Prevention of cachexia-like syndrome development and reduction of tumor progression in inhibin-deficient mice following administration of a chimeric activin receptor type II-murine Fc protein

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Inhibin is a secreted tumor suppressor, and inhibin α null mice develop gonadal sex cord-stromal tumors with 100% penetrance at an early age. Inhibin-deficient mice die of a severe wasting syndrome due to increased activin signaling through activin receptor type II. The current study was designed to assess the in vivo effects of an activin antagonist, a chimeric activin receptor type II fused to the Fc region of a murine IgG2a (ActRII-mFc), administered transiently to the inhibin-deficient mice. Results showed that the severe weight loss was prevented in the ActRII-mFc-treated mice, FSH levels were reduced, and an extended life span was observed for these mice compared with phosphate-buffered saline-treated controls. Although ActRII-mFc treatment did not seem to prevent the formation of gonadal tumors, tumors were smaller in the majority of experimentally treated mice and were characterized by the presence of variable numbers and sizes of cysts in contrast to the solid hemorrhagic tumors that typically developed in the controls. Moreover, the ActRII-mFc-treated mice were less anemic, and their livers and stomachs were histologically normal. In summary, this study demonstrated that in vivo administration of the activin antagonist, ActRII-mFc, not only prevents the cachexia-like symptoms in the inhibin-deficient mouse model, but also reduces tumor progression. These results support an essential role of activins in the cachexia-like syndrome development and implicate activins as growth-promoting factors in gonadal tumor progression. The current findings have potential implications in the design of new drugs or strategies for the treatment of ovarian and testicular tumors and other conditions where ligands signal through ActRII.

Keywords: activin receptor type II; inhibin; cachexia; tumor

Introduction

Activins and inhibins are members of the TGF-β superfamily that comprises a large group of structurally related polypeptide growth factors essential in normal physiologic and developmental processes (Massague, 1998; Pangas and Woodruff, 2000; Chang et al., 2002; Pangas et al., 2002; Brown et al., 2003; Burdette et al., 2005). Activins are homodimers and heterodimers of disulfide-linked β subunits that include activin A (βA: βA), activin B (βB: βB) and activin AB (βA: βB) (Risbridger et al., 2001). Three additional activin β subunits (βC, βD and BE) have been recently identified (Oda et al., 1995; Fang et al., 1996; Lau et al., 1996; Schmitt et al., 1996). Although activin βC and βE are primarily expressed in adult liver (Fang et al., 1997), they are not essential for liver function (Lau et al., 2000). In contrast, activin βD was characterized in Xenopus laevis and represents a new dorsal mesoderm-inducing factor (Oda et al., 1995). Activins signal through their type II receptors (ActRI and ActRIIB) and type I receptor (ActRI), leading to the phosphorylation and activation of the receptor-regulated SMADs (R-SMADs; SMAD2 and SMAD3) (Mathews and Vale, 1991; Massague, 1992, 1998; Attisano et al., 1993; Mathews, 1994; Matzuk et al., 1995c; Derynck and Zhang, 2003). A heteromeric complex is formed between R-SMADs and the common SMAD (Co-SMAD; SMAD4), which then translocates to the nucleus to regulate activin-responsive gene transcription (Moustakas et al., 2001; Chang et al., 2002; Massague, 2000; Mehr and Wrana, 2002). In contrast, inhibins are heterodimers of disulfide-linked subunits of α and β [inhibin A (α: βA) and inhibin B (α: βB)]. In many cell types, inhibins have counter-regulatory roles to activins, acting as activin receptor antagonists (Xu et al., 1995).

Although the initial role of activins and inhibins was found in the regulation of FSH secretion, where activins promote and inhibins inhibit FSH production (Pangas and Woodruff, 2000), additional diverse roles of activins and inhibins in cell proliferation and differentiation have been identified (Brown et al., 2003). In contrast to their postulated roles in mesoderm induction in X. laevis (Smith et al., 1990) and fish (Wittbrodt and Rosa, 1994), activins are not essential for mesoderm formation in mice (Matzuk et al., 1995c). Instead, an
obligatory role of activins during craniofacial development has been unraveled in mammals (Matzuk et al., 1995a,c). Our previous studies identified inhibin as a secreted tumor suppressor specific to the gonads by using embryonic stem (ES) cell technology to functionally inactivate inhibin α (Inha) in vivo (Matzuk et al., 1992). Mice homozygous for the Inha null allele develop gonadal sex cord-stromal tumors at an early age with 100% penetrance (Matzuk et al., 1992). The inhibin-deficient mice have elevated activin levels in the serum (Matzuk et al., 1994, 1996) as well as overexpressed activin βA mRNA in the testis (Trudeau et al., 1994). These mice succumb to a cachexia-like syndrome (Matzuk et al., 1994), the primary cause of lethality. Activin signaling through ActRII directly causes the wasting syndrome since the cachexia-like symptoms were minimized in double homozygous mutant mice lacking both inhibin and ActRII (Coerver et al., 1996).

The genetic studies described above provide powerful evidence that the activin–ActRII signaling axis is a critical mediator of many of the activin-induced pathological changes in the inhibin-deficient mice (e.g. depletion of gastric parietal cells and hepatocellular necrosis around the central vein). Nonetheless, these studies do not necessarily differentiate between effects that originate in the developing embryo and those effects that are subject to activin regulation in the postnatal animal and therefore amenable to treatment. We thus attempted to design a study by using an activin antagonist to probe the in vivo effects of activins on the development of the cachexia-like syndrome and gonadal tumors in inhibin-deficient mice. As previously reported, truncation of the serine/threonine kinase cytoplasmic domain of the ActRII is able to inhibit activin signaling in Xenopus (Hemmati-Brivanlou and Melton, 1992; De Winter et al., 1996). Furthermore, the extracellular domain (ECD) of ActRII has the intrinsic ability to bind activins with high affinity and is functionally active in competing with cellular ActRII (Donaldson et al., 1999; del Re et al., 2004). Therefore, we selected a chimeric ActRII fused to the Fc region of murine IgG2a (ActRII-mFc) to use as an activin antagonist for the administration to inhibin-deficient mice.

In the current study, we found that treatment with ActRII-mFc protected inhibin-deficient mice from suffering the severe weight loss and early death by preventing the pathological changes in the livers and glandular stomachs observed in control mice. Furthermore, previously unidentified roles of activin signaling in gonadal tumor development in the absence of inhibin were revealed.

Materials and Methods

Gene targeting and generation of inhibin α (Inha) knockout mice

Targeting of the inhibin α gene in ES cells and generation of Inha null mice were described previously (Matzuk et al., 1992). Mice were maintained on a mixed C57BL/6J/129S6/SvEv genetic background. All animals used in this study were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Genotyping of Inha null mice was conducted by Southern blot (Matzuk et al., 1992) using genomic tail DNA.

Construction, production and administration of ActRII-mFc

ActRII-mFc was constructed by joining the ECD of ActRII to the Fc region of a murine IgG2a and was generated by Acceleron Pharma, Inc., Cambridge, MA, USA. The ActRII-mFc protein has very high affinity (Kd 2 × 10⁻¹⁰ M) for activin A (unpublished data) (del Re et al., 2004). Administration of ActRII-mFc to inhibin-deficient mice was as follows: (i) ActRII-mFc-treated group (experimental group): inhibin-deficient mice at 3 weeks of age (n = 9 for male and n = 11 for female) were injected with ActRII-mFc intraperitoneally (i.p.) (10 mg/kg body weight) in phosphate-buffered saline (PBS) twice a week; (ii) PBS-treated controls: inhibin-deficient mice (n = 14 per sex) were injected i.p. with 100 μl of PBS at 3 weeks of age following the same injection schedule as the ActRII-mFc-treated group.

Body weights and survival rate

Body weights were measured and recorded twice a week for a period of 3–12 weeks (males) or 3–19 weeks (females), and the development of cachexia-like symptoms (weight loss, sunken-eye appearance, lethargy, pale periphery, kyphoscoliosis, etc.) was closely monitored (Matzuk et al., 1994). Animals were sacrificed for further studies (morphology, histology, hormonal assays, etc.) when overt signs of wasting syndrome developed (i.e. body weight loss of ≥20% of peak body weight, kyphoscoliosis, severe lethargy, etc.) or at 12 weeks in males or 19 weeks in females.

Histological analysis

Ovaries/testes, liver and stomach were collected from mice at the time of sacrifice and fixed in 10% neutral buffered formalin (NBF) overnight. Samples were then transferred to 70% ethanol and further embedded and sectioned for hematoxylin and eosin (liver and stomach) or periodic acid Schiff-hematoxylin (ovary and testis) staining using standard procedures of the Pathology Core Services Facility at Baylor College of Medicine.

Hematocrit analysis

Briefly, periorbital blood from isoflurane-anesthetized mice was collected into heparinized microhematocrit capillary tubes (Baxter Healthcare Corp., Deerfield, IL, USA) followed by 15 min spinning in a microhematocrit centrifuge. The value was then read on a microcapillary reader and recorded.

Serum activin and FSH analyses

Cardiac puncture was performed to collect blood samples from mice anesthetized by isoflurane inhalation at the time of sacrifice. Blood was allowed to clot at room temperature in serum separator tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Serum was then separated by centrifugation and kept at −20°C until assayed. Total activin A was measured using a specific enzyme-linked immunosorbent assay (Knight et al., 1996) according to the manufacturer’s instructions (Oxford Bio-Innovations, Oxfordshire, UK) with modifications as described previously (O’Connor et al., 1999). The average intra-plate coefficient of variation (CV) was 5.1% and the inter-plate CV was 6.6% (n = 2 plates). The limit of detection for activin A was 10 pg/ml.

Serum FSH was analysed by the Ligand Assay and Analysis Core at the Center for Research in Reproduction, University of Virginia. The limit of detection for FSH was 2 ng/ml (detailed information is at http://www.healthsystem.virginia.edu/internet/crr/ligand.cfm).

Statistical analysis

Data were expressed as mean ± SEM and a Student’s t-test was used to analyse the differences of means (testis/ovary/liver weights, hematocrit, FSH and activin A) between treatment (ActRII-mFc-treated) and control (PBS-injected) groups. Statistical significance was reported at P < 0.05.

Results

Cachexia-like symptoms were prevented and survival increased in inhibin-deficient mice treated with ActRII-mFc protein

Weight loss in inhibin-deficient mice starts after ~6–7 weeks of age accompanied by several cachectic symptoms (i.e. dorsal kyphoscoliosis, a sunken-eye appearance, pale periphery, lethargy, etc.) due to increased activin signaling (Matzuk et al., 1994). As expected, the above cachectic symptoms including early weight loss were observed in PBS-treated Inha null mice of both sexes (Fig. 1). However, ActRII-mFc treatment prevented the dramatic weight loss in both males (Fig. 1A) and females (Fig. 1B) during the respective 12- and 19-week experimental period. The survival curve showed that 71% (10/14) of PBS-treated inhibin-deficient male mice died by 12 weeks of age (9.5–12 weeks) due to the development of the cachexia-like syndrome, whereas 100% (9/9) of the ActRII-mFc-treated males survived past 12 weeks (Fig. 2A). In the PBS-treated inhibin-deficient
pared with PBS-treated controls. In the inhibin-deficient males (et al., 1994), we expected that treatment of inhibin-deficient mice with ActRII-mFc would lead to normal morphology/histology of the livers and stomachs. Depletion of gastric parietal cells and hepatocellular death around the central vein are two histological hallmarks of the livers and stomachs. However, ActRII-mFc treatment abolished the activin-induced pathological changes in the livers and glandular stomachs of inhibin null mice.

**Reduction of tumor progression in inhibin-deficient mice following ActRII-mFc administration**

To determine if the increased survival in ActRII-mFc-treated mice was caused by the attenuation of the inhibin-deficient tumor phenotype, gonads were examined grossly and histologically at the end time-points when both male (12 weeks) and female (19 weeks) mice were sacrificed for further analyses. In the inhibin-deficient males, although ActRII-mFc administration did not prevent testicular tumor formation, a pronounced effect of ActRII-mFc treatment on tumor growth/progression was found. Overall, the testicular tumors that developed in ActRII-mFc-treated mice were smaller than those in PBS-treated controls, as evidenced by comparison of average testis weights of ActRII-mFc-treated mice (12 weeks) with PBS controls (9.5–12 weeks) (Fig. 3F; \( P < 0.05 \)) as well as analysis of testis histology (Fig. 3B–D). Morphological and histological analyses showed that PBS-treated control mice developed large, solid and hemorrhagic tumors as expected (Fig. 3A). In contrast, smaller testicular tumors were found in all of the experimental mice examined, and more seminiferous tubules were present in these testes, although with a block in spermatogenesis (Fig. 3B–D). Interestingly, testes from the majority of ActRII-mFc-treated mice appeared to be more cystic (Fig. 3C), and cysts were usually found in both testes with variable sizes and numbers.

In the females, tumor formation and development in the experimental mice appeared to be limited in some mice; there were minimal tumor foci in the ovaries which continued to be comprised of follicles and corpora lutea as well as cysts (Fig. 4A–C). In some experimental mice, ovarian follicles and corpora lutea continued to be present despite the development of solid tumors (Fig. 4D). In contrast, normal ovarian architecture was inevitably destroyed and replaced by tumors in the PBS-treated inhibin-deficient mice (Fig. 4F). However, exceptions were found in two ActRII-mFc-treated females that developed large hemorrhagic tumors (Fig. 4E); histologically indistinguishable from those observed in the PBS-treated control mice (Fig. 4F).

**ActRII-mFc treatment abolished the activin-induced pathological changes in the livers and glandular stomachs of inhibin null mice**

Based on the evidence that infusion of recombinant activin A caused similar liver and stomach pathology as that of inhibin-deficient mice which have highly elevated activin levels (Schwall et al., 1993; Hully et al., 1994), we expected that treatment of inhibin-deficient mice with ActRII-mFc would lead to normal morphology/histology of the livers and stomachs. Depletion of gastric parietal cells and hepatocellular death around the central vein are two histological hallmarks of cachectic inhibin-deficient mice (Matzuk et al., 1994). In the PBS-treated control mice, the above pathological features were apparent in the livers (Fig. 5A; hepatocellular death and lymphocytic infiltration around the central vein) and stomachs (Fig. 5C; mucosal atrophy and depletion of parietal cells). However, ActRII-mFc treatment prevented these pathological changes in the livers (Fig. 5B) and stomachs (Fig. 5D) of inhibin-deficient mice of both sexes. Consistent with the normal liver histology, the weights of livers from ActRII-mFc-treated mice (12 weeks for males and 19 weeks for females) were increased.
compared with the PBS-treated controls (9.5–12 weeks for males and 11.5–19 weeks for females) (Fig. 5E and F; \(P, 0.01\)).

Hematocrit analysis

In addition to the severe weight loss and the pathological lesions in the liver and stomach, inhibin-deficient mice become anemic at the advanced cachectic stage of tumor development (Matzuk et al., 1994, 1996). Hematocrit analysis showed that the inhibin-deficient female mice (\(n = 7\)) treated with ActRII-mFc had higher hematocrit compared with mice (\(n = 9\)) injected with PBS alone (44.6 ± 3.2% versus 28.5 ± 1.1%; \(P < 0.01\)). The hematocrit of inhibin-deficient male mice treated with ActRII-mFc (38.7 ± 3.2%; \(n = 3\)) was also higher compared with that of the cachectic inhibin-deficient mice at 7–12 weeks of age (28.8 ± 2.6%; \(n = 7\)) (Matzuk et al., 1994).

Serum activin and FSH levels following ActRII-mFc treatment

Inhibins and activins are critical regulators of pituitary FSH production, and mice deficient in inhibin have elevated serum levels of activins (Matzuk et al., 1994; Coerver et al., 1996) and FSH (Matzuk et al., 1992). In the current study, serum activin A was measured to examine the potential effect of ActRII-mFc treatment on circulating activin levels (Fig. 6A). The results showed that PBS-treated inhibin-deficient mice have a dramatic elevation of serum activin A levels (24.55 ± 3.52 ng/ml for males and 35.31 ± 4.20 ng/ml for females) over adult WT males (0.05 ± 0.01 ng/ml) and females (0.04 ± 0.00 ng/ml) (Li et al., 2007). Significantly higher serum total activin A levels were observed in ActRII-mFc-treated male (12 weeks; \(P, 0.01\)) and female (19 weeks; \(P < 0.05\)) mice compared with PBS-treated controls (9.5–12 weeks; \(n = 12\)) and ActRII-mFc-treated (12 weeks; \(n = 5\)) inhibin-deficient male mice. Data are shown as mean ± SEM, and bars without a common letter are significantly different at \(P < 0.05\). Scale bars: A, 200 μm; B and C, 400 μm; D and E, 50 μm

Discussion

Inhibin-deficient mice develop focally invasive sex cord-stromal tumors (Matzuk et al., 1992), and the death was primarily attributed
to the activin-induced wasting syndrome (Matzuk et al., 1994), a common feature present at later stages of these cancers. The above syndrome is gonadal dependent since gonadectomized inhibin-deficient mice failed to develop the cachexia-like symptoms (Matzuk et al., 1994). Thus, the inhibin-deficient mice represent a useful model for studying the mechanisms of tumorigenesis and testing the effects of anti-tumor reagents on gonadal tumor development. We demonstrate in this study that many of the phenotypic consequences of inhibin deficiency in the inhibin-deficient mice can be prevented by treatment with ActRII-mFc, a chimeric ActRII joined to the Fc region of a murine IgG2a. These data further indicate that elevated levels of activins in inhibin-deficient mice not only cause the severe cachexia-like symptoms, but also may promote tumor growth and development. Of note, it is possible that ActRII-mFc affects the inhibin-deficient phenotype through inhibition of factors other than activins. However, ActRII-mFc preferentially binds to activins versus other ligands (unpublished data), and therefore the primary effect of ActRII-mFc treatment is most likely due to activin antagonism.

In support of the roles of activins in hepatocytes (Yasuda et al., 1993; Clotman and Lemaigre, 2006) and the observed effects of recombinant activin A on hepatocellular death both in vivo and in vitro (Schwall et al., 1993), ActRII mRNA transcripts are present in the liver (Coerver et al., 1996). Interestingly, the majority of ActRII and Inha double homozygous mutant mice has histologically normal livers and stomachs and suffer no weight loss (Coerver et al., 1996). It is thus suggested that activin signaling through ActRII contributes to the wasting syndrome via induction of pathological changes in the liver and glandular stomach in inhibin-deficient mice. Further studies demonstrated that the differentiation of preparietal cells to acid-producing parietal cells as well as the differentiation of several other gastric epithelial cells types are blocked in the presence of supra-physiological levels of activins in the absence of inhibin (Li et al., 1998). In the present study, ActRII-mFc administration in inhibin-deficient mice prevented the dramatic weight loss and pathological changes in the liver and stomach and ameliorated the anemia. These findings indicate that elevated activins in the inhibin-deficient mice contribute to the weight loss as well as anemia secondary to the nutritional deficiencies induced by the pathological lesions in the liver and glandular stomach.

The mechanisms of tumor development in inhibin-deficient mice are complex, and a number of genetic modifiers have been identified in our previous studies including gonadotropin releasing hormone (Kumar et al., 1996), FSH (Kumar et al., 1999), anti-Mullerian hormone (Matzuk et al., 1995b) and its receptor (Mishina et al., 1996), follistatin (Cipriano et al., 2000), androgen receptor (Shou et al., 1997), cyclin D2 (Burns et al., 2003a), p27 (Cipriano et al., 2001) and estrogen receptors (Burns et al., 2003b). However, the

Figure 4: Histological analysis of ovary/ovarian tumors from inhibin-deficient mice treated with PBS or ActRII-mFc

(A) Histopathology of an ovary from a 19-week-old inhibin-deficient female treated with ActRII-mFc. Note the presence of follicles (arrow), corpora lutea (**) and multiple cysts (c) in the ovary. (B) High power view of the follicles (arrow) and corpora lutea (**) shown in (A). (C) Histopathology of a cystic ovary from a 19-week-old inhibin-deficient female treated with ActRII-mFc. Note the presence of multiple large cysts (c) in this ovary. (D) Histopathology of an ovary from a 19-week-old inhibin-deficient female treated with ActRII-mFc. Note the presence of a solid tumor (arrow head), follicles (arrow), and corpora lutea (**). (E) Histopathology of a sex cord-stromal tumor from a 19-week-old inhibin-deficient female treated with ActRII-mFc. Note the presence of the hemorrhage (*) in the solid tumor. (F) Histopathology of a gonadal tumor from a 19-week-old inhibin-deficient female treated with PBS. A hemorrhagic region (*) is indicated. Scale bars: A, C and D, 200 μm; B, 100 μm; E and F, 50 μm.
roles of activins in gonadal tumorigenesis remain unknown. A number of studies have identified an antiproliferative role of activins in extra-gonadal cancer cell lines (Burdette et al., 2005; Wang et al., 1996) as well as tumor cells from a variety of organs including thyroid (Matsuo et al., 2006) and adrenal (Beuschlein et al., 2003) glands. In contrast, results of the current study provide novel in vivo evidence supporting a role of activins in stimulating gonadal tumor development. Although our results do not provide compelling evidence that activins are essential for tumor formation in inhibin-deficient mice (since tumors are still present in most of the ActRII-mFc-treated mice), they do support the finding that activins act as tumor growth factors by showing that treatment of inhibin-deficient mice with ActRII-mFc appeared to reduce/suppress the tumor progression. Reduced testis weight and tumor size/foci as well as more testicular cysts were phenotypic characteristics of ActRII-mFc-treated males. Although more variable effects of ActRII-mFc injection were observed on the tumor development in the females, the majority of experimental female mice also had smaller tumors or demonstrated minimal tumor foci in their ovaries with variable numbers of cysts. Evidence corroborating the hypothesis that elevated activin levels positively affecting tumor growth and development also stems from in vitro studies indicating that activins function as autocrine factors in stimulating the proliferation of gonadal tumor cell lines derived from inhibin-deficient mice (Shikone et al., 1994) as well as ovarian clear cell adenocarcinoma cells (Mabuchi et al., 2006).

The current findings are consistent with two of our genetic studies demonstrating that tumor development was compromised in inhibin-deficient mice with reduced/suppressed activins (Kumar et al., 1999; Cipriano et al., 2000). One study showed that gain-of-function transgenic mice overexpressing follistatin develop less aggressive tumors and have low circulating activin levels due to local binding of follistatin to activins (Cipriano et al., 2000). In the other case, mice deficient in FSH have dramatically reduced activins in the inhibin-deficient background. These mice demonstrated minimal/milder cachexia-like symptoms and developed slower-growing tumors (Kumar et al., 1999). Interestingly, FSH levels were decreased in inhibin-deficient mice following ActRII-mFc treatment in the present study. However, the significance of reduction of FSH in the tumor development of ActRII-mFc-treated inhibin-deficient mice is not clear. More recently, we found that Inha and Smad3 double knockout males are protected from early tumorigenesis and associated cachectic symptoms, and SMAD3 deficiency also reduces ovarian tumorigenesis but to a much milder degree in comparison to males (Li et al., 2007). Collectively, the above results suggest that elevated
activins contribute to the fast growing and aggressive nature of gonadal tumors in inhibin-deficient mice. The mechanisms of tumor development and the downstream signaling pathways and proteins that mediate activin action in vivo, including the potentially redundant roles of SMAD2 and SMAD3 in the ovary, await further investigation.

Analysis of activin levels in the present study was hampered by potential accumulation of activin–ActRII-mFc complexes. Activin levels are expected to decrease in inhibin-deficient mice treated with ActRII-mFc, since ActRII can bind activins, and phagocytosis will be initiated by an interaction between the Fc region of the immunoglobulin and receptors primarily presented on effector cells (macrophages, monocytes, etc.). However, higher serum total activin A levels were observed in ActRII-mFc-treated mice compared with PBS-treated controls. Of note, while formation of the activin–ActRII-mFc complex and subsequent phagocytosis reduce free activin levels, it might simultaneously stimulate the secretion of activin via a negative feedback mechanism (see Fig. 7 for details).

The efficiency of ActRII-mFc clearance might also be compromised due to the binding of activins to the chimeric receptor as well as to potentially reduced phagocytosis in inhibin-deficient mice via unknown mechanisms. Therefore, the high serum activin A levels in ActRII-mFc-treated mice may reflect the accumulation of circulating nonfunctional activin–ActRII-mFc complex. As indirect evidence supporting the reduction of functional/free activins following ActRII-mFc treatment, serum FSH was markedly decreased in the experimental males, although statistical significance was not achieved in the females due to low animal numbers and variations in the control group.

Interestingly, our previous ActRII knockout mouse model revealed that absence of activin signaling through ActRII in the gonads has a minimal effect on the development of gonadal sex cord-stromal tumors in vivo (Coever et al., 1996). The seemingly anomalous results may be explained by the fact that both ActRII and ActRIIB are present in a variety of reproductive tissues including ovary and testis (Matzuk and Bradley, 1992; Wu et al., 1994). Thus activin signaling through ActRIIB or other unidentified receptors in the gonads may contribute to mediate the tumor-promoting effect of activins in ActRII and Inha double knockout mice.

In summary, using the inhibin-deficient mouse as a model, we show that administration of a chimeric ActRII-mFc protein in vivo reduced tumor growth/progression and protected inhibin-deficient mice from suffering severe weight loss via preventing the pathological changes in livers and stomachs. These findings have important potential clinical implications in designing new drugs/strategies in alleviating or preventing the cachexia-like syndrome and gonadal tumor development as well as in alleviating other conditions in which TGFβ superfamily signaling through ActRII causes disease in humans.

**Figure 7:** Hypothetical schematic of circulating activin changes in inhibin-deficient mice following ActRII-mFc administration and the effects on the cachexia-like symptoms and tumor development. Activin levels are elevated in the absence of inhibin. ActRII-mFc will bind free activin to form activin–ActRII-mFc complexes in the circulation, thus reducing free activin levels. Reduction of free activin levels may simultaneously stimulate the secretion of activins via a negative feedback mechanism. On one hand, more complexes may be formed due to the accelerated secretion of activins, leading to suppressed functional activins (free activins) in the circulation. On the other hand, the activin complex can be cleared through phagocytosis, the efficiency of which depends on that of the immune system. Consequently, total activin (free and bound) levels may be increased due to the accumulation of activins-ActRII-mFc complexes. Protection of ActRII-mFc-treated inhibin-deficient mice from the cachexia-like syndrome and the relatively normal liver and glandular stomach are supposed to be the results of suppression of functional activin in the circulation, as indicated by the reduced FSH levels in the ActRII-mFc-treated male mice. Reduction of functional activins may also contribute to the slower-growing and less hemorrhagic tumors observed in ActRII-mFc-treated inhibin-deficient mice versus PBS-treated controls.
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ActRII-mFc reduces cachexia and tumor development

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