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Review

Genetically modified mouse models in studies of luteinising hormone action

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Abstract

Numerous genetically modified mouse models have recently been developed for the study of the pituitary–gonadal interactions. They include spontaneous or engineered knockouts (KO) of the gonadotrophin-releasing hormone (GnRH) and its receptor, the gonadotrophin common-α(Cα), luteinising hormone (LH) β and follicle-stimulating hormone (FSH) β subunits, and the two gonadotrophin receptors (R), LHR and FSHR. In addition, there are also transgenic (TG) mice overexpressing gonadotrophin subunits and producing supraphysiological levels of these hormones. These models have offered relevant phenocopies for similar mutations in humans and to a great extent expanded our knowledge on normal and pathological functions of the hypothalamic–pituitary–gonadal (HPG) axis. The purpose of this article is to review some of our recent findings on two such mouse models, the LHR KO mouse (LuRKO), and the hCG overexpressing TG mouse (hCG+).

Keywords: Luteinising hormone (LH); Choriongonadotrophin (hCG); LH receptor; Knockout mice; Transgenic mice; Pituitary–gonadal function; Reproduction; Sexual development

Contents

1. Introduction

The HPG axis forms the backbone of the endocrine regulation of reproductive functions. Disturbances in the regulation of the key hormones and receptors functioning along this axis are common causes of hypogonadism and infertility in humans, and inactivating and activating mutations have recently been detected in the genes encoding GnRH, gonadotrophins and their receptors (Themmen and Huhtaniemi, 2000; de Roux and Milgrom, 2001; Huhtaniemi and Themmen, 2005). Our knowledge about the molecular pathogenesis of the disturbances caused by these mutations has been greatly increased by

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recently developed genetically modified mouse models. In most cases, they provide accurate phenocopies for respective human mutations (Themmen and Huhtaniemi, 2000; Huhtaniemi and Themmen, 2005). However, also differences exist, which provide examples of interesting species differences in the regulation of reproductive functions. In addition, some novel phenotypic features of the mouse models have provided hypotheses for more detailed studies on the physiology and pathophysiology of gonadotrophin function in humans. The mouse models may also predict phenotypes of mutations that have not yet been detected in humans. The purpose of this short review is to summarise some of the findings made recently in our laboratory using two genetically modified mouse models, the LHR KO mouse (LuRKO) and the hCG overproducing TG mouse (hCG+).

2. The LHR KO mouse (LuRKO)

2.1. General features

The LHR KO was described independently by two laboratories in 2001 (Lei et al., 2001; Zhang et al., 2001a). The former model was created by targeted deletion of the proximal part of the LHR promoter region and exon 1, the latter by targeted disruption of the long 11th exon of LHR, encoding the transmembrane and intracellular domains of the receptor. Both models produce complete elimination of functional LHR in the −/− mice. In principle the phenotypes observed in the two models are identical, although the two laboratories producing the mice interpret their findings somewhat differently, in particular as concerns the evidence for or against the functional significance of extragonadal LH/hCGR action (see below). A very similar phenotype is found in the recently produced LHβ subunit KO mouse (Ma et al., 2004).

Most phenotypic features of the female and male LuRKO mice are expected and in line with the existing knowledge about LH function (Fig. 1). The intrauterine sex differentiation of both sexes is normal, which is expected in the female, since the ovarian LHR expression starts only after birth and the fetal ovaries have no significant endocrine activity (Jost et al., 1973; Sokka et al., 1996; O'Shaughnessy et al., 1997). In the male rat and mouse, it has been shown before with many experimental models that the fetal testicular testosterone (T) production that is essential for the intrauterine masculinisation is not dependent on gonadotrophin stimulation (Cattanach et al., 1977; Kendall et al., 1995; El-Gehani et al., 1998). For this very reason the normal masculinisation of LuRKO mice at birth was expected. However, this finding is at variance with the human, where completely inactivating LHR mutations totally block the male fetal masculinisation (Themmen and Huhtaniemi, 2000).

In the adult age, female LuRKO mice present with anovulatory infertility and hypo-oestrogenemia, as demonstrated by their poorly developed accessory sex glands, including thin uteri (Fig. 1B). There is a total lack of follicular maturation beyond the antral stage in these mice, which indicates, besides the essential role of LH in ovulation, that the final preovulatory maturation of follicles from antral to preovulatory stage, also needs LH stimulation. The male LuRKO mice have total lack of postnatal

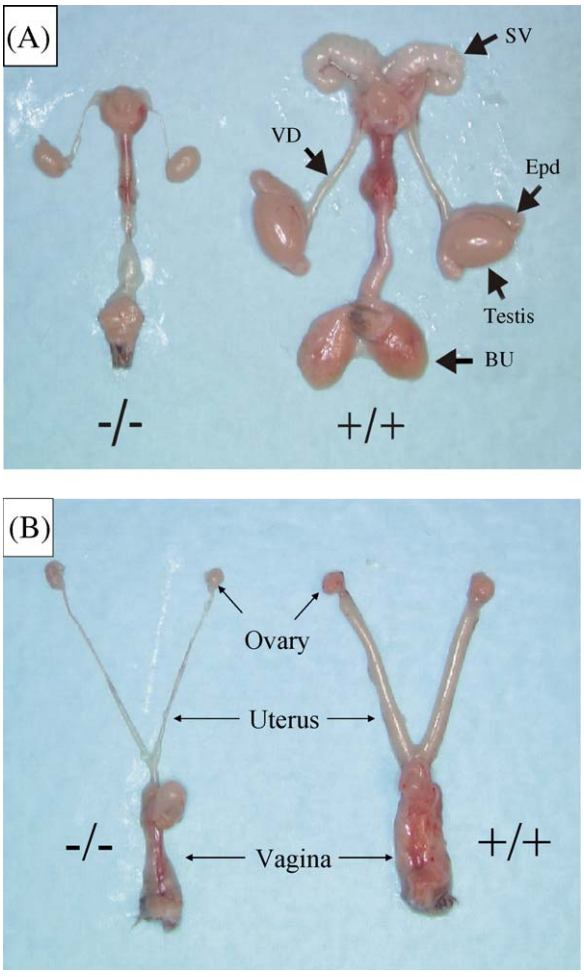

Fig. 1. Panel A: Testes and accessory sex organs of a LuRKO mouse (−/−) and a WT littermate (+/+). VD, vas deferens: SV, seminal vesicle; Epd, epididymis; BU, bulbourethral gland. Panel B: The ovaries, uterus and vagina of a LuRKO (−/−) mouse and a WT littermate (+/+). Modified from Zhang et al. (2001a).

sexual maturation with hypogonadism, cryptorchidism, poorly developed accessory sex glands and spermatogenic arrest at the round spermatid stage (Zhang et al., 2001a).

2.2. Further characteristics of male LuRKO mice

Detailed follow-up of postnatal testicular development in LuRKO mice (Zhang et al., 2004) revealed that the testicular histology of the KO and control wild-type (WT) mice was similar until about 2 weeks of postnatal life, but in adulthood the former were devoid of mature Leydig cells and showed thin seminiferous tubules with arrested spermatogenesis (Fig. 2). Testicular T concentration at birth was indistinguishable between WT and LuRKO testes. When the expression levels of several Leydig cell and steroidogenesis specific genes were followed, they initially showed similar level of expression in WT and LuRKO mice. But after puberty, most of them (such as cytochrome P 450 side chain cleavage, 17α-hydroxylase cytochrome P 450, 17β-hydroxysteroid dehydrogenase [HSD] III, steroidogenic acute regulatory protein [StAR], insulin like factor 3) were expressed at very low level in LuRKO testes in accordance with the lack of

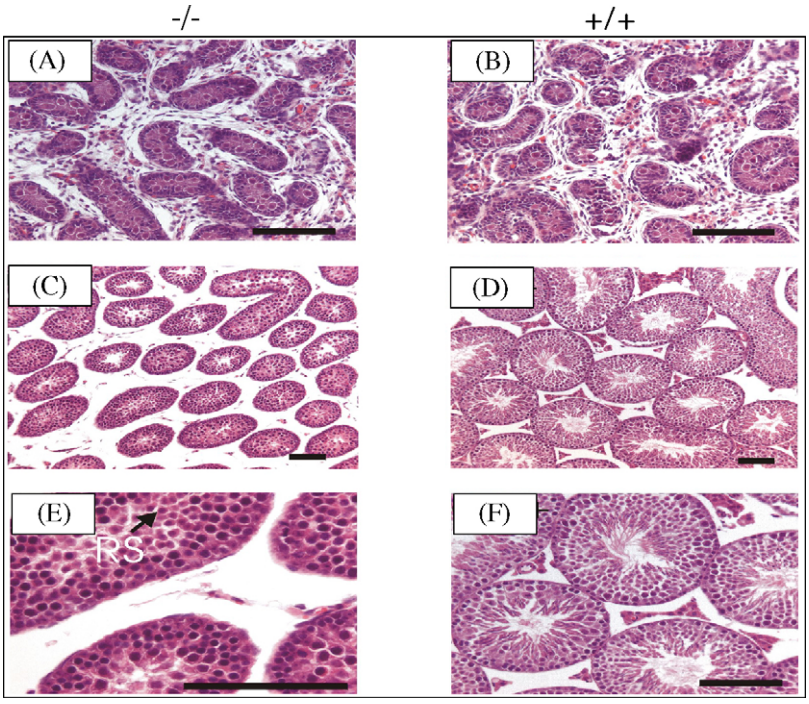

Fig. 2. Testicular histology of a 1-day-old LuRKO mouse (−/−) and a WT littermate (+/+) in panels A and C, of a 45-day old (−/−) and (+/+) mouse in panels B and D, and the same samples at higher magnification in panels E and F. Arrow indicates round spermatid (RS). The bar in the panels is 100 μm. Modified from Zhang et al. (2001a).

growth and maturation of the adult Leydig cell population. The only steroidogenic gene that made an exception was *3*β-HSD I, which was equally highly expressed in adult WT and LuRKO testes. The reason for the constant level of this enzyme may be that it is expressed already in mesenchymal and peritubular Leydig cell precursors (Haider and Servos, 1998). Hence, these studies confirmed the previous knowledge that fetal Leydig cell steroidogenesis, although responsive to LH stimulation, is not dependent on this action (Huhtaniemi, 1994). Full steroidogenesis of adult Leydig cells, in contrast, is not possible without sufficient gonadotrophin support.

Because the male LuRKO mice are hypogonadal after birth due to the lack of adult Leydig cell differentiation, this model made it possible to study to what extent T treatment alone, when started before puberty, is able to restore the male phenotype (Pakarainen et al., 2005a). High-dose T replacement therapy was initiated in LuRKO mice at the age of weaning (21 days) and continued until the age of 60 or 90 days. The treatment induced in the LuRKO mice a male phenotype that was indistinguishable from WT controls, including testicular descent and full spermatogenesis. Hence, T priming before the age of 21 days is not necessary in male LuRKO mice for their subsequent normal sexual maturation. The mice have normal T levels at birth, but become gradually hypoandrogenic after 10 days of age, when the fetal Leydig cell population, active after birth, cannot be replaced by steroidogenically active adult Leydig cells (Zhang et al., 2004). Conspicuously, some abnormalities including vigorous inflammation were found in the epididymides and accessory sex glands of the T treated LuRKO mice. Interestingly, the fertility of these mice was found to be reduced despite apparently normal spermatogenesis and normal sexual behaviour. The mice were found to have low ejaculatory frequency and low sperm counts in cauda epididymidis. In conclusion, the T treated mice remained subfertile due to combined effect of the above abnormalities in sperm production, transport and deposition. The T dose needed to induce the onset of spermatogenesis had to be so high that it increased peripheral T concentration about 10-fold above normal, which could be responsible, though by unknown mechanism, for the accessory sex gland inflammation. Whether it was responsible for the other abnormalities, or whether they were due to the lack of proper T priming of the prepubertal mice before the onset of T treatment at 21 days of age is not readily apparent.

In another study we addressed in detail the question on how far the spermatogenesis can advance in mice in the absence of LH stimulation, but in the presence of normal FSH level (Zhang et al., 2003). FSHβ and FSHR KO mice have shown previously that, despite somewhat suppressed testis weight and spermatogenesis, LH/T action is sufficient to maintain spermatogenesis at a level sufficient to maintain normal fertility (Kumar et al., 1997; Dierich et al., 1998; Abel et al., 2000). As young adults the spermatogenesis of LuRKO mice was arrested at the round spermatid stage (Zhang et al., 2001a), in keeping with previous information about the progression of spermatogenesis in the absence of proper androgen production (Plant and Marshall, 2001). However, if we followed the LuRKO males until the age of 12 months, qualitatively full spermatogenesis was observed in their testes with appearance of elongated spermatids of late stages 13–16. Quite unexpectedly, this took place at intratesticular T levels that were only 2% of those in WT controls. However, the mice

remained cryptorchid and their epididymides and accessory sex glands were severely underdeveloped, which would have prevented their normal fertility. When the mice were treated with the antiandrogen flutamide, to block the action of the remaining low intratesticular T levels, spermatogenesis stopped at the round spermatid stage, indicating that the very low constitutively produced T level was sufficient to advance postmeiotic spermatogenesis. The normal to slightly elevated FSH levels of the LuRKO mice apparently stimulated spermatognensis to the round spermatid stage, as has been observed in gondotrophin-deficient mice upon FSH supplementation (Allan et al., 2001).

The finding that qualitatively complete spermatogenesis is possible in the absence of the LH-stimulated high intratesticular T production contradicts the current dogma. If extrapolated to humans it may offer the explanation why all men do not reach azoospermia when treated in male contraceptive trials with T to suppress gonadotrophin secretion (Kamischke and Nieschlag, 2004). It may also be that in those men not reaching azoospermia, there is a gonadotrophin-independent component of androgen production that is able to maintain spermatogenesis. Therefore, in order to develop an effective hormonal contraceptive method for man, we should be able to achieve total ablation of intratesticular T levels. How this can be achieved by simultaneously maintaining normal peripheral androgen levels, to prevent the loss of libido and potency, poses a real challenge.

2.3. Further characteristics of female LuRKO mice

The LuRKO females show follicular maturation only until the antral, but never to the preovulatory stage, and they never ovulate (Fig. 3) (Lei et al., 2001; Zhang et al., 2001a). This indicates that besides the known mandatory role of LH for ovulation this hormone is also needed for the final follicular maturation before ovulation. There are earlier studies on hypophysectomised rats and mice indicating that ovulation can be induced with recombinant FSH without any LH activity. Besides the regulation of follicular maturation, the studies indicated a role for FSH in ovulation and luteinisation, and showed that ovulation is possible without the LH surge (Galway et al., 1990; Tapanainen et al., 1993; Wang and Greenwald, 1993). Although these findings are quite convincing, it still remains uncertain whether the FSH stimulated ovulation is mediated purely via FSHR activation, or whether some level of LHR stimulation is also necessary. It is possible that the permissive effect of a low degree of constitutive LHR activation is needed for the unexpected FSH stimulated ovulation. Furthermore, evidence for the role of the epidermal growth factor (EGF) family members, amphiregulin, epiregulin and betacellulin, as mediators of the LH action in follicle maturation, has been recently shown (Park et al., 2004). These proteins have been demonstrated to mediate the LH action by acting as paracrine mediators between mural granulosa and cumulus cells, and to be necessary for the formation of cumulus–oocyte complexes and oocyte maturation.

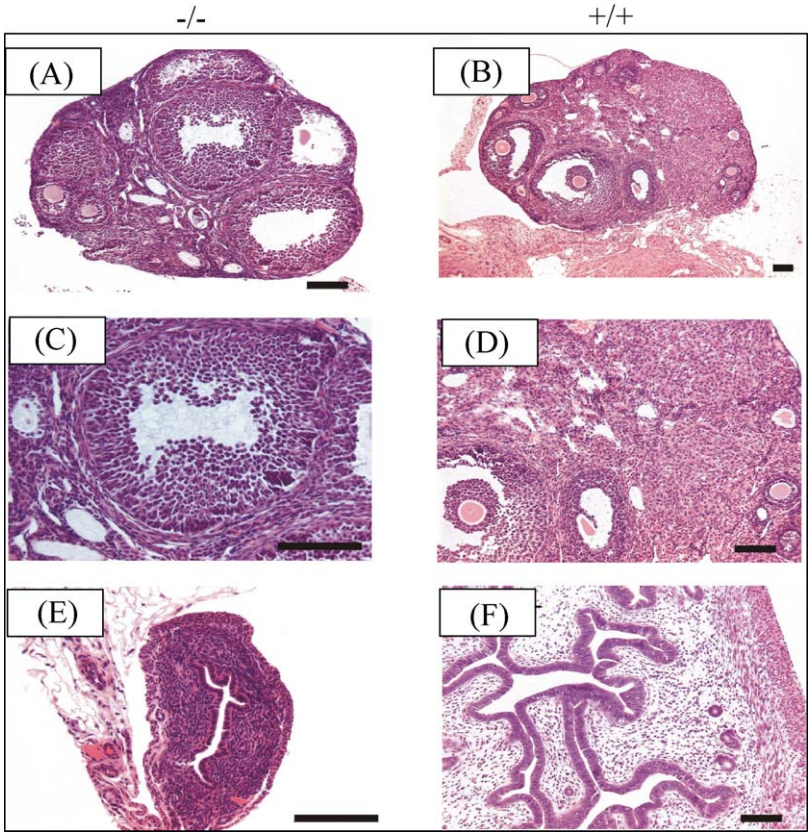

Fig. 3. Ovarian histology of a 45-day-old LuRKO mouse (−/−) and a WT littermate (+/+) in panels A and B, and at higher magnification in panels C and D. Panels E and F show uterine histology in (−/−) and (+/+) mouse. The bar in the panels is 100 μm. Modified from Zhang et al. (2001a).

Hence, even the LH action on oocyte maturation appears to be indirect.

To revisit the question of FSH stimulated ovulation, we assessed in LuRKO mice the progression of folliculogenesis and induction of ovulation by hCG and recombinant human FSH treatments (Pakarainen et al., 2005b). As expected, hCG had no effect in LuRKO mice, and the results provided clear evidence that follicular development and ovulation could not be induced by high doses of FSH in the absence of functional LHR.

Ovarian histology indicated that follicular maturation did not advance in LuRKO mice beyond the antral follicle stage, whether or not they were treated with FSH. Neither were ovulations detected in the LuRKO ovaries after any of the gonadotrophin treatments. The ovarian resistance to FSH treatment in the absence of LHR was confirmed by real-time RT-PCR and immunohistochemical analyses of a number of gonadotrophin-dependent genes, which only responded to the treatments in WT mice. Neither did oestradiol priming preceding the gonadotrophin stimulations alter the negative outcome. Hence, this study showed that LHR expression is essential, besides the ovulation, also for final follicular maturation from the antral to the preovulatory stage. The discrepancy between the findings in hypophysectomised and LuRKO mice in their FSH responsiveness is puzzling and not readily explainable. A very recent finding by (Urizar et al., 2005) offers an intriguing explanation. These authors found that FSH and LH receptors when expressed in the same cell may form heterodimers. Because luteinising granulosa cells do express both receptors in situ before ovulation, there is a possibility that such heterodimers could form during the preovulatory period, have a physiological function, and explain why FSH stimulation can induce ovulation in hypophysectomised but not in LuRKO mice. Gondotrophin receptor expression still persists in gonads after hypophysectomy, and ligand-bound FSHR could form functional dimers with non-liganded LHR during FSH treatment, but this is not possible in LuRKO ovaries. It will be intriguing to carry out a similar experiment with FSH treatment in LHβ KO mice (Ma et al., 2004).

Quite unexpectedly, a number of studies have recently reported that LH/hCG receptors are expressed in a variety of extragonadal tissues, including the uterus, oviduct, cervix, placenta, mammary gland, sperm, certain areas of the brain, and many others (Rao, 2001; Shemesh, 2001; Zhang et al., 2001b; Fields and Shemesh, 2004). Many of these findings were made on human tissues, but similar observations have been reported on a number of other mammalian species, including baboons, bovines, pigs, rats, and mice (Ziecik et al., 1986; Fazleabas et al., 1999; Shemesh et al., 2001; Zhang et al., 2001b; Apaja et al., 2004). On the basis of these observations, a shift in the old paradigm that gonadotrophins only have gonadal actions has been proposed (Rao, 2001). A number of extragonadal LH/hCG actions have been suggested, including effects on brain development and sexual behaviour, regulation of uterine blood flow, survival of early pregnancy, decidualisation, and regulation of uterine growth and enzyme levels. However, although many of the effects appear at face value convincing, there are several caveats to be considered: (1) often only fragments of the LHR mRNA have been identified using RT-PCR, without evidence for full-length message, (2) the size of immunoreactive LHR protein detected by immunoblotting has not always agreed with the genuine gonadal receptor, and often heterologous antibodies have been used, (3) most of the data have been obtained in vitro, and there are no compelling in vivo data, (4) human inactivating mutations of LHR have only gonadal phenotype, and the extragonadal effects can be explained by gonadal dysfunction, and (5) all extragonadal phenotypes so far observed in LuRKO or LH/hCG overexpressing mice can be explained by alterations in gonadal function.

With the above doubts in mind, we set out to assess the effect of ovarian transplantation on mouse reproductive maturation, fertility, pregnancy and lactation (Pakarainen et al., 2005c). Prepubertal (23 day old) LuRKO females were orthotopically replaced with pieces of WT ovary, using similarly transplanted WT mice as controls (Fig. 4). Most ovarian transplants attained normal endocrine function in both groups of mice, as demonstrated by normal age at vaginal opening, estrous cycle, and sexual behaviour. Both the LuRKO and WT control mice became repeatedly pregnant (9/16 versus 16/20 after first mating; difference not significant) and delivered litters of similar size, which grew normally after birth, as a sign of normal lactation. In conclusion, fertility was fully restored in LuRKO mice by transplantation of WT ovarian tissue, the only site of functional LHR in these animals, indicating that extragonadal LH/hCG action is not necessary for normal sexual maturation, sexual behaviour, maintenance of pregnancy, delivery or lactation in mice. Thus the findings indicate physiological redundancy for these receptor sites at least in the mouse. The group of Rao (Chudgar et al., 2005), using the other LHR KO mouse model, were unable to achieve pregnancies in their mice after similar orthotopic transplantations of WT ovaries. The reason for this discrepancy remains open, but it may be technical.

Novel information is continuously mounting about extragonadal effects of gonadotrophins (Filicori et al., 2005). Our finding that LuRKO mice with orthotopically transplanted WT ovarian tissue gain normal fertility speaks against the physiological importance of such effects. We have also observed that the effects on bone that we found in LuRKO and hCG+ mice, were totally abolished when the mice were gonadectomised (Yarram et al., 2003). However, the findings of extragonadal LH/hCG receptor are apparently genuine, and several effects, even though mostly observed in vitro, speak for such a possibility. Further information is needed before this conundrum can be solved.

3. hCG overproducing TG mouse (hCG+)

3.1. General features

Higher than normal gonadotrophin secretion is usually due to primary gonadal failure. In addition, there are pituitary gonadotroph cell tumours, but they are usually endocrinologically silent (Losa et al., 2001). Ectopic gonadotrophin production, especially of hCG, occurs in numerous non-trophoblastic tumours. Other pathological conditions with inadvertently high gonadotrophin secretion include central precocious puberty

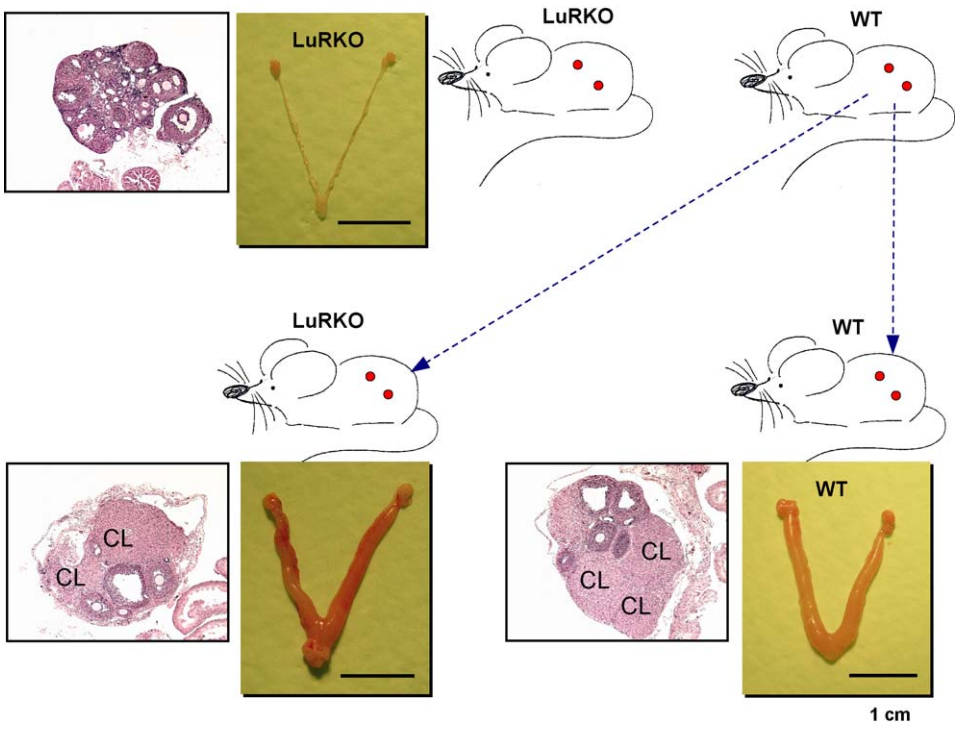

Fig. 4. The strategy of orthotopic ovarian transplantations between WT and LuRKO mice (at 23 days of age), and macroscopic and microscopic appearance of the ovaries and uteri 12 weeks after the operation. Please note similar presence of corpora lutea (CL) in the ovaries transplanted to WT and LuRKO mice, and absence of these structures in ovaries of the intact LuRKO mice. Modified from Pakarainen et al. (2005c).

and polycystic ovarian syndrome (high LH). Finally, the postmenopausal state represents a condition with persistently high levels of gonadotrophin secretion. A number of genetically modified mouse models with chronically elevated gonadotrophin levels have been recently developed (Rulli and Huhtaniemi, 2005), and they provide relevant models to study the pathophysiology and molecular mechanisms involved in similar human conditions.

The first mouse model with elevated LH/hCG action was the one expressing the bovine LHβ subunit fused in frame with the 24 amino acid C terminal peptide (CTP) of hCGβ, and driven under regulation of the pituitary-specific bovine Cα promoter (Risma et al., 1995). Expression of the transgene was thus targeted to pituitary gonadotroph cells, and when coupled with endogenous Cα, the transgene expression increased bioactive LH levels in circulation in female mice 5–10-fold. However, because of poor activity of the Cα promoter in the male, no elevation in LH bioactivity was found in male mice.

In order to achieve higher LH/hCG levels in both sexes, we produced recently a mouse model expressing high physiological–pharmacological levels of hCG. This model was also expected to unravel pathological phenotypes that would not be prominent in models with less striking elevation of hormone action (Rulli et al., 2002; Rulli et al., 2003; Ahtiainen et al., 2005). We first produced a TG mouse expressing the hCGβ subunit under the human ubiquitin promoter (hCGβ+). In both sexes, the circulating LH/hCG bioactivity was moderately elevated through association of the TG hCGβ protein with the endogenously expressed Cα subunit in the pituitary gland. LH/hCG bioactivity was increased about 30-fold in female animals (Rulli et al., 2002), but only 3–4-fold in males (Rulli et al., 2003). In this model, the availability of endogenous Cα apparently becomes rate-limiting when the TG hCGβ subunit is produced in excess. Consequently, the sexual dimorphism observed in the level of bioactive hCG would be attributed to a differential sex steroid feedback regulation of Cα expression, as was also observed with the bLHβ-CTP mouse (Risma et al., 1995). To achieve higher hCG levels in both sexes, we subsequently produced a Cα expressing TG mouse, using the same ubiquitin promoter (Rulli et al., 2003). Now most of the tissues expressed both transgenes, and when they were dimerised, the consequence was about 1000-fold elevation of serum LH/hCG bioactivity in both sexes.

3.2. Male phenotype of hCG+ mice

The hCGβ+ males were fertile and showed only a mild phenotype, in agreement with their moderate increase in bioactive hCG. These mice had smaller testes in the face of full spermatogenesis and normal sperm quality, thus failing to demonstrate an adverse effect on male fertility (Rulli et al., 2003). As expected, the Cα overproducing mouse has no phenotype in either sex, in keeping with the necessity of α/β dimerisation for the biological activity of hCG. In contrast, the double-TG α/β expressing males (hCG+) were infertile and their reproductive organs showed severe alterations with smaller testes, enlarged seminal vesicles and prostate, dilated vasa deferentia and urinary bladder, as well as kidney defects in adulthood (Rulli et al., 2003). Testicular steroidogenesis was enhanced despite a clear down-regulation of LH/hCG receptor expression.

Functional obstruction of vas deferens due to overproduction of secretory fluids or impaired emptying of the glands appears to be the main cause for the infertility observed in these animals. Progressive degeneration of seminiferous tubules and epididymides was associated with the obstruction. These changes may be due to the hormonal imbalance, in accordance with the male phenotype of oestrogen receptor-α (KO) mice with elevated androgen levels (Eddy et al., 1996).

As in previous studies on long-term LH/hCG treatments in vivo (Risbridger et al., 1982; Gaytan et al., 1994), these mice developed focal Leydig cell hyperplasia/hypertrophy, but failed to present with testicular tumours. This was intriguing, since in humans, a specific activating mutation of the LHR ($Asp^{578}His$) is associated with Leydig cell adenomas (Liu et al., 1999; Richter-Unruh et al., 2002). Interestingly, we subsequently found in postnatal hCG+ males clear Leydig cell adenomas of fetal Leydig cell origin, but these tumours disappeared at puberty (Ahtiainen et al., 2005).

3.3. Female phenotype of hCG+ mice

The female phenotypes of the hCGβ+ and hCGα/β+ mice were rather similar, presenting with precocious puberty, disrupted oestrous cycles and infertility due to ovarian and uterine pathologies (Rulli et al., 2002). The ovaries presented with occasional hemorrhagic cysts and massive luteinisation, resembling luteomas (Fig. 5). Cells of the latter structures were filled with lipid droplets indicating active steroid synthesis as a consequence of LH/hCG hyperstimulation. Accordingly, transiently high levels of oestradiol, and persistent elevation of T and progesterone were observed in the hCGβ+ ovaries. Strong luteinization apparently was the reason why oestrogen production was high only at the time of puberty.

Another interesting phenotype of this model was the development of pituitary adenomas and mammary gland tumours in older age (Rulli et al., 2002). The pituitary tumours turned out to be macroprolactinomas, probably due to overexposure of the mice to oestrogens during peripuberty, followed by persistently elevated levels of androgens, and possibly progesterone, as a source of locally produced oestrogens (Carretero et al., 1999). Due to its luteotrophic properties (Freeman et al., 2000), high prolactin (PRL) levels secreted by the pituitary tumours may augment the maintenance of ovarian luteinisation and progesterone overproduction. In addition, the role of PRL in mammary gland development and tumourigenesis in mice has been established (Wennbo and Tornell, 2000). Consequently, the indirectly orchestrated high hCG production, along with increased oestrogen, progesterone and PRL levels, thus brought about mammary gland proliferation and differentiation in hCGβ+ mice. The response originally resembled lactating state of the gland, but was subsequently transformed into metastatic adenocarcinomas in older age. Due to its complex and multistep hormonal dysregulation, this is a good animal model to understand the hormone-dependent pathogenesis of the pituitary and mammary gland. Conspicuously, the extragonadal tumours of the hCGβ+ mouse tumours were strictly dependent of ovarian function, since ovariectomy prevented both responses despite persistently high levels of hCG.

The phenotype of the hCG+ females, despite much higher LH/hCG level that in hCGβ+ females, was rather similar. Apparently the 30-fold elevation of LH/hCG bioactivity of the single-TG mice already induced near-maximal effect. The only clearly novel phenotype we observed in the double-TG hCG+ mice, with 1000–2000-fold elevation of LH/hCG levels, were ovarian teratomas (Rulli et al., Unpublished). We are currently characterising this phenotype in further detail.

More recently, another transgenic model for hCG was reported where either one or both subunits of hCG were overexpressed using a mouse metallothionein promoter (Matzuk et al., 2003). In this model, hCGβ overexpressing females were

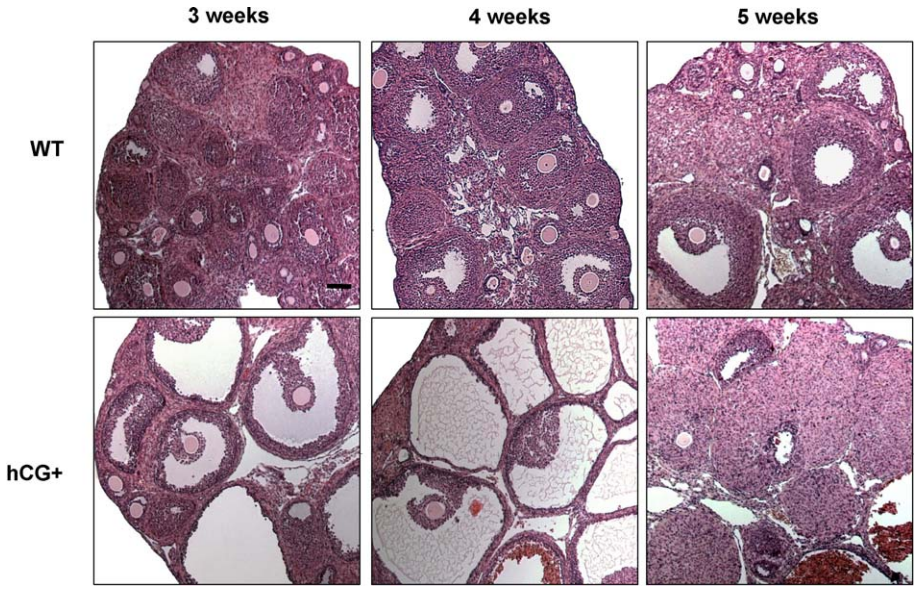

Fig. 5. Histological appearance of littermate WT and hCG+ ovaries at the age of 3, 4 and 5 weeks. The bar in the 3 week/WT panel is 100 μm, and magnitude of enlargement is the same in each panel.

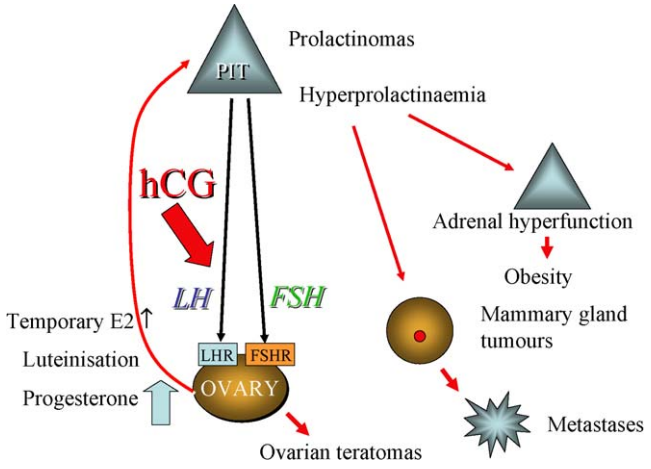

Fig. 6. Schematic presentation of the multiple tumourigenic effects induced by chronically elevated hCG production in the female TG hCGβ+ mice.

infertile and progressively developed cystic ovaries, whereas males were infertile despite no discernible phenotype. In contrast, the TG male mice co-expressing Cα- and hCGβ-subunits showed multiple reproductive defects resembling those found in our previous model, such as infertility, Leydig cell hyperplasia, increased testosterone, reduced testis size and enlarged seminal vesicles. Double-TG females were infertile, had elevated oestradiol levels, developed cystic ovaries with thecal layer enlargement and stromal cell proliferation and degenerating kidneys (Matzuk et al., 2003). No evidences for tumourigenesis in gonadal or extragonadal tissue was reported in this model. A TG mouse model expressing a constitutively active yoked hormone–receptor complex was recently generated, where hCG was covalently linked to the N-terminus of rat LHR in a fusion protein (Meehan et al., 2005). Males exhibited prepubertal increases in T levels and seminal vesicle weights, and decreases in serum FSH, LH, testes weight, and the size of the seminiferous tubules. Females presented with precocious sexual development and progressive ovarian lesions, from enhanced follicular development to degenerating follicles and hemorrhagic cysts.

Taken together, these studies indicate that chronically elevated hCG leads to multiple gonadal and extra-gonadal defects in males and females, including ovarian and testicular tumours as a primary effect, whereas the effects found in the pituitary and mammary glands in females are due to secondary effects of the aberrant gonadal function (Fig. 6). The distinct phenotypes emerging from the existing mouse models overexpressing hCG/LH in vivo may be related to the level of hCG/LH production, age, characteristics of the transgene expression, and the genetic background. However, the phenotypic similarities among the different models further emphasize the role of gonadotrophin action on the reproductive physiopathology.

4. Conclusions and future perspectives

The inactivation and overstimulation of LH action in genetically modified mice have brought lots of new information about functions of this gonadotrophin. Besides the expected phenotypes in line with existing knowledge about the functions of LH, also novel phenotypic features were found. The most intriguing of these are the spermatogenic potential of the testis in the absence of LH stimulation and high intratesticular T, the apparent redundancy of extragonadal LH/hCG action, and the tumourigenic potential of persistently elevated LH/hCG levels. The relevance of these findings to the human belong to the future challenges of gonadotrophin research.

It appears that seemingly similar genetic modifications do not always bring about identical phenotypes. One example is the differences in phenotypes and responses to various treatments between the two existing LHR KO models (Lei et al., 2001; Zhang et al., 2001a). The genetic background of the mice used is critical and can explain the differences. It is therefore important that multiple models of apparently similar genetic manipulations are investigated, to single out the effects of the genetic manipulation and genetic background.

The rather robust TG and KO models currently available seldom simulate pathologies of human genetic aberrations of gonadotrophin action, which are usually more subtle due to point mutations in gonadotrophin or gonadotrophin receptor genes. Besides simple constitutive inactivation or activation of gonadotrophin action, the human mutations often bring about qualitative alterations in hormone action. Examples of such situations are the tumourigenic effect of the particular constitutively activating LHR mutation, Asp^{578}His (Liu et al., 1999; Richter-Unruh et al., 2002), and the recently discovered FSHR mutations that render the receptor responsive to hCG, explaining the molecular pathogenesis of pregnancy-associated ovarian hyperstimulation syndrome (Delbaere et al., 2005). Animal knock-in models for human point mutations are therefore an apparent next step in refining our knowledge about the structure–function relationships of gonadotrophin action.

In conclusion, the genetically modified mouse models, including our LuRKO and hCG+ mice, have clarified the classical concepts of the physiology and pathophysiology of gonadotrophin action. They have also shed light on novel and controversial topics of this field. Lots of work, including improved models simulating more closely human genetic diseases, is still needed before a complete picture about the multitude of gonadotrophin actions, their role in various diseases and the therapeutic potentials can be reached.

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