0013-7227/96/$03.00/0
Endocrinology

Vol. 137, No. 10
Printed in U.S.A.

Gonadotropins Are Essential Modifier Factors for Gonadal Tumor Development in Inhibin-Deficient Mice*

T. RAJENDRA KUMAR, YAN WANG, AND MARTIN M. MATZUK

Departments of Pathology (T.R.K., Y.W., M.M.M.), Cell Biology (M.M.M.), and Molecular and Human Genetics (M.M.M.), Baylor College of Medicine, Houston, Texas 77030

ABSTRACT

We previously demonstrated that mice deficient in inhibin develop gonadal sex cord-stromal tumors with nearly 100% penetrance. These ovarian and testicular tumors develop as early as 4 weeks of age and eventually cause cachexia-like symptoms and death in the inhibin-deficient mice. Gonadectomized inhibin-deficient mice initially do not develop this wasting syndrome, but eventually will develop adrenal cortical tumors with similar penetrance. These studies have demonstrated that inhibin is a secreted type of tumor suppressor in the gonads and adrenal glands. Gonadotropins are implicated to influence gonadal tumor development in humans as well as experimental animals, and in inhibin-deficient mice, serum FSH levels are elevated. To determine whether gonadotropins influence the development and/or progression of the tumors in the inhibin-deficient mice, we took advantage of a naturally occurring mutant mouse, hypogonadal (*hpg*); *hpg/hpg* mice lack a functional GnRH gene and, therefore, have suppressed FSH and LH levels. Heterozygous *hpg/+* mice were crossed to heterozygous inhibin mutant mice to generate compound homozygous mutant mice that lack both inhibin and GnRH. These compound homozygous mutant mice do not develop a wasting syndrome, do not exhibit gonadal or adrenal tumors, and can survive for more than 1 yr. These results demonstrate that gonadotropins are essential modifier factors for tumor development in inhibin-deficient mice. (*Endocrinology* **137:** 4210–4216, 1996)

GONADAL GROWTH and differentiation are complex processes mediated by interactions between several regulatory molecules. Alterations within this network may lead to aberrant growth and differentiation of the gonads, including the development of testicular or ovarian tumors. The development of human gonadal cancers often results in secondary infertility (1, 2). Compared to prostate cancer, testicular cancers result in fewer deaths in males (1, 2). In contrast, ovarian cancer is the major cause of death among women affected with gynecological cancers (1, 2). These common testicular and ovarian tumors are classified as germ cell and epithelial cell tumors, respectively. In contrast, the incidence of sex cord-stromal tumors is less frequent. Identification of the molecular and genetic basis of ovarian and testicular tumor development and the rational chemotherapeutic approaches to prevent and/or treat different types of gonadal cancers have been limited due to the lack of appropriate model systems.

Inhibins are members of the transforming growth factor-β superfamily that includes proteins with diverse functions such as activins, Mullerian inhibiting substance (MIS), and bone morphogenetic proteins. Inhibins were originally identified as gonadal peptides based on their ability to suppress pituitary FSH synthesis and secretion (3). Inhibins were later found to be expressed in diverse tissues and cell types (3). The major sites of inhibin production in the gonads are the Sertoli cells in testis and the granulosa cells in ovary. To study gonadal growth and differentiation, we previously generated an animal model in which mice deficient in inhibin develop multiple sex cord-stromal tumors (*i.e.* granulosa and/or Sertoli cell tumors) with nearly 100% penetrance (4). Male mice deficient in inhibin develop multifocal, hemorrhagic, bilateral testicular tumors as early as 4 weeks. Similarly, female mice deficient in inhibin develop multifocal, hemorrhagic ovarian tumors as early as 5 weeks. Histological analysis indicated the presence of incompletely differentiated or mixed cell populations of both granulosa and Sertoli cells in the gonads of both sexes. As the tumors progress in males, Leydig cells also decrease in number (5, 6). In the gonads of inhibin-deficient male and female mice, the germ cells are initially unaffected. Inhibin-deficient male and female mice that develop gonadal tumors eventually die of a severe wasting syndrome. Interestingly, gonadectomized, inhibin-deficient male and female mice live longer, eventually develop adrenal tumors, develop a nearly identical wasting syndrome, and eventually die (7). These studies have identified inhibin as a novel secreted tumor suppressor protein with specificity for the gonads and adrenal glands. It is not known, however, whether mutations in the inhibin α-subunit gene in humans can cause similar gonadal sex cord-stromal tumors.

LH and FSH are heterodimeric glycoprotein hormones synthesized and secreted by pituitary gonadotropes (8). Expression of the gonadotropin common α-subunit and the hormone-specific β-subunits is regulated by the hypothalamic peptide, GnRH; steroids; and the gonadal peptides, inhibin and activin (9). Both LH and FSH bind to distinct receptors in the gonads and regulate several aspects of gonadal growth, differentiation, and steroidogenesis (8). Several studies suggest that the gonadotropins play important roles as tropic factors for gonadal tumor development. Gonadotropins are important cell survival factors in the testis of

Received May 2, 1996.
Address all correspondence and requests for reprints to: Dr. Martin M. Matzuk, Department of Pathology, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030. E-mail: mmatzuk@bcm.tmc.edu.
* This work was supported in part by NIH Grant CA-60651 (to M.M.M.).

male rats (10), and targeted overexpression of a LH analog in transgenic mice leads to ovarian tumors (11). In elderly women, elevated FSH levels are associated with the development of ovarian cancer (12). Serum FSH levels are also elevated in inhibin-deficient mice, consistent with its known role to suppress FSH synthesis (4). In a series of experiments, Beamer and colleagues have successfully used the SWR and SWXJ recombinant inbred strains of mice to study ovarian cancer at a genetic level. These mice develop heritable, pubertal-onset granulosa cell tumors (13, 14). As in the inhibin-deficient mice, the early onset and histological characteristics of these tumors are similar to human juvenile granulosa cell tumors (13, 14). Beamer and colleagues have also shown that ovarian androgens synthesized in response to normal gonadotropin stimulation may be required for granulosa cell tumorigenesis (14).

In the present study, we have generated compound homozygous mutant mice that lack both inhibin-α and GnRH. The hypogonadal (*hpg/hpg*) mouse has a deletion in the GnRH gene leading to suppressed LH and FSH levels and complete infertility in males and females (15). As *hpg/hpg* mice have suppressed levels of FSH and LH, compound homozygous mutant mice obtained by this selected genetic cross allowed us to determine whether endogenous gonadotropins influence the development and/or progression of gonadal tumors in inhibin-deficient mice.

Materials and Methods

Generation of mice

Generation of inhibin heterozygote (*inha*^{m1}/+) and homozygote (*inha*^{m1}/*inha*^{m1}) mice was described previously (4). Male mice heterozygous for the hypogonadal mutation (*hpg*) at the GnRH locus were obtained from the Jackson Laboratories (Bar Harbor, ME). Compound heterozygous male and female mice were generated initially; subsequently, they were intercrossed to obtain *inha*^{m1}/*inha*^{m1}, *hpg/hpg* compound homozygous mutant mice. The compound homozygous mutants were obtained at an expected Mendelian frequency of 1:16. All animals were maintained and treated according to the NIH Guide for the Care and Use of Laboratory Animals.

Southern blot analysis

Southern blot analysis was performed on tail DNA samples using ^{32}P-labeled probes. The methods were previously described (4). The hypogonadal mutation was detected using a 300-bp rat GnRH complementary DNA probe as previously described (16).

Histological analysis

The compound homozygous mutant mice and control littermate mice were weighed weekly. Histological analysis was performed on the gonads of mice that were more than 6 months of age. Ovaries were carefully dissected under a microscope, fixed in 10% buffered formalin, subjected to dehydration in a graded series of ethanol, embedded in paraffin wax, and cut at 3 μm using a microtome. Testes were decapsulated and fixed in 10% buffered formalin or fixed in Bouin's solution overnight then placed in lithium carbonate-saturated 70% ethanol. Testes were processed, embedded, and sectioned as described above. The testis and ovary sections were stained with either hematoxylin and periodic acid-Schiff (PAS) reagent or hematoxylin and eosin using standard procedures. In each case, tissues from at least five mice were examined.

Results

inha^{m1}/inha^{m1}, hpg/hpg compound homozygous mutant mice fail to develop a wasting syndrome

The first overt sign of ovarian and testicular tumor development in inhibin-deficient mice was severe weight loss. Beginning at approximately 6–7 weeks of age, the majority of the male and female inhibin-deficient mice began to lose weight (Fig. 1) and eventually died (4). The inhibin-deficient

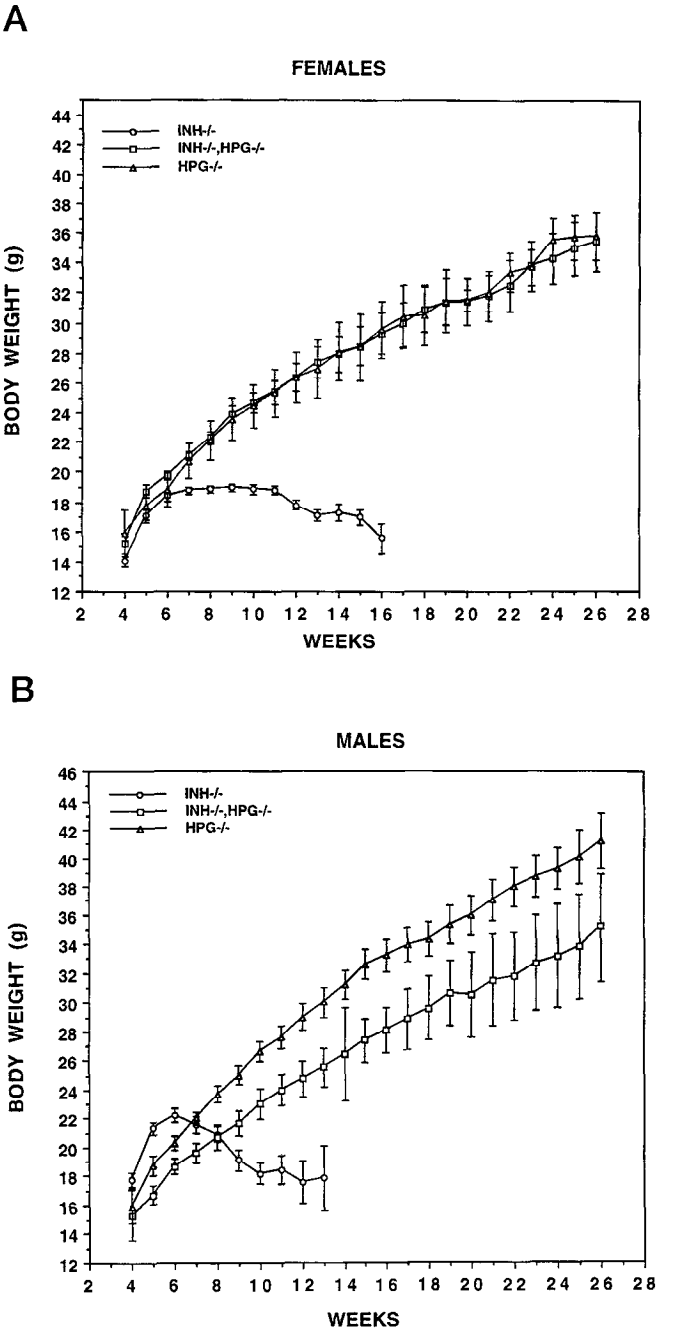

FIG. 1. Body weights (mean ± SEM) of *inha*^{m1}/*inha*^{m1}; *inha*^{m1}/*inha*^{m1}, *hpg/hpg*; and *hpg/hpg* female (A) and male (B) mice. Mice were weighed weekly. The following numbers of mice were used: *inha*^{m1}/*inha*^{m1}, 23 females and 40 males; *inha*^{m1}/*inha*^{m1}, *hpg/hpg*, 16 females and 10 males; and *hpg/hpg*, 11 females and 13 males.

mice developed a characteristic hunchback and sunken eye appearance. This cachexia-like or wasting syndrome, secondary to gonadal tumor development, allowed us to monitor tumor development in the compound homozygous mutant mice. In contrast to a deficiency of only inhibin, male and female mice deficient in both inhibin and GnRH (*inha*^{m1}/*inha*^{m1}, *hpg/hpg*) failed to display any of the overt wasting symptoms. The compound homozygous mutants continued to appear healthy even after 6 months, and their weights were comparable to those of *hpg/hpg* control mice (Fig. 1) or mice that are wild type at the inhibin locus and heterozygous at the GnRH locus (data not shown). Compound homozygous mutant mice caged for more than 1 yr failed to exhibit any symptoms of tumor development. As expected, mice that were inhibin deficient (*inha*^{m1}/*inha*^{m1}) but heterozygous mutant at the GnRH locus (+/hpg) developed gonadal sex cord-stromal tumors and continued to die of the characteristic wasting syndrome. The above results for the compound homozygous mutants indicate that LH and/or FSH might be influencing gonadal tumor onset and/or the progression of the tumors in the inhibin-deficient mice.

inha^{m1}/inha^{m1}, hpg/hpg compound homozygous mutant mice do not develop gross gonadal tumors

Essentially every inhibin-deficient (*inha*^{m1}/*inha*^{m1}) mouse more than 4 weeks of age demonstrated macroscopic or microscopic evidence of gonadal tumors (4). Detailed histological analysis identified these tumors as mixed or incompletely differentiated gonadal sex cord-stromal tumors. The testicular tumors were evident as early as 4 weeks of age in inha^{m1}/*inha*^{m1} males as small foci of nodular proliferation and appeared to be clonal. The tumor foci progressed very rapidly and became focally hemorrhagic and invasive. Analysis of the ovaries from 7- to 12-week-old inha^{m1}/*inha*^{m1} females demonstrated similar hemorrhage and disruption of the normal follicular architecture by the invasive tumors. These tumors often contained masses of granulosa cells, undifferentiated gonadal stromal derivatives, or clusters of mitotically active stromal cells reminiscent of seminiferous tubules (*i.e.* mixed granulosa/Sertoli cell tumors).

As the adult compound homozygous mutant mice (*inha*^{m1}/*inha*^{m1}, *hpg/hpg*) failed to demonstrate the characteristic cachexia-like symptoms (7) of the mice deficient in inhibin alone (*inha*^{m1}/*inha*^{m1}), we hypothesized that they also failed to develop gonadal tumors. To prove this hypothesis, the gonads from these compound homozygous mutant mice were examined grossly. The gonads of adult (>6 months of age) compound homozygous mutant mice were grossly small, similar to those of hypogonadal mice (*hpg/hpg*) as previously described (15). The ovaries from the compound mutant mice continued to be present in the bursal sac, embedded deep within the abdominal fat pads. The ovaries from compound homozygous mutant (as well as *hpg/hpg* ovaries) had to be dissected carefully from the bursal sacs using a dissection microscope. The ovaries from the compound mutants were small, lacked hemorrhage, and were essentially indistinguishable grossly from the ovaries of age-matched hypogonadal (*hpg/hpg*) control female mice. Histological analysis, however, revealed several differences between the ovaries of *hpg/hpg* and the *inha*^{m1}/*inha*^{m1}, *hpg/hpg* mice. Although the ovaries from *hpg/hpg* adult females were very small in contrast to wild-type ovaries (Fig. 2A), the *hpg/hpg* ovaries continued to demonstrate histologically normal follicular development through the secondary follicle stage (Fig. 2B). In contrast, ovaries from the *inha*^{m1}/*inha*^{m1}, *hpg/hpg* adult female mice showed relatively little follicular development beyond the primary follicle stage (Fig. 2C). Furthermore, there were seminiferous tubule-like structures observed within all of the compound homozygous mutant ovaries processed (Fig. 2D). Although reminiscent of seminiferous tubules in the testes, these tubule-like structures lacked germ cells. The nuclei of these cells contained very prominent nucleoli. These tubule-like structures were nearly identical to many of the malignant Sertoli-cell tumor lesions observed in the ovaries of mice deficient in inhibin alone (4) (Fig. 2, E and F). Histological analysis of ovaries from compound homozygous mutant mice showed similar findings at all ages examined (data not shown).

In contrast to those from the compound homozygous mutant females, testes from the *inha*^{m1}/*inha*^{m1}, *hpg/hpg* mutant male mice did not demonstrate any premalignant or malignant changes. The testes of the compound homozygous mutant mice were grossly small compared to the testes of wild-type males (Fig. 3A) and were similar in size to testes from *hpg/hpg* males (data not shown). The testes of the compound homozygous mutant mice were histologically similar to those of the *hpg/hpg* mice, except for the presence of PAS-positive secretions in the tubules of these mice (Fig. 3, C–F). Although spermatogenesis and Leydig cell hypoplasia were noted in the *inha*^{m1}/*inha*^{m1}, *hpg/hpg* testes, similar to those in *hpg/hpg* or *inha*^{m1}/*inha*^{m1} mice, there were no signs of granulosa cell tumors within the tubules of the compound homozygous mutants (Fig. 3, E and F). In addition, the adrenal glands in both sexes of compound mutant mice looked grossly normal, without any signs of tumor or hemorrhage (data not shown). Genotypes of all of the mice studied histologically were reconfirmed by Southern blot analysis at the time of death.

Discussion

We have previously generated inhibin-deficient mice using gene-targeting strategies in embryonic stem cells. Inhibin-deficient mice develop focally invasive gonadal sex cord-stromal tumors of granulosa or Sertoli cell origin and adrenal cortical tumors and eventually die due to a severe wasting syndrome. Thus, inhibin is identified as the first secreted tumor suppressor specific for the gonads and adrenals. Because the tumors initiate as foci and not all cells of the gonads develop to form a tumor, this suggests that another secondary event(s) is necessary for malignant growth. These may include altered regulation/mutation of several important growth factors, steroids, or tumor suppressor proteins such as p53 (Fig. 4). Identification of these modifier loci/factors is critical to understanding the complex process of gonadal sex cord-stromal tumor formation and progression.

To dissect out the individual components involved in the cascade of events that leads to the formation of gonadal tumors in inhibin-deficient mice, we have systematically be-

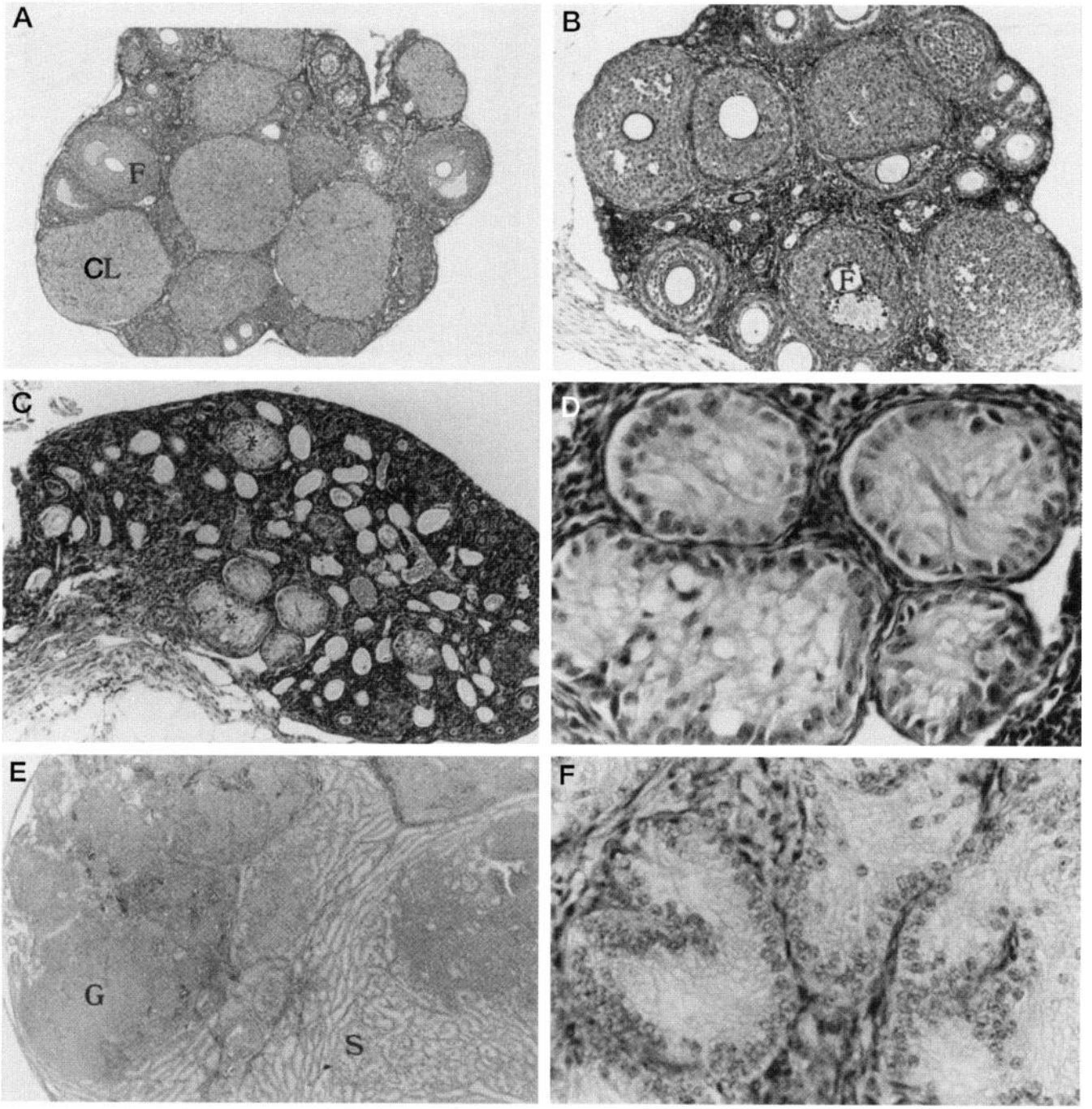

FIG. 2. Histological analysis of the ovaries of wild-type and mutant mice. A, Section through a wild-type adult ovary at 12 weeks of age at low power, showing normal corpora lutea (CL) and antral follicles (F). B, Section through an adult *hpg/hpg* ovary at ~12 weeks of age. Folliculogenesis is essentially normal up through the secondary follicle stage, and one antral follicle (F) is seen in this section. However, no mature (tertiary) follicles or corpora lutea are present. Magnification at low power (2.5-fold higher magnification than A). C and D, Sections through an adult $inha^{m1}/inha^{m1}$, *hpg/hpg* compound homozygous mutant ovary at ~30 weeks of age. Although some primary follicles with normal oocytes are apparent in this section, more advanced folliculogenesis is absent. Sertoli cell-like tubules are also present (indicated by an *asterisk*). The cells lining the tubules have prominent nucleoli, as seen at high power. C, Low power (identical magnification to B above). D, High power. E and F, Sections through a mixed granulosa/Sertoli cell tumor of an inhibin-deficient adult female at ~12 weeks of age. The granulosa (G) cell tumor-like component (undifferentiated component) is obvious as more densely cellular regions. The Sertoli-cell tubule(s) tumor component of this mixed neoplasm is shown at low power (E) and high power (F) and resembles the premalignant lesions seen in C and D above.

gun generating mice with multiple genetic lesions by selective cross-breeding. Using this approach, we have generated compound homozygous mutant mice that are deficient in both inhibin and GnRH. Using these mice as models, we have tested the hypothesis that the pituitary gonadotropin hormones LH and FSH are critical modifiers of gonadal sex cord-stromal tumor development and/or progression. Our hypothesis is based on the following observations. First, in-

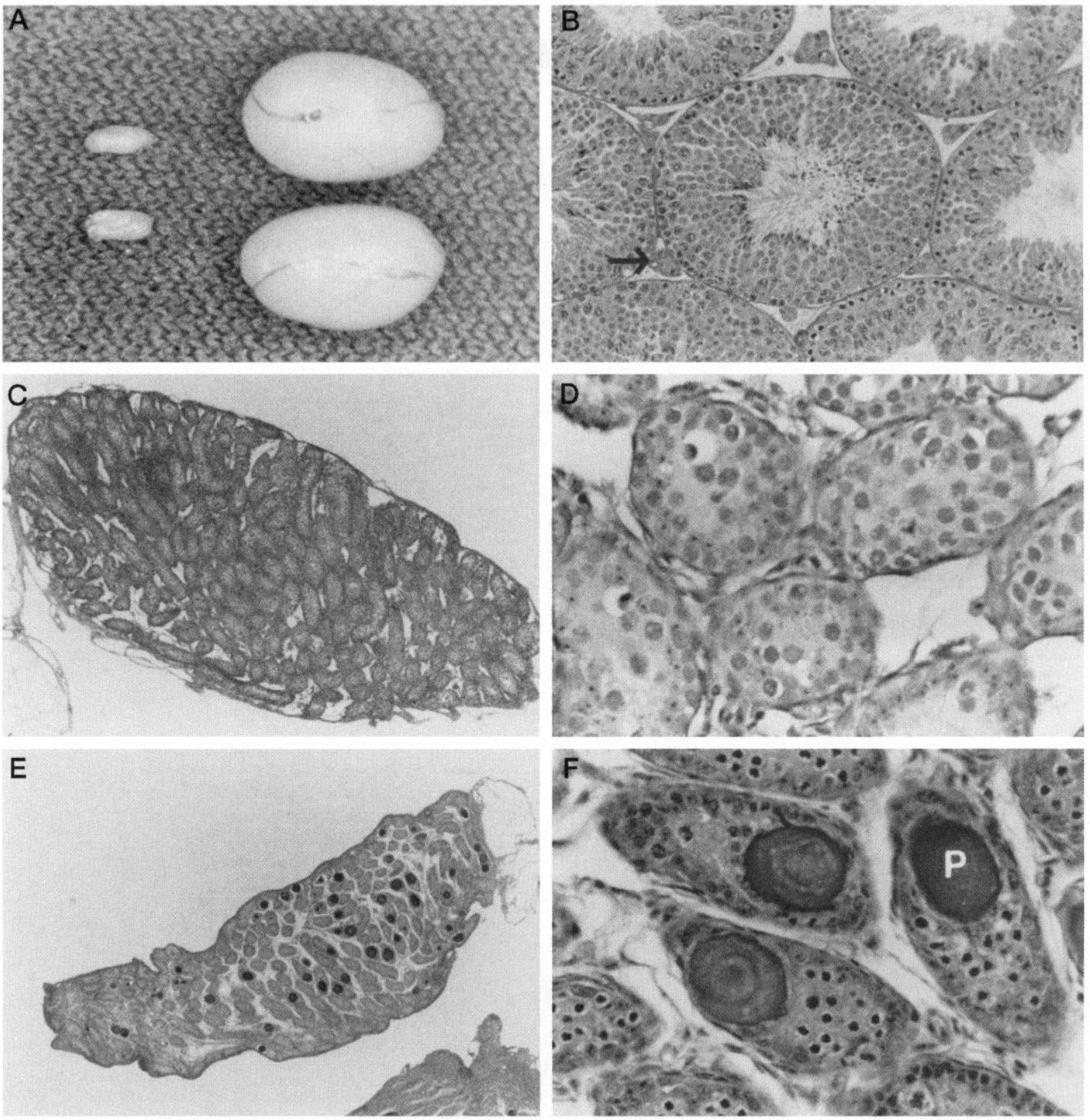

FIG. 3. Gross and histological analysis of the testes of wild-type and mutant mice. All sections were stained with PAS-hematoxylin. A, Gross analysis of the testes of wild-type (*left*) and $inha^{m1}/inha^{m1}$, *hpg/hpg* (*right*) adult male mice at 22 and 39 weeks of age, respectively. Note the lack of any gross hemorrhage or enlargement in the compound mutant testes consistent with the pathological analysis below. B, Sections through the testis of a wild-type adult male mouse taken at high power. Normal spermatogenesis is evident in the seminiferous tubule in the *center*. Leydig cell (*arrow*) islands are also apparent between the tubules. C and D, Sections through the testis of an *hpg/hpg* adult male mouse. C, Low power. D, High power. As described previously (13) and evident at high power, there is an arrest of spermatogenesis in the smaller diameter seminiferous tubules in this hypogonadal mutant. Leydig cells are not easily distinguished in the mutant. The magnification of D is 2-fold greater than that of B above. E and F, Sections through the testis of a 46-week-old $inha^{m1}/inha^{m1}$, *hpg/hpg* compound mutant mice. E, Low power. F, High power. The magnification of E is the same as that of C above. The magnification of F is the same as that of D above. Testis size in this compound homozygous mutant mouse is similar to that in the *hpg/hpg* mutant above. Note the presence of prominent PAS-positive material (P) in the lumen of many of the seminiferous tubules.

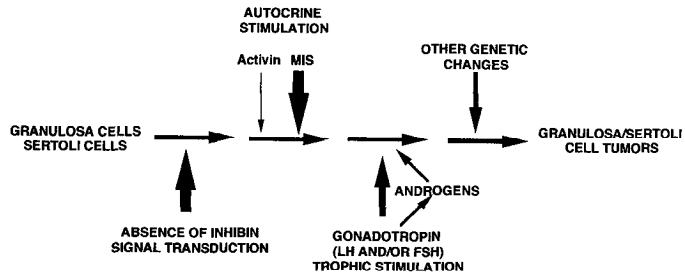

FIG. 4 A hypothetical model for granulosa/Sertoli cell tumorigenesis in inhibin-deficient mice. The primary event is the absence of inhibin or mutations in the inhibin signal transduction pathway. Several modifier factors, such as activins (positive effector) or MIS (negative effector), or yet unidentified genetic events may influence the malignant progression of the granulosa/Sertoli cells. In this manuscript, we demonstrate that the gonadotropins (FSH and/or LH) are key tropic factors that influence tumor progression/development.

hibin-deficient mice demonstrate elevated serum FSH levels (4); this elevated FSH may stimulate the development and/or progression of the tumors. Second, elderly postmenopausal women, who are most prone to develop ovarian epithelial cancer, exhibit increased LH and FSH levels in their serum (12). Third, targeted overexpression of a LH analog in transgenic mice leads to infertility, polycystic ovaries, and granulosa cell tumors (11). Fourth, several *in vitro* studies on ovarian epithelial cancer cell lines demonstrate increased binding of FSH and hCG, a LH analog, and stimulation of DNA synthesis and cell proliferation by these tumors (17, 18). Fifth, chronic treatment of male rats with hCG results in testicular (Leydig cell) tumors (19).

Our present study using a genetic approach proves that gonadotropins are important modifier factors for gonadal sex cord-stromal tumor development in inhibin-deficient mice. Although the incidence of sex cord-stromal tumors in humans is only 5–8% of all ovarian cancers, these studies suggest that perhaps suppression of gonadotropins in women with the most commonly occurring ovarian epithelial cancers may also influence tumor growth (see below). For example, Wimalasena *et al.* (20) showed that hCG and FSH regulate ovarian epithelial cancer cell growth *in vitro*. This suggests that gonadotropins may be critical modulators of the development and/or progression of multiple types of gonadal cell tumors, although these hormones might be acting through different independent pathways depending on the cell type.

Why do the ovaries of compound homozygous mutant female mice fail to demonstrate a progression beyond the premalignant stage (*i.e.* the initiation of development of tubules) in the absence of gonadotropins? Several possibilities exist. Even though inhibin is the major tumor suppressor protein, the malignant transformation of a given stromal cell in the absence of inhibin protein might be critically dependent on the proliferation status of that cell. Because LH and FSH are well known mitogenic factors for granulosa, Sertoli, Leydig, and thecal cells during the growth and development of gonads, the absence of both of these hormones may render the affected cell types essentially mitotically inactive. This would then prevent the formation and/or development of invasive foci in the gonads. Alternatively, in inhibin-deficient mice, gonadotropins might be stimulating a factor from a distinct cell type other than the stromal cells within the gonads, which will then act on the cancer cell. Such existence of paracrine interactions between distinct cell types within gonads is evident from a number of studies (21). The only noticeable feature in compound mutant male mice testes is the presence of a PAS-positive substance within the tubules. It is unclear what this PAS-positive substance was or why the absence of both inhibin and GnRH would cause such secretion.

Several agonists and antagonists of GnRH have shown promise in treating human ovarian epithelial and testicular cancers (22). We have not used this strategy to suppress gonadotropin levels because the administration of analogs is cumbersome. In addition, administration of GnRH analogs would complicate the issue due to the direct dual actions of these analogs in both the pituitary and the gonads (*i.e.* GnRH receptors are present in both sites). Further, the analogs can be injected into mice only at restricted time points. The advantage of our genetic approach is to avoid these complications. The neoplastic cell transformation in the gonads of inhibin-deficient mice might occur at an early stage. If this is true, the influence of the gonadotropins is essentially abolished in our model during embryonic and neonatal life (when gonadal differentiation occurs), a situation difficult to achieve with the GnRH injection regimens. Although the absence of gonadal tumor development in inhibin- and GnRH-deficient mice identifies gonadotropins as important factors that influence this process, the exact roles that LH and FSH individually play are difficult to delineate. To resolve this, we are generating independent lines of mice that are deficient in inhibin and either LH or FSH.

Are the gonadotropins influencing the gonadal tumors in inhibin-deficient mice via their steroidogenic output, as suggested by Beamer and colleagues (13)? Antisteroids have been shown to be effective in suppressing ovarian epithelial tumor cell growth (23). To determine whether androgens influence the development of testicular tumors in inhibin-deficient mice, we are generating mutant male mice deficient in inhibin and harboring a mutation in the androgen receptor ($inha^{m1}/inha^{m1}$, X^{tfm}/Y; Shou, W. S., and M. M. Matzuk, unpublished data). It will be interesting to determine whether estrogens secreted from the tumors also influence tumorigenesis in males and females (5). To determine this, we will intercross mice carrying a mutation in the estrogen receptor gene (ER^{m1}) (24) with inhibin mutant mice to generate $inha^{m1}/inha^{m1}$, ER^{m1}/ER^{m1} mice.

Recently, transgenic mice that harbor a polyoma viral large T antigen sequence linked to an early promoter have been generated (25). These mice develop Sertoli cell tumors. These tumor cells when injected into athymic mice resulted in secondary tumors, representing the progression into a mixed germ cell-sex cord cell proliferation (26). Transgenic mice in which the expression of simian virus large T antigen is directed to the testis using the regulatory sequences of MIS (27) also develop Sertoli cell tumors. It will be interesting to determine whether these tumors are also influenced by gonadotropins and/or steroids.

The $inha^{m1}/inha^{m1}$, *hpg/hpg* mutant mice did not develop any adrenal tumors beyond 1 yr of age. The absence of

adrenal tumors in these compound homozygous mutant mice may suggest that gonadotropins are perhaps also essential for their development in gonadectomized inhibin-deficient mice. However, no reports have documented the direct binding of gonadotropins to the adrenal glands. It will be worthwhile to study the long term effects of gonadectomy on the compound mutant mice to address this issue.

Lastly, although the majority of human gonadal cancers are epithelial or germ cell derived, the inhibin- and GnRH-deficient mice are important models for understanding gonadal sex cord-stromal tumor development and may prove valuable in testing the efficacy of recombinant gonadotropin analogs as antigonadal tumor drugs. These models have been useful in further deciphering the molecular aspects of gonadal sex cord-stromal cancers and will continue to aid us in formulating and testing a generalized mechanism of gonadal growth and differentiation.

Acknowledgment

We thank Ms. Shirley Baker for excellent help in the preparation of the manuscript.

References

1. **Damjanov I** 1993 Pathology of Infertility. Mosby, St. Louis, pp 1–42
2. **Marchbanks PA, Peterson HB, Rubin GL, Wingo PA** 1989 Cancer and Steroid Hormone Study Group. Research on infertility: definition makes a difference. Am J Epidemiol 130:259–267
3. **Vale W, Bilezikjian LM, Rivier C** 1994 Reproductive and other roles of inhibins and activins. In: Knobil E, Neill JD (eds) The Physiology of Reproduction. Raven Press, New York, pp 1861–1878
4. **Matzuk MM, Finegold MJ, Su J-GJ, Hsueh AJW, Bradley A** 1992 α-Inhibin is a tumour-suppressor gene with gonadal specificity in mice. Nature 360:313–319
5. **Matzuk MM, Kumar TR, Shou WS, Coerver KA, Lau AL, Behringer RR, Finegold MJ** 1996 Transgenic models to study the roles of inhibins and activins in reproduction, development and oncogenesis. Recent Prog Horm Res 51:123–157
6. **Matzuk MM, Finegold MJ, Mishina Y, Bradley A, Behringer RR** 1995 Synergistic effects of inhibins and Müllerian-inhibiting substance on testicular tumorigenesis. Mol Endocrinol 9:1337–1345
7. **Matzuk MM, Finegold MJ, Mather JP, Krummen L, Lu H, Bradley A** 1994 Development of cancer cachexia-like syndrome and adrenal tumors in inhibin-deficient mice. Proc Natl Acad Sci USA 91:8817–8821
8. **Bousfield GR, Perry WM, Ward DN** 1994 Gonadotropins: chemistry and biosynthesis. In: Knobil E, Neill JD (eds) The Physiology of Reproduction. Raven Press, New York, pp 1749–1792
9. **Gharib SD, Wierman ME, Shupnik MA, Chin WW** 1990 Molecular biology of the pituitary gonadotropins. Endocr Rev 11:177–199
10. **Tapanainen JS, Tilly JL, Vihko KK, Hsueh AJW** 1993 Hormonal control of apoptotic cell death in the testis: gonadotropins and androgens as testicular cell survival factors. Mol Endocrinol 7:643–650
11. **Risma KA, Clay CM, Nett TM, Wagner T, Yun J, Nilson JH** 1995 Targeted overexpression of luteinizing hormone in transgenic mice leads to infertility, polycystic ovaries, and ovarian tumors. Proc Natl Acad Sci USA 92:1322–1326
12. **Godwin AK, Perez RP, Johnson SW, Hamaguchi K, Hamilton TC** 1992 Growth regulation of ovarian cancer. Hematol Oncol Clin North Am 6:829–841
13. **Tennent BJ, Shultz KL, Beamer WG** 1993 Genetic susceptibility for C19 androgen induction of ovarian granulosa cell tumorigenesis in swxj strains of mice. Cancer Res 53:1059–1063
14. **Beamer WG, Shultz KL, Tennent BJ, Shultz LD** 1993 Granulosa cell tumorigenesis in genetically hypogonadal-immunodeficient mice grafted with ovaries from tumor-susceptible donors. Cancer Res 53:3741–3746
15. **Mason AJ, Hayflick JS, Zoeller RT, Young III WS, Phillips HS, Nikolics K, Seeburg PH** 1986 A deletion truncating the gonadotropin-releasing hormone gene is responsible for hypogonadism in the hpg mouse. Science 234:1366–1371
16. **Kumar TR, Low MJ** 1995 Hormonal regulation of human follicle-stimulating hormone-β subunit gene expression: GnRH stimulation and GnRH- independent androgen inhibition. Neuroendocrinology 61:628–637
17. **Simon WE, Albrecht M, Hänsel M, Dietel M, Hölzel F** 1983 Cell lines derived from human ovarian carcinomas: growth stimulation by gonadotropic and steroid hormones. J Natl Cancer Inst 70:835–845
18. **Simon WE, Hölzel F** 1979 Hormone sensitivity of gynecological tumor cells in tissue cultures. J Cancer Res Clin Oncol 94:307–323
19. **Neumann F** 1991 Early indicators for carcinogenesis in sex-hormone-sensitive organs. Mutat Res 248:341–356
20. **Wimalasena J, Dostal R, Meehan D** 1992 Gonadotropins, estradiol, and growth factors regulate epithelial ovarian cancer cell growth. Gynecol Oncol 46:345–350
21. **Pescovitz OH, Srivastava CH, Breyer PR, Monts BA** 1994 Paracrine control of spermatogenesis. Trends Endocrinol Metab 5:126–131
22. **Parmer H, Phillips RH, Lightman SL, Schally AV** 1988 Therapy of advanced ovarian cancer with D-Trp-6-LH-RH (decapeptyl) microcapsules. Biomed Pharmacother 42:531–538
23. **Slotman BJ, Rao BR** 1989 Response to inhibition of androgen action of human ovarian cancer cells *in vitro*. Cancer Lett 45:213–220
24. **Lubahn DB, Moyer JS, Golding TS, Couse JF, Korach KS, Smithies O** 1993 Alteration of reproductive function but not prenatal sexual development after insertional disruption of the mouse estrogen receptor gene. Proc Natl Acad Sci USA 90:11162–11166
25. **Paquis-Flucklinger V, Michiels J-F, Vidal F, Alquier C, Pointis G Bourdon V, Cuzin F, Rassoulzadegan M** 1993 Expression in transgenic mice of the large T antigen of polyoma virus induces Sertoli cell tumors and allows the establishment of differentiated cell lines. Oncogene 8:2087–2094
26. **Paquis-Flucklinger V, Rassoulzadegan M, Michiels J-F** 1994 Experimental Sertoli cell tumors in the mouse and their progression into a mixed germ cell-sex cord proliferation. Am J Pathol 144:454–459
27. **Peschon JJ, Behringer RR, Cate RL, Harwood KA, Idzerda RL, Brinster RL, Palmiter RD** 1992 Directed expression of an oncogene to Sertoli cells in transgenic mice using Müllerian inhibiting substance regulatory sequences. Mol Endocrinol 6:1403–1411