MINIREVIEW

Activins and Activin Receptors in Cell Growth[1] (44077)

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Abstract. **Activin and inhibin, members of transforming growth factor–β (TGFβ) superfamily, have diverse and widespread effects within living organisms at many stages during growth and development. From the initial isolation of these growth factors based on their effects of FSH secretion, the study of these factors, as well as of the activin-binding protein follistatin, has progressed from the localization of the expression of the inhibin α subunit, activin βA and βB subunits, and activin receptors in the tissues of various organisms to the examination of activin and inhibin as autocrine and paracrine agents in cell proliferation and differentiation. The inhibitory effects on cell growth and differentiation that have been observed upon treatment of cells with activin suggest that further understanding of the bioactivity of this molecule and its characterization on a molecular level may aid in a more complete understanding of cell growth and differentiation. This minireview discusses the roles of activin, inhibin, and follistatin in the arenas of cell proliferation, differentiation, and embryogenesis, as well as the roles of these molecules in cancerous cells.**

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Background

Inhibins and Activins. Inhibin and activin are polypeptides originally isolated from ovarian fluid based on their effect on pituitary follicle-stimulating hormone (FSH) production. Inhibins are heterodimers consisting of a common α subunit and one of two highly homologous β subunits (βA and βB), while activins are either hetero- or homodimers of inhibin β subunits (βAβA and βBβB, and βAβB). The isolation of inhibin and activin, their chemical properties, and their bioactivity have been previously reviewed (1, 2). Recently, two new inhibin β subunits, βC and βD, have been cloned from humans and Xenopus, respectively (3, 4). These two subunits have the same general structure as inhibin βA and βB subunits, with nine cysteine residues conserved. The cDNA of the human βC subunit contains a single open reading frame encoding a 352–amino acid precursor protein. The mature C-terminal polypeptide is 116 amino acids long according to the putative cleavage site. The mature βC subunit shows 51% amino acid homology to the inhibin βA subunit and 53% homology to the βB subunit. The precursor of the βD subunit of Xenopus is a 367–amino acid protein, and proteolytic cleavage of the precursor yields a mature C-terminal mature peptide of 114 amino acids. The amino acid homology of the inhibin βD subunit to the βC subunit is 63%. Although injection of inhibin βD subunit mRNA into ventral blastomeres of Xenopus led to the formation of secondary body axis and injection into two-stage embryos caused expression of mesodermal markers in isolated animal caps, general information about these two newly discovered inhibin subunits,

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such as dimer formation with other inhibin subunits, receptor binding, and bioactivity, is unavailable at this time.

Binding Proteins for Inhibins and Activins. *In vitro,* inhibins and activins bind to follistatin, α_2-macroglobulin, and activin receptors. Follistatin is a single-chain polypeptide isolated from ovarian fluid which inhibits pituitary FSH secretion (5). It was found by double-ligand blotting that follistatin binds to both activin and inhibin through a common β subunit and neutralizes some activities of activin but not those of the inhibin (6). In addition to follistatin, inhibins and activins can also bind to a large–molecular weight serum protein, α_2-macroglobulin, which has been reported to bind other cytokines and growth factors (7). The binding of α_2-macroglobulin to activins and inhibins does not affect their function. Activins act through binding to cell plasma membrane receptors. Four types of high-affinity activin receptors (type I, IB, II, and IIB) have been identified. They are all transmembrane proteins containing specific serine/threonine kinase activities. Both activin type I and type II receptors are required for the signal transduction of activin. Activin receptors have been extensively reviewed elsewhere (8). Although the binding sites of inhibin on the cell membrane have been observed, the inhibin receptor has not yet been isolated. Inhibin can bind to activin receptors with low affinity (9, 10).

Genomic Structure and Chromosomal Mapping of Inhibin, Activin, and Follistatin. Inhibin subunits seem to be encoded by different genes. Restriction enzyme digestion and Southern blot analysis showed that inhibin subunit genes are present in the human genome as single copies and are not adjacent. Comparisons of the inhibin subunit genomic clones with their cDNAs reveal that human inhibin α, βA, and βB subunit genes each consist of two exons separated by an intron of 1.7, 9.7, and 2.5 kb, respectively (11, 12). The conservation of inhibin subunit genomic structure suggests that they come from a common precursor. Inhibin subunits in other species, such as bovine, mouse, and rat, have similar genomic organizations (13–15). By primer extension, nuclease mapping assay, DNase I footprinting, and DNA sequencing analysis, five contiguous transcription sites were found in the mouse inhibin α subunit promoter, but, as in the rat, TATA and CAAT boxes, as well as GC-rich sequences, were absent from the mouse promoter. In the 5′ flank region of mouse and rat inhibin α subunits, several cAMP response elements, AP-1 sites, and uEBP-E–binding sites were observed, indicating that inhibin α subunit expression may be regulated by a different mechanism. The characteristics of the inhibin α subunit promoter place them in a class of promoters that is not constitutively active, but rather one that is regulated during differentiation or development. Like the inhibin α subunit promoter, the inhibin βB subunit promoter contains neither TATA nor CAAT box elements. The inhibin βB subunit promoter has GC-rich sequences and Sp1-binding sites, but no uEBP-E–binding sites, while the inhibin βA subunit promoter has multiple transcription sites and is the only one of three inhibin subunit promoters containing TATA and CAAT boxes. Transcription factor AP-2 sites are found in the promoters of all three inhibin subunits, suggesting that the potential signal may be transduced through the PKC pathway. In addition, the presence of cAMP response elements in inhibin α and βB subunit promoters implies that inhibin and activin may be under the regulation of PKA as well. Different DNA sequences present in the promoters of three inhibin subunits suggest that their transcriptions are independently regulated (11–15). Follistatin, the binding protein for inhibins and activins, possesses different 3′ open reading frames which generate two precursor proteins of 317 and 344 amino acids by omission or addition of 27 amino acids encoded by the last exon at the C terminus. Restriction enzyme mapping and DNA sequencing reveal that the follistatin gene contains six exons separated by five introns (16). Primer extension and RNase protection assays reveal that there are eight cap sites in the follistatin gene. The follistatin promoter contains a TATA-like sequence but lacks a CAAT element. In the 5′ flank region, several potential *cis*-elements such as Sp1- and AP-2–binding sites, as well as sequences similar to the cAMP response element and AP-1–binding site were found. By the CAT transcription assay, two segments in the 5′ flank region of follistatin revealed the ability of negative control of basal transcription. Stimulation with forskolin and TPA increased the expression of follistatin (17). Through the use of somatic cell hybridization, Southern blot, and *in situ* hybridization, inhibin subunits have been mapped to the chromosomes of human, mouse, and sheep (18–20). The inhibin α subunit was assigned to human chromosome 2q33 to 2qter, mouse chromosome 1, and sheep chromosome 2q. Additionally, the inhibin βA subunit was mapped to human chromosome 7p15 to 7p14, mouse chromosome 13, and sheep chromosome 4q26, while the inhibin βB subunit was mapped to human chromosome 2cen to 2q13 and mouse chromosome 1. A Taq polymorphism for inhibin α and βA-subunits, a *Pst*I polymorphism for ovine follistatin, a dinucleotide repeat polymorphism for inhibin βA subunit, and a *Bam*HI polymorphism for the inhibin βB subunit in humans have been reported (21–25).

Assays for Inhibins and Activins. The radioimmuno assay (RIA) was a standard method to measure the concentration of inhibin and activin in various biological fluids. RIAs usually employ the use of synthetic peptides and antibodies against them or purified protein and antibodies raised to them. The problems with most of these assays are the relatively low sensitivity of the measurements of inhibin and activin in serum and the interference of biologically inactive forms of inhibin present, including a pro-α form and free α subunit. Recently, several two-site assays have brought the solution to this problem. Both the two-site immunoradiometric assay (IRMA) and two-site enzyme-linked immunosorbent assay (ELISA) use two antibodies, one raised against the N-terminal region of inhibin α subunit, and the other specific for the C-terminal region of the

inhibin βA subunit (26, 27). The specificity of the assays was highly increased with a cross-reaction with free α inhibin subunit of less than 0.1%. In a recently developed IRMA using monoclonal antibodies instead of antisera, the sensitivity of this assay was increased to 5 pg/ml for inhibin A and 0.1 pg/ml for activin A (28). A specific competitive protein binding assay for activin A using follistatin as a binding protein was also developed with a sensitivity of 0.5 ng/ml (29).

Activins, Inhibins, Follistatin, and Proliferation

The multiplicity of activin action and production suggests that mechanisms may exist to control the bioavailabilty of this protein *in vivo* and to allow appropriate and timely expression of activin bioactivity at local tissues. Follistatin has been implicated in this function in *in vitro* and *in vivo* studies using cultured pituitary cells (30). Thus, the presence of follistatin may act as a regulator in an *in vivo* biological system.

Gonads. Recently, inhibin-α–deficient mutant mice were generated through the targeted deletion of the inhibin-α gene through homologous recombination in murine embryonic stem cells (31). Homozygous mutant mice developed gonadal sex cord–stromal tumors. The predisposition of inhibin-deficient mice to gonadal tumors identifies inhibin as the first extracellular (ligand) protein that can function as a tumor suppressor protein. It was speculated that the lack of inhibin activity at the pituitary and/or gonadal level led to some form of endocrinological imbalance that resulted in uncontrolled gonadal cell proliferation. Activins have been suggested as potential local hormones in the regulation of gonadal cell growth and differentiation (32, 33). To better understand the physiological roles of inhibins and activins in the gonads, cell lines from these gonadal sex cord–stromal tumors derived from mice deficient in both inhibin-α and p53 were established (34). These cell lines express inhibin βA and βB mRNA and secrete the βA subunits of activin A. This is consistent with the high levels of activin A and B found in the serum of tumor-bearing homozygous mice mutants (35). Treatment with activin A stimulated cell growth. On the other hand, follistatin inhibited cell proliferation in a dose-dependent fashion, reaching 30% inhibition with an 8 n*M* dose after 5 days of treatment by blocking endogenous activin. Treatment with recombinant inhibin A alone or together with activin had no significant effect on tumor cell growth *in vitro*. Sex cord–stromal tumors are known to contain Sertoli, granulosa, Leydig, or thecal cells of gonadal stromal origin, either singly or in combination in any degree of differentiation (36). Activin thus acts as an autocrine/paracrine factor in the regulation of gonadal tumor cell proliferation *in vitro*. The development of gonadal tumors in inhibin-α–deficient mice demonstrates that inhibin plays an important role in the gonads as a tumor-suppressor protein. However, the lack of an antiproliferative effect on the part of inhibin in the tumor cell lines on the tumor cells suggest (i) that the tumor suppressor activities of inhibin require an intact p53 pathway; (ii) that inhibin signal transduction pathways are defective in the tumor cells; or (iii) the inhibin protein does not inhibit mitosis. The activin-inhibin-follistatin pathway in these gonads is essential for gonadal cell growth.

Spermatogenesis. Recent data have demonstrated that activins and inhibins act as regulators of spermatogonial proliferation (37). Testicular Leydig cells produce activin (38, 39) and Sertoli cells produce inhibin (40). Messenger RNA and protein subunits of the α- and β1-chains have also been localized in multiple cell types in the testis of rats of various ages (41–43). This suggests that inhibin and activin may play a role in regulating Sertoli-Leydig cell interaction (39, 44–47) or Sertoli-germ cell interaction (43, 48). To explore the local actions of activins and inhibins in the testis, Sertoli and germ cells were isolated from immature rats and cocultured *in vitro* (37). While inhibin had no effect on cell growth, activin A and activin B stimulated germ cell proliferation in these cocultures as determined by [^{3}H]thymidine incorporation. It has been shown that activin binds in a specific and stage-dependent manner to receptors as opposed to inhibin, which is capable of binding to testicular cells throughout development (30). A recent study demonstrated that activin receptors II and IIB were expressed in the testis of mid- to late-gestation rat embryos, suggesting an autocrine role for activin in the male reproductive system (49). Apparently, activin and inhibin may act at different levels to regulate the proliferation of testicular cells such as germ and Sertoli cells. During the period of sexual differentiation where cell division is most active in the rat model, activin was shown to inhibit testicular DNA synthesis as well as male mesonephroi (50). Interestingly, *in vivo* studies demonstrate a decrease in spermatogenesis in hamster testis injected locally with both impure and purified inhibin (48). This is opposite to the results observed *in vitro* (37). Thus inhibin may be acting on other cell types in the testis or on stages of spermatogenesis not represented in the co-culture or may require another hormone or growth factor which is not present in the *in vitro* system.

Oogenesis. mRNAs encoding activin subunits and type II receptors are expressed in the rat ovary, indicating an autocrine role in the female reproductive system (49, 51). Studies performed on oogenesis have indicated that activin acts as a meiotic inhibitor in oocytes (52). While activin was shown to inhibit testicular DNA synthesis and the development of mesonephroi in males, ovarian DNA synthesis and the development of mesonephroi in females were stimulated in studies performed at the height of active cell division in the rat model (50). The experimentation presented here demonstrates that activin and inhibin play important roles in the regulation of gonadal cell proliferation during sexual differentiation.

Pituitary. Research has been performed to determine the effects of activin upon extragonadal organs. It has long been known that activin exhibits an effect on the pituitary gland. Activin A was linked to inhibition of cell prolifera-

tion in GH_4 cells of a rat pituitary cell line (53) as well as the corticotropic cells of the anterior pituitary in the mouse pituitary cell line, ATt20 (54). Treatment of pituitary somatotrophs with activin inhibited the mitogenic effects of GH-releasing factor (55), however, activin A was shown to increase the number of FSH cells of the anterior pituitary gland (56, 57). Type II activin receptors were found to be expressed in embryonic pituitary glands of rats, while only activin receptor IIB (ActRcIIB) was found to be expressed in the adult (51, 58). These findings are a sharp indication that activin plays an important autocrine role in the regulatory action of cell proliferation in the anterior pituitary and supports the explanations of certain functions of this extragonadal structure.

Other Endocrine Organs. The βA dimer has been shown to inhibit fetal adrenal growth *in vitro* in a dose-dependent manner (59). While the three combinations of βA and βB subunit dimers as well as activin type II receptors have been identified in the term human placenta, no published reports are available that characterize the endocrine role of activin in this organ (49, 51, 60). Additionally, activin A was found to act in the negative regulation of cell proliferation in rat pancreatic acinar AR42K cells (61). Experiments have demonstrated that activin receptor type IIB is expressed in rat embryos, thus suggesting an autocrine role for activin A in the developing rat (51). In the thyroid gland, activin A was observed to increase DNA synthesis and thyroid growth (62).

Other Organs. In a recent experiment, mRNA transcripts encoding βA and βB subunits were detected in human fetuses *via* Northern analysis using ^{32}P-labeled cDNA probes (63). In the nervous system, both βA and βB subunits were identified in the cerebrum and spinal cord. In muscular tissues, the βA subunit was expressed in the heart, skeletal muscle, and smooth muscle from stomach samples. Additionally, the βA subunit showed expression in hemopoetic tissues of the human fetus in bone marrow, spleen, and liver samples. Another experiment showed expression of activin A in vascular smooth muscle cells (64). βB was shown to be highly expressed in developing rat salivary glands (51, 63). Therefore, it is probable that activin exerts a paracrine/autocrine effect upon these tissues dependent upon concomitant activin receptor expression. Based on previous experimentation, an autocrine effect for activin has been demonstrated by the localization of activin subunits as well as the following activin receptors in these tissues: Activin receptor II (ActRcII) in fetal bovine heart endothelial cells (65), ActRcIIB in the salivary glands of rat embryos (51), ActRcII and IIB in both adult and embryonic rat brains (51, 58), ActRcIIB in the smooth muscle of embryonic rat stomachs (51), and both type II receptors in the spinal cords of adult rats (58). Furthermore, in the liver, activin was shown to inhibit hepatocyte growth in an autocrine fashion *via* the pathway of programed cell death (66–70). On the other hand, activin may have a paracrine function in skeletal muscle (58).

Experiments have demonstrated that activin regulates cell proliferation in hematopoietic stem cell systems (71). Marrow stromal cells and monocytes produce activin A as well as osteoclasts, where activin also exerts a mitogenic effect (72–74). Activin A increases the proliferation of erythroid progenitors from both bone marrow and peripheral blood *via* an indirect mechanism mediated by accessory cells—both monocytes and T lymphocytes (71, 75, 76). In another battery of experiments performed on peripheral blood, the proliferation of interleukin-3–responsive granulocyte macrophage colony–forming erythroid progenitors was inhibited by activin A, while the proliferation and differentiation of interleukin-3–responsive erythroid burst-forming progenitors was stimulated (77). These findings cast light on the role of activin A as a regulator of erythropoiesis. Additionally, it was found that chondrogenesis in chick limb bud mesodermal cells was inhibited upon treatment with activin A (78, 78a). A study of lipopolysaccharide (LPS)-induced lymphocyte proliferation showed that treatment with activin A caused dose-dependent inhibition (79).

Activin has been known to inhibit neural cell differentiation (80), while activin A produced by the mouse macrophage cell line $P388D_1$ acts to induce growth inhibition of B lineage cells resulting in apoptosis (81). Activin has also been found to be expressed in cultured human retinal pigment epithelial cells, where treatment of activin inhibits cell growth (82). Thus, it is seen that activin is expressed at various stages of development and that it acts in the modulation of proliferation in a paracrine/autocrine manner for various tissues at many stages of development.

Cells Derived from Human Cancer. A major focus of the effects of activin has been on its role in the proliferation of tumor cells. Activin exhibits a mitogenic effect on embryocarcinoma P19 cells (64) and stimulates the differentiation of Friend erythroleukemia cells (83, 84). Activin A inhibited cell growth in HepG2 cells, a line developed from hepatoma (85). However, inhibin has been observed as an antagonist to the activity of activin in HepG2 cells (86). We previously have shown that activin A has exhibited an inhibitory effect on the proliferation of Y-79, a cell line developed from retinoblastoma (87). Similarly, we have seen the inhibitive effects of activin upon the prostatic carcinoma cell line LNCaP (unpublished data), which expresses activin subunits and receptors (88). However, two other prostate cancer cell lines that we studied previously, PC3 and DU145, while they were found to co-express activin subunits and activin receptors (89, 90), activin did not inhibit cell proliferation (unpublished data). We have reported the expression of activin and its receptors in the following breast cancer cell lines: MDA-MB 231, MCF-7, T4-70, HS-578T, and ZR-75-1 (91). We have also reported that the rat osteogenic sarcoma cell line UMR-106 expresses both activin subunits and receptors (92). The ability of activin to act as an inhibitory factor in certain normal and

tumorigenic cell environments merits further investigation into the mechanism of this negative regulatory action.

Embryogenesis

Embryogenesis in Amphibians. The effects of activin on embryogenesis have been characterized fairly well in amphibians. In the past few years, many studies have been published that demonstrate that activin and the activin-binding protein follistatin play an important role in the induction of mesoderm and neural tissues. The majority of this work has been performed on Xenopus, in which activin and its receptors have been found to be expressed in the embryo during induction. Recent studies have finally begun to tackle the role of activin in mammalian systems. Interestingly, studies in mice have indicated that the embryos lack activin and express defective forms of either follistatin or type II activin receptors, yet there is no observed hindrance in mesodermal formation or neural differentiation.

Mesoderm induction is the first of many inductive interactions that occur during amphibian development. The early embryo of Xenopus is radially symmetrical and divided into two germ layers: ectoderm in the animal hemisphere and endoderm in the vegetal hemisphere. A third germ layer, the mesoderm, is induced in the equatorial region from ectodermal cells. These cells are stimulated to acquire mesodermal fate and respond to signals from endodermal cells during the early blastula stage (93, 94). It has been shown that ectodermal cells retain the ability to respond to this inductive effect until gastrulation begins (94).

This process can be mimicked *in vitro* by the addition of several peptide growth factors, also called "mesoderm-inducing factors" (MIFs), to cultured Xenopus animal cap explants. Animal cap cells would normally follow an ectodermal fate and become epidermis, but in the presence of MIFs, they form mesodermal tissues including notochord and muscle, as well as neural tissue and eyes. The MIFs that have been identified so far are either members of the FGF family or the TGFβ family. As the most potent inducers, activins can induce mesoderm in an axial pattern when applied to cultured animal caps and can induce the formation of a partial second body axis in Xenopus embryos when expressed ectopically (95).

In vivo experimentation has shown that the expression of a dominant negative receptor for activin blocked the formation of mesoderm for axial structures (96), suggesting that "activin-like" proteins, which are present maternally, act as mesoderm inducers (97). These maternal "activin-like" proteins are three isoforms of activin (A, AB, and B) but with slight variation in cellular content. Activin mRNA is expressed in the follicle cells which are arranged as an epithelial layer around growing oocytes but not in oocytes. Additionally, maternal activin mRNA is not detectable in fertilized eggs (95), which implies that activin proteins are synthesized by other cells including follicular cells and then transferred to the oocytes. We know that mesoderm induction is initiated around the early blastula stage (95), when neither the activin βA nor βB gene have been expressed; thus, it is probable that maternal activin molecules are mobilized at the crucial moment to start mesoderm induction. Furthermore, mRNA encoding follistatin—a protein that when bound to activin forms an inactive complex—is expressed in Spemann's organizer and can be found during the gastrula stage of development (98, 99). It is thought that follistatin may play a role in suppression of the action of activins in early embryos.

A Xenopus activin receptor, XAR1, was found to be expressed maternally and zygotically. In the early stages of embryogenesis, the maternal RNA is distributed equally throughout the embryo. Injection of synthetic mRNA coding for the activin receptor disturbs mesoderm induction and axis formation, demonstrating that regulated expression of XAR1 is essential for normal development (96).

Embryogenesis in Mammals. The well-established role of activin as a regulator of early amphibian development introduced the possibility that activin might also play a regulatory role in the development of higher vertebrates. Activin induced the homolog of the homeobox gene goosecoid in the 6.4-day mouse epiblast, which suggests that activin has a regulatory function in early mammalian gastrulation (100). In mice, both activin βA and activin βB subunits are expressed zygotically before implantation (101) and expression declines after implantation. The level of transcription is high in the deciduum, the maternal region of the placenta, when the mesoderm is formed (10). During organogenesis in 12- to 20-day *post coitum* rat embryos, the βA subunit of activin was localized in the heart, brain, skin, and skeletal tissues, while the βB subunit was found in the brain, salivary glands, and gonads (103). Messenger RNA coding of the βA subunit has also been found in bone marrow (104). Three activin subunits are expressed in the midgestational human fetal adrenals (105) and testes (106), but contain predominantly the α subunit. Recently, it has been demonstrated that activin receptor II and IIB transcripts are expressed in developing neural and muscular tissues and some exocrine glands in humans. This suggests that these tissues are targeted by activin (107). In addition, further studies have reported that mRNA encoding activin βA and βB subunits and follistatin are co-expressed with the receptors in several extragonadal tissues. Specifically, in the nervous system activin subunit transcripts are expressed in cerebrum and spinal cord. The βA subunit of activin was expressed intensely in heart muscle, while in skeletal muscle this expression was less intense. Follistatin was found to be expressed strongly in the spinal cord but weakly in the cerebrum and cerebellum. In heart and skeletal muscle, the activin-binding protein also exhibited an expression pattern opposite to that of activin. Activin subunits and follistatin were also found to be located in developing kidneys, salivary glands, and livers. These data suggest that an activin-follistatin system is acting locally and at multiple sites during early development (100).

In vivo investigations into the function of mammalian activins have been performed on mice with mutations in the activin βA, βB, or in both βA and βB subunits. Activin βA-deficient mice were observed to develop to term but died within 24 hr of birth. These mice lacked whiskers and lower incisors, and had cleft palates, demonstrating the possible role of the activin βA subunit in craniofacial development (108) and that zygotic expression of activin is not essential to mesoderm induction. Mice lacking the βB subunit suffered from distinct developmental and reproductive defects. βB-deficient mice did not develop normal eyelids; eye lesions resulted from the failure of eyelid fusion in late development. The reproductive ability of female βB-deficient mice was greatly impaired, while male mutants bred normally (109). The results of these data indicate that neither maternal nor zygotic expression of activin are necessary for mesoderm induction. The absence of both activin βA and βB subunits results in manifestation of the individual defects of the subunits without any additional defects (108). In contrast to lower vertebrates such as Xenopus, the zygotic expression of activins is not a prerequisite for mesoderm formation in mice. Thus, from this series of recent experiments we see that (i) activin, in any form, is not required for mesoderm induction; and (ii) the lack of one activin subunit is not compensated for by another subunit.

Tests could not be performed on female mice deficient in the βA subunit to determine whether they produce embryos lacking mesoderm because the mutant mice died within 24 hr of birth. Activin A derived from the deciduum, which is activin A from a maternal origin, could still act as an inducer. A test of this hypothesis was performed by generating a null mutation in the activin type II receptor (ActRcII); if activin A was necessary for mesoderm induction, then an embryo lacking a type II activin receptor would not bind activin and therefore no mesoderm would form. If, however, decidual activin was required for mesoderm formation, then these embryos would exhibit defects similar to those of activin-deficient mice. A series of experiments of this nature resulted in mice that formed mesoderm but had defects different from those of activin-deficient mice. A small number of ActRcII-deficient mice had skeletal and facial abnormalities similar to Pierre-Robin syndrome in humans, but the majority of these mice developed into adults. The effects seen in adulthood were the suppression of FSH secretion and defective reproduction. These findings confirm the role of ActRcII in activin signaling pituitary gonadotrophs, but the lack of phenotypic congruence between ActRcII-deficient mice and activin-deficient mice suggests that activins are not signaling through type II activin receptors during embryonic development (110).

However, overexpression of activin subunits, receptors, and truncated receptors in Xenopus has been shown to induce mesoderm by virtue of interactions with other TGFβ-related proteins and their receptors (108). Thus, the identification of the specific receptors expressed at the time of mesoderm induction will elucidate which TGFβ superfamily members are involved in this process. Additionally, the lack of overlap between phenotypes of activin-deficient and ActRcII-mutated mice raises the question of relevance of activin to its receptors and calls for a better understanding of the biological specificities of these molecules.

Follistatin. Follistatin is an activin-binding protein and activin antagonist *in vitro* (111). *In vivo,* follistatin may act in the presentation of activin to its receptors (112). In mice, messenger RNA is first expressed in the deciduum and then later in the hindbrain, somites, vibrissae, teeth, epidermis, and muscle (99). In studies on Xenopus, the overexpression of follistatin results in the induction of neural tissue (113). A recent study using mice homozygous for a mutation in follistatin has shown that follistatin-deficient mice experience retarded growth, exhibit decreased mass in the diaphragm and intercostal muscles, have shiny, taut skin, and manifest several defects of the hard palate and the thirteenth pair of ribs. Additionally, whiskers and teeth develop abnormally and the mice have difficulty with breathing, resulting in death shortly after birth. These defects are much more wide-ranging than the defects in activin-deficient mice, and this indicates that follistatin may be involved in the modulation of other members of the TGFβ family as well (99).

Despite the analysis of mesoderm-inducing factors and gene knock-out experiments, the need has been expressed for a comprehensive understanding of mesoderm induction and the signals for its initiation. These questions must be addressed and sufficiently answered to complement the numerous studies of embryo development (114).

Conclusion

Activin, inhibin, and follistatin are widely expressed and biologically diverse in their effects on cell growth and differentiation in many organisms. The pervasive nature of the expression of the α, βA, and βB-subunits and activin receptors has called for further investigation into the characterization of these growth factors in cell proliferation and differentiation at various stages of development. These experiments have demonstrated that activins and inhibins, along with follistatin, act in the modulation of cell growth and differentiation. The observed inhibitory effect of activin in particular warrants continued investigation in order to characterize the mechanisms of these cellular effects in normal and cancerous cells and to better understand the roles that these growth factors play in the autocrine and paracrine stimulation of cell growth, especially at the molecular level.

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