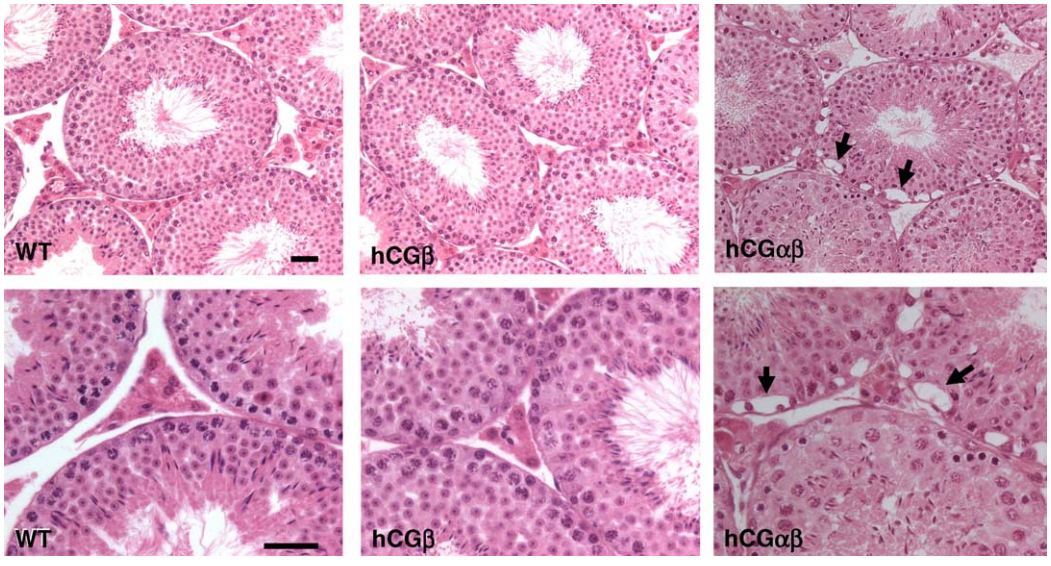

Fig. 5. Testicular histology of wt (6-month-old), hCGβ (6-month-old) and hCGαβ (8-month-old) mice. No difference is seen between the wt and hCGβ mice, whereas slight degeneration of seminiferous epithelium is seen in the hCGαβ testes (arrows). The upper and lower rows represent two different magnifications; bars = 50 μm (modified from Rulli et al., 2003).

marker, and of 3β-hydroxysteroid dehydrogenase type VI and prostaglandin D synthase, adult Leydig cell markers (O'Shaughnessy et al., 2002a,b), were not changed as compared with wt males. These findings suggested that the Leydig cell adenomas found in 10-day-old TG males were derived from the fetal Leydig cell population. Fetal Leydig cells thus appeared to be the only cell type in male mice that responds to supraphysiological LH/hCG stimulation with neoplastic transformation. With respect to the formation of Leydig cell adenomas, the hCGαβ males are a good phenocopy of the males with the specific activating LHR mutation (A578H) inducing Leydig cell adenomas (Liu et al., 1999) and may offer a mouse model for further exploration of the molecular mechanisms responsible for this intriguing condition. As with the A578H LHR mutation, the highly elevated hCG levels may have activated alternative signalling cascades (phospholipase C/inositol trisphosphate or MAP kinase), which were considered the cause of tumorigenesis in the afore mentioned mutation.

8. Extragonadal phenotypes of the TG mice: direct or indirect hCG action?

Many of the phenotypes detected in the hCG overexpressing mice occurred in extragonadal organs, such as the pituitary, mammary and adrenal glands, as well as male urogenital structures. Because there is a considerable amount of information about extragonadal LH/hCG receptor expression in humans and other mammalian species (Rao, 2001; Shemesh et al., 2001; Licht et al., 2001) the question arises whether the extragonadal phenotypes of our mouse model were due to indirect actions through altered gonadal hormone production or to direct effects. In fact, LH/hCG receptors have been found in all tissues presenting extragonadal phenotypes in the hCG overexpressing mice; the adrenal gland (Pabon et al., 1996; Kero et al., 2000), pituitary gonadotroph cells (Huang et al., 1995), mammary gland (Hu et al., 1999; Meduri et al., 2003; Rao et al., 2004), male urogenital organs (Reiter et al., 1995; Dirnhofer et al., 1998; Tao et al., 1998). In addition, there are numerous reports on LH/hCG receptor expression in the uterus (Ziecik et al., 1986; Lei et al., 1992; Rao, 2001; Shemesh et al., 2001; Licht et al., 2001; Srisuparp et al., 2003). The level of extragonadal LH/hCG receptor expression is in general low, about 10% of that measured in gonadal tissue, and functional data in particular on these receptors are limited. Perhaps the most convincing data exist on direct LH/hCG effects on uterine function.

When we gonadectomised the hCGβ or hCGαβ mice, all the extragonadal phenotypes disappeared, although the circulating levels of transgenic hCG stayed unaltered, and were even fortified by the post-gonadectomy increase of levels of pituitary LH. We must therefore conclude that the current mouse model provides no convincing evidence for direct extragonadal actions of hCG. All the phenotypes detected must be considered consequences on altered gonadal endocrine function. In the female, elevated oestradiol and progesterone production could have caused some of the effects, but also the secondary elevation of PRL was apparently responsible for some of the phenotypes, for instance the mammary gland tumours.

In another study (Yarram et al., 2003), we compared bone density in female wt, hCGαβ TG and LH receptor knockout (LuRKO) mice. Bone density was highest in the hCGαβ mice, intermediate in wt, and lowest in LuRKO mice. This finding would naturally fit with a direct effect of hCG on bone density, which would then directly correlate with the level of LH/hCG action. However, when these mice were gonadectomised, all groups presented with bone densities indistinguishable from the LuRKO mice with very low ovarian hormone production. Therefore, although the hCGαβ mice produce intriguing extragonadal phenotypes, we have to be very cautious when considering the possibility of extragonadal LH/hCG effects. Comparison of the findings with gonadectomised control showed that no solid evidence for direct extragonadal actions could be obtained.

9. Concluding remarks

It may look unphysiological that the phenotypes of the hCGβ and hCGαβ mice were evoked by hormone levels that exceeded the physiological levels 40- and 1000-fold, respectively. However, we trust that our observations have good "pathophysiological" relevance. They may simulate mechanisms that prevail in humans in conditions with pathologically elevated gonadotropin and/or sex steroid action. The latter hormones, more modestly elevated, were apparently responsible for the phenotypes.

Our finding of hCG overproducing mice show clearly that female mice are very sensitive to tumorigenic effects of the elevated LH/hCG action, and consequent elevation of ovarian steroidogenesis. A multitude of tumours and al-

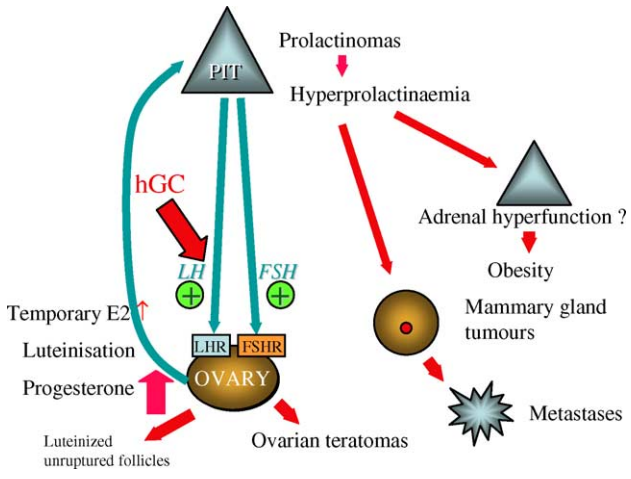

Fig. 6. Schematic presentation of the different pathologies induced by chronic TG elevation of circulating hCG level in mice.

tered endocrine functions were observed in gonads, pituitary gland, mammary gland and adrenal cortex (Fig. 6). The effects were apparently secondary responses to increased gonadal hormones, because they were lost when the animals were ovariectomised. Hence, no evidence for direct extragonadal actions of hCG could be obtained. Interestingly, the adult male mice appeared very resistant to effects of chronically elevated hCG. Besides mild Leydig cell hyperplasia, reduced testis size and signs of elevated androgen production in accessory sex organs, no evidence for tumorigenesis was observed. However, Leydig cell adenomas in prepubertal testes, derived from the fetal Leydig cell population, were apparent. The findings thus corroborate some clinical data on tumorigenic effects of chronically elevated gonadotropin and sex steroid levels, in particular in females, and call for additional studies on molecular pathogenesis of the tumours and in general the clinical significance of these experimental data.

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