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Activins and Inhibins in Endocrine and Other Tumors

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Inhibin and activin are members of the TGFβ superfamily of growth and differentiation factors. They were first identified as gonadal-derived regulators of pituitary FSH and were subsequently assigned multiple actions in a wide range of tissues. More recently, the inhibin α subunit was considered as a tumor suppressor based on functional studies employing transgenic mouse models. This review evaluates the functional and molecular evidence that the inhibin α subunit is a tumor suppressor in endocrine cancers. The evaluation highlights the discrepant results from the human and mouse studies, as well as the differences between endocrine tumor types. In addition, we examine the evidence that the activin-signaling pathway is tumor suppressive and identify organ-specific differences in the actions and putative roles of this pathway in endocrine tumors. In summary, there is a considerable body of evidence to support the role of inhibins and activins in endocrine-related tumors. Future studies will define the mechanisms by which inhibins and activins contribute to the process of initiation, promotion, or progression of endocrine-related cancers. (*Endocrine Reviews* 22: 836–858, 2001)

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I. Introduction

INHIBIN AND ACTIVIN subunits are present in numerous human tissues of both endocrine and nonendocrine organs. The dimeric proteins formed from the subunits are members of the TGFβ superfamily of growth and differentiation factors and were isolated and characterized as gonadal-derived regulators of FSH synthesis and secretion. Inhibins are dimers of an α subunit and either a β_A or β_B subunit, whereas activins are homo- or heterodimers of the β_A or β_B subunits. To add to the complexity of this subgroup, three additional activin β subunit proteins were described, and there is a growing family of activin and inhibin binding proteins, receptors, and signaling molecules.

Since the first description of these proteins as regulators of FSH, multiple actions were assigned to them in a variety of tissues. In addition, the role of the inhibin α subunit gene as a tumor suppressor was investigated in mouse models. Measurement of inhibin is used clinically to detect and monitor human ovarian tumors. The aim of this review is to evaluate the evidence suggesting that the inhibin α subunit gene is tumor suppressive. The contribution of activin and its signaling pathway to malignant progression is also evaluated. Evidence from transgenic mouse models is presented, together with a summary of the genetic mutations in human cancers that involve the chromosomes on which the activin and inhibin subunit genes reside. The literature reporting the detection, action, and role of activin and inhibin in endocrine tumorigenesis, including the prostate and gonads, is reported. The conclusion from this review is that there is a substantial body of evidence to support the hypothesis that both inhibin and activin contribute to tumorigenesis.

Abbreviations: ActRI, Type I activin receptor; ActRII, type II activin receptor; BMP, bone morphogenetic protein; FAST, forkhead activin signal transducer; h, human; GCT, granulosa cell tumor; InhBP, inhibin binding protein; LOH, loss of heterozygosity; R-smad, receptor-regulated Smad; SARA, Smad anchor for receptor activation; SV40, simian virus 40.

II. Activin and Inhibin: Members of the TGFβ Superfamily

Inhibin and activin are members of the TGFβ superfamily of growth factors, which includes bone morphogenetic proteins (BMPs) and Müllerian inhibitory substance. Currently, over 45 members of this family have been identified (1, 2). Structural similarities between inhibin, activin, and other members of the TGFβ superfamily are based on the conservation of the cysteine spacing within each subunit and the

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disulphide linkages between two subunits that form the characteristic cysteine knots. Other similarities relate to dimer formation, the location of the bioactive peptide in the carboxyl-terminal region of the precursor molecule, and the similarities in their intracellular signaling mechanisms (3, 4).

Inhibin consists of two partially homologous disulfide-linked subunits (α and β_A or β_B, thus inhibin A and B), whereas activin is a dimer of disulfide-linked β subunits (activin A, B, AB). Another subset of activin β subunits (β_C, β_D, and β_E subunits) were identified, based on their homology to the β_A and β_B subunits (5–9). The β_C subunit dimerizes with itself and the β_A and β_B subunits *in vitro* to form activin C, AC, and AB (10). The formation of inhibin C ($\alpha\beta_C$ dimers) requires both cellular colocalization and dimerization of α and β_C subunits, but *in vitro* studies have shown that the β_C subunit does not dimerize with the α subunit (10). Activin β_D (6) and β_E (9) subunits were isolated from *Xenopus* and mouse cDNA libraries, respectively. The activin β_E subunit shows close similarity to the activin β_C subunit in terms of genomic organization and chromosomal localization, amino acid sequence identity, and tissue expression patterns (8, 9). In addition to the structural similarities, a common *in vitro* bioactivity, namely mesoderm induction, was identified (6), although mice bearing functional deletion of the activin β_C and/or β_E subunit genes did not show developmental defects and were phenotypically normal (11).

Inhibin and activin were originally isolated from follicular fluid as a range of molecular weight forms, consisting of variously processed precursor forms (12). The α and β inhibin and activin subunits are synthesized as full-length precursor proteins. The inhibin α subunit precursor protein consists of three regions, Pro, α_N, and α_C, whereas the activin β subunit precursors consist of two regions, Proβ and β. Proteolytic cleavage at dibasic or polybasic proteolytic cleavage sites occurs intracellularly as well as in serum (13, 14).

Studies in which the proteolytic cleavage sites in both α and β_A subunits were modified by site-directed mutagenesis showed that the full-length inhibin or activin dimers were inactive in a pituitary cell bioassay, whereas the truncated dimers were bioactive (15). Intermediate processed forms of the precursor α or β_A subunits showed some activity. *In vitro* studies demonstrated that high molecular weight forms of inhibin present in serum were processed to 30-kDa inhibin (14). Activin A was present in serum as the mature 25-kDa dimeric form (16).

Sensitive immunoassays are now available for detecting all α subunit-containing forms of inhibin, including Pro-αC, as well as dimeric inhibin A, inhibin B, and activin A, in serum (17–20). These assays have been used to explore the role of inhibin and activin in normal physiological processes and in endocrine diseases (see *Section IV*). Immunoassays for examining activins B–E in serum or tissue are not currently available.

A. Activin receptors and signaling

At the cell surface, activin ligands interact with a dual receptor system involving a family of transmembrane serine/threonine kinase receptors classed as type I or type II receptors (Fig. 1 and Ref. 21). Activin binding to the type II receptor (ActRII) leads to the recruitment of the type I receptor (ActRI) and the formation of a heteromeric complex. Formation of this complex induces phosphorylation of the ActRI, which leads to activation of the receptor-regulated

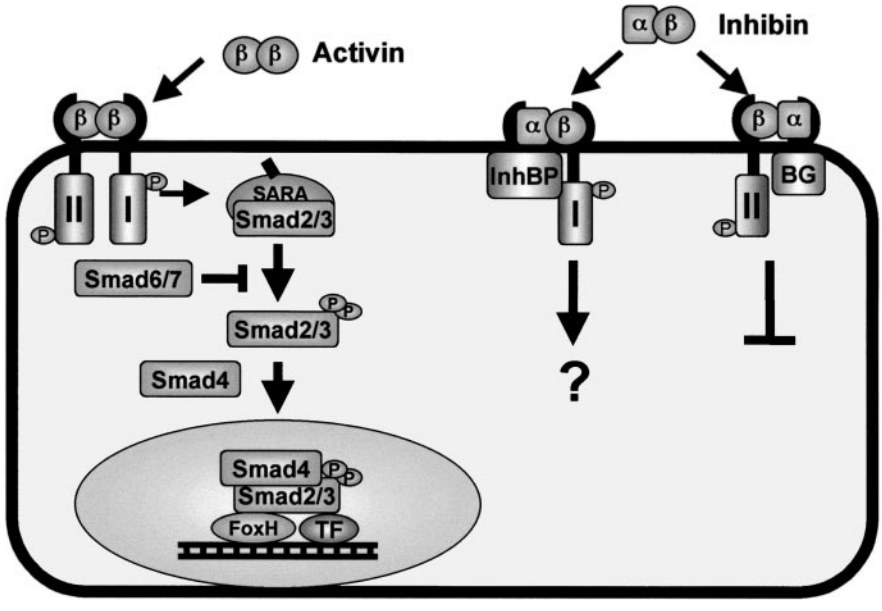

FIG. 1. Activin and inhibin signaling pathways. The binding of activin ligand ($\beta\beta$) to the type II receptor (II) initiates the activin signaling pathway and leads to recruitment of the type I receptor (I). SARA brings Smad2 into position, and phosphorylation of Smad2 by the activin/receptor complex liberates it from SARA. Smad2 recruits Smad4 and the complex locates to the nucleus, where it binds FAST/FoxH and other transcription factors to regulate the transcription of activin responsive genes. Inhibin potentially interferes with activin signaling by forming complexes with the activin receptors; the low affinity of inhibin for ActRII is enhanced by binding to betaglycan (BG). This complex does not activate a known signal cascade. It was suggested that binding of inhibin to the inhibin receptor (InhBP) leads to recruitment of the activin type I receptor. This complex may be responsible for initiation of a unique inhibin response.

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Smad [R-Smad (22–25)]. R-Smads are ligand specific, with Smad2 and Smad3 mediating activin and TGFβ signaling, and Smad1, -5, and -8 mediating BMP signaling (22). The interaction between the R-Smads and the receptor complex involves a membrane-bound protein named Smad anchor for receptor activation [SARA (26)]. After phosphorylation, the R-Smads are released and form heteromeric complexes with the Co-Smad, Smad4. The R-Smad and Co-Smad complex then translocates to the nucleus to regulate gene expression. A third class of Smads, the inhibitory Smads (Smad6 and Smad7), can antagonize the signaling events described above and can prevent access and phosphorylation of the R-Smads or interfere with the formation of the R-Smad/Smad4 complexes (2, 27, 28).

In the nucleus, Smads target specific gene promoters with low DNA binding affinity, and Smad binding alone is not sufficient for gene activation. Smads use members of the forkhead activin signal transducer (FAST) family (also called FoxH) as DNA binding partners to regulate gene transcription (29–31). Other transcription factors are likely to be involved in the Smad pathway, including *fos, jun*, and the vitamin D receptor (for review see Itoh *et al.*, Ref. 23). However, in contrast to FAST/FoxH, many of the other DNA binding partners can function independently of Smads, whereas FAST/FoxH target genes require Smad function for transcriptional activation (32). Thus, the binding of activin ligands to the membrane-bound receptor initiates a cascade of protein-protein interactions that controls gene expression and specific biological responses.

B. Inhibin receptors and signaling

The mechanism of inhibin action is still controversial, and the downstream signaling proteins in the inhibin signal transduction cascade are not as well characterized as those for activin. Although it was suggested that inhibin signals through its own receptor (33, 34), the identity of specific cell surface receptors for inhibin has been difficult to demonstrate. It was proposed that inhibin antagonized the action of activin through a dominant-negative mechanism involving the binding of inhibin to the activin receptor (35, 36). Betaglycan, a type III receptor for TGF-β, binds inhibin with high affinity and specificity, and together with ActRIIA can enhance the cell-membrane binding of inhibin (37). Theoretically, the binding of inhibin and betaglycan can block the effects of activin via its receptor (Fig. 1). More recently, Chong *et al.* (38) isolated another inhibin binding protein (InhBP or p120), which is expressed in the pituitary and testis. A key question to resolve from all these studies is whether or not betaglycan, InhBP, and ActRIIs are physiological receptors for inhibin. If this is the case, and if a cell expresses all of these proteins, there are three predictable outcomes or responses. First, activin binding to its receptor initiates activin signaling events leading to a biological response specific for activins. Secondly, inhibin binds to either the activin receptor or to betaglycan and blocks the activin response by generating a nonfunctional receptor complex. Thirdly, inhibin binds to InhBP and activates an inhibin transduction pathway, which elicits a response specific for inhibin ligands (39, 40). However, the evidence to support these predictions remains to be determined.

C. Binding proteins

The activin binding protein, follistatin, is a key inhibitor of activin action and is the subject of previous reviews (4, 41, 42). Essentially, follistatin was isolated on the basis of its inhibin-like ability to suppress FSH activity *in vitro.* This inhibitory activity was subsequently shown to be due to its ability to bind and neutralize activin with high affinity. Follistatin is structurally dissimilar to members of the TGFβ family. It is a product of a single gene forming a series of molecular weight forms by alternate splicing of the mRNA. The 288-amino acid and 315-amino acid forms of follistatin are the most common. Follistatin has structural homology with epidermal growth factor and a group of enzyme inhibitors of the Kazal family, which include secreted protein, acidic and rich in cysteine (SPARC), SC1/hevin, QR1, agrin, testican, and tsc36/FRP. These proteins contain a distinctive, follistatin-like, 10-Cys-containing module followed by an extracellular calcium binding domain. Follistatin prevented the mesoderm-inducing activity of activin in *Xenopus*, and by blocking activin signaling via activin receptors, follistatin induced neural differentiation (43). Follistatin also neutralized the mesoderm-regulating activities of other cytokines of the TGFβ superfamily (BMP-2, BMP-4, and BMP-7) that bind follistatin (43).

More recently, a novel follistatin-like protein (FSLP) with two, rather than three, follistatin domains was identified in the mouse (44). Follistatin-like protein binds activin and BMPs with high affinity and is expressed in a similar range of tissues, including liver and testis, to follistatin. Its biological role has not yet been determined.

Several other binding proteins were identified that may also influence inhibin and activin action. In addition to betaglycan and InhBP (discussed above), which bind inhibin with high affinity but not activin, α_2 macroglobulin forms high molecular weight complexes with inhibin and activin (45). However, no influence on the *in vitro* activities of either inhibin or activin was reported, and its potential role *in vivo* is not established.

D. Functional activities of inhibin and activin

Inhibin was originally isolated based on its ability to suppress FSH production and secretion by rat pituitary cells *in vitro*. FSH regulation appeared to be major biological role for inhibin, although at high concentrations, inhibin antagonized the actions of activin. Activin, in contrast, has a range of activities and is involved in bone growth, mesoderm induction in *Xenopus laevis* embryos, reproduction through the regulation of pituitary FSH production, nerve cell survival, wound healing, and tissue differentiation in pancreas, kidney, and heart. In some instances, activin action opposes that of inhibin; for example, activin is a potent stimulator of FSH release, suggesting that inhibin may be an antagonist of activin. Within the ovary, FSH in combination with activin caused

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a dose-related increase in DNA synthesis, suggesting that FSH in the presence of activin is mitogenic (46, 47).

It is generally considered that inhibin is an endocrine factor with a primary function of regulating FSH. Conversely, activin is believed to have local effects as a paracrine and/or autocrine factor (48). For further details on actions of inhibin and activin, the reader is referred to various reviews (3, 4).

III. Activin and Inhibin in Tumors

A. Functional evidence for a role of activin and inhibin in tumors

Matzuk *et al.* (49) made a significant contribution to our understanding of the role of activin and inhibin in cancer by reporting that the inhibin α subunit is a tumor suppressor gene with gonadal and adrenal specificity. Given the similarities in the TGFβ and activin transduction pathways and the recognized role of the TGFβ pathway in tumor suppression (50), it is reasonable to consider that the activin receptors and downstream signaling proteins are tumor suppressors. In the following sections, the evidence suggesting that both the inhibin α subunit gene and the activin signaling pathway are tumor suppressive will be reviewed.

1. Inhibin α subunit as a tumor suppressor. Both sexes of inhibin-deficient mutant mice generated by targeted deletion of the inhibin α subunit gene developed gonadal sex-cord stromal tumors with very high penetrance (49). The development of gonadal tumors was rapidly followed by a cancer cachexia-like wasting syndrome, which was associated with severe weight loss and pathology of the stomach and liver (51). After gonadectomy of these mice, tumors of the adrenal gland occurred (51). Both FSH and activin A levels were significantly increased in the serum.

In the absence of inhibin α subunit expression, the role of activin in tumor initiation and/or the onset of cachexia required consideration. The symptoms associated with cachexia were attributed to activin, mediated by the ActRIIA receptor signaling pathway (51, 52). Mutant mice deficient in both the inhibin α subunit and the ActRIIA genes developed tumors but did not suffer from an unusual weight loss and the stomachs and livers were histologically normal (52). This finding was consistent with the previous observation that systemic administration of activin A promoted similar cancer cachexia-like wasting symptoms (53). Furthermore, Cipriano *et al.* (54) showed that inhibin α subunit-deficient mice that also overexpressed follistatin continued to develop tumors, but the cachexia-like symptoms were reduced. Thus, the weight of evidence suggests that activins play a significant role in the onset of cachexia in inhibin-deficient mice.

The role of activin in tumor development is unclear. In the ovary, it was suggested that a combination of elevated gonadotropins and activins promoted tumor formation. Gonadotropins stimulate activin production by human (46) and rat (47) granulosa cells *in vitro*, and activin is mitogenic in sex cord-stromal tumor cell cultures from inhibin α subunit- and p53-deficient mice (55). However, the experimental evidence supporting a role for activin in tumor formation is unconvincing. First, mice deficient for both FSH and inhibin developed ovarian tumors, despite severely reduced serum activin levels (56). Second, overexpression of activin β subunits alone in male mice did not result in testicular tumors (57). Third, ovarian tumor formation was evident in inhibin α subunit and ActRIIA double mutant mice (52).

Ovarian transplant experiments provide additional evidence that activin is not essential for the initiation of gonadal tumors. The experiment described by Matzuk *et al.* (58) was designed to determine whether tumor initiation required an increased production of activin or the absence of inhibin in the circulation. Ovaries from 3-wk-old inhibin-deficient female mice were transplanted into the bursa of 3- to 4-wk-old wild-type immunocompatible female recipients. Either one or both of the ovaries from the recipients were removed from the bursa in which the inhibin-deficient ovaries were transplanted. If both normal ovaries were removed so that circulating levels of inhibin fell, the transplanted inhibin-deficient ovaries developed tumors and the mice became cachexic. If an inhibin-deficient ovary was transplanted into a mouse bearing a contralateral ovary so that circulating inhibin levels were maintained, no tumors developed. These results showed that the local production of activin within the transplanted inhibin-deficient ovaries was not sufficient for tumor development. Inhibin is an endocrine hormone that regulates gonadotropins; FSH levels were elevated in inhibin-deficient mice, and therefore, the role of gonadotropins requires consideration. Gonadotropins were essential for tumor formation, as mutant mice that lack both inhibin α and GnRH did not develop gonadal tumors (56, 59), and roles for both FSH and LH were considered.

Although inhibin α subunit mutant mice have elevated FSH levels, mice deficient in both inhibin α subunit and FSH showed evidence of ovarian tumor formation, albeit at a reduced level compared with α-deficient mutant mice (56). In addition, overexpression of FSH did not result in ovarian tumor formation (56). Alternatively, LH might be important in tumorigenesis because overexpression of LH *in vivo* was shown to cause ovarian granulosa and thecal tumors (60).

Huhtaniemi and colleagues (61) examined the role of gonadotropins in an alternate transgenic mouse model system that incorporated the simian virus 40 (SV40) T-antigen under the regulation of the inhibin α subunit gene promoter. The inhibin α subunit promoter targeted expression of the T-antigen to the gonads, and gonadal tumors arose. Removal of the gonads resulted in the formation of adrenal tumors. Huhtaniemi and colleagues (62–64) showed a role for gonadotropins in the development and growth of the T-antigen-induced tumors. Tumors did not develop after the withdrawal of gonadotropins using GnRH antagonists, by crossbreeding with gonadotropin-deficient *hpg* mice, or by long-term treatment with T. Further studies specifically implicated LH in adrenal tumorigenesis in this mouse model. After gonadectomy, tumors arose in the LH sensitive X zone of the adrenal gland, where inhibin α subunit is normally expressed (62–65).

Although the Matzuk and Huhtaniemi transgenic mouse models both illustrate that gonadotropins modify the role of inhibin in tumor formation, it is difficult to make direct comparisons between the two models. In one instance, loss of inhibin α subunit expression caused tumor formation in

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inhibin-deficient mice and was associated with elevated levels of activin and FSH in serum. On the other hand, tumor formation in the Hutaniemi model was driven by expression of the SV40 T antigen, under the regulation of the inhibin α subunit promoter. Therefore, the latter is not a model of inhibin deficiency (serum inhibin levels were elevated and FSH levels reduced); rather, it is a model in which the regulation of the inhibin α subunit promoter was studied. Likewise, it does not contradict the hypothesis that inhibin α is a tumor suppressor because tumorigenesis was driven by the SV40 T antigen.

Other factors were considered as modifiers of the role of inhibin in tumorigenesis. In male mice, the influence of androgens on tumor development was examined in transgenic mice that were inhibin α deficient and carried the testicular feminization mutation, *i.e.*, an inactivating mutation of the androgen receptor (66). Although testicular tumors continued to develop, multifocal lesions were observed at an earlier age and were less hemorrhagic at later stages. In contrast to the modifiers described above, Müllerian inhibitory substance synergized with the effects of inhibin α subunit loss to influence and promote a more rapid development of testicular tumors (67). Similarly, enhanced gonadal tumorigenicity occurred in double mutant mice lacking both the inhibin α subunit gene and the $p27^{Kip1}$ tumor suppressor gene. These mice developed ovarian and testicular tumors and died earlier than those mice lacking inhibin α alone (68).

Thus, the mouse models support the hypothesis that the inhibin α subunit is tumor suppressive. In addition, these studies reveal a complex network of interactions involving inhibin, activin, and other modifiers in the development and progression of gonadal and adrenal tumors.

2. Activin receptors and signaling proteins as tumor suppressors. The previous section examined the role of activin ligands in inhibin-deficient mice and identified an essential contribution of activins to the onset of cachexia rather than to the initiation of tumor formation. In this section, we consider the hypothesis that the activin signaling pathway is tumor suppressive and contributes to malignant progression. Activins share many common elements with the TGFβ signaling cascade, members of which are considered tumor suppressors, *e.g.*, Smad4/DPC4 (69). Several studies showed that the development of resistance to TGFβ by tumor cells represents a key event in the progression to malignancy, and in colon cancers, resistance to TGFβ was acquired through mutational inactivation of the TGFβ type II receptor (70).

However, little is known about alterations in activin signaling events during the development and progression of endocrine-related cancers. Overexpression of the activin β_A subunit was recorded in inhibin-deficient mice (71), but in normal mice, overexpression of this subunit alone did not lead to tumor formation (57). Up-regulation of TGFβ ligand expression occurred in colon tumors, but up-regulation of ligand expression, *per se*, was not considered to be a key event in malignant progression. Instead, malignancy was associated with the presence of inactivating mutations of the receptors, so that the tumor cells were insensitive to the growth-inhibitory actions of TGFβ. van Schaik *et al.* (72) investigated the levels of activin receptor expression in prostate carcinoma and showed that expression levels were reduced in malignant progression, but they did not determine whether inactivating mutations that might render the cells resistant to activin ligands were present. Although mutations of ActRIB were recently described in pancreatic cancers, it is not known whether these confer resistance to activin (73). It may prove insightful to compare changes to the activin signaling pathway with those of the TGFβ signaling pathway. Many studies on activins focused on the changes to ligand expression. For example, down-regulation of activin subunit production by N-*myc* was reported recently in neuroblastoma cell lines (74), and the authors concluded that this would deprive the cells of a signal from this growth-inhibitory factor. Whether there is up- or down-regulation of activin β subunit expression and therefore activin ligand levels, as described above, it is unlikely that ovarian tumor formation is primarily due to elevated levels of activin A or B in the inhibin-deficient mouse model.

The role of the other subset of activin β subunits, β_C, β_D and β_E, in tumorigenesis remains unknown. Although it was demonstrated that the activin β_C subunit dimerized with the activin β_A and β_B subunits (75), a functional role for these heterodimers has yet to be established. Mice with null mutations in either the individual activin β_C or β_E subunit genes or both genes develop normally and have no obvious abnormalities in liver or reproductive function (11). The resultant effects of overexpressing these subunits are not known.

B. Molecular genetics of activin and inhibin gene loci

Malignant transformation and progression involves a complex series of genetic alterations that change the gene expression profile of the cells and promote tumor survival. Chromosomal regions harboring tumor suppressor genes are often deleted or down-regulated in cancer, whereas oncogenes and genes that promote cell survival are often localized to chromosomal regions over-represented in human cancers.

In the previous section, we reviewed the evidence suggesting that the inhibin α subunit gene and/or the activin signaling pathway are tumor suppressive. To support this hypothesis, it would be predicted that genetic alterations to the chromosomal regions housing these genes would occur in malignancy. The chromosomal localization of the human inhibin- and activin-related genes was determined over the last decade. This section of the review provides a synopsis of the chromosomal localization of the inhibin and activin subunit genes and the genes that encode follistatin and members of the activin signaling cascade (Table 1). The recent literature is reviewed to determine whether mutations were commonly observed for these chromosomal regions in endocrine cancers and cancers of the liver and kidney (those discussed in *Section IV*).

The long (q) arm of chromosome 2 is of particular relevance to inhibin and activin because, among other genes, it houses the genes for the inhibin α subunit (76), activin β_B subunit (76), ActRI (77), and ActRII (76), and mutations on this chromosomal arm have the potential to alter the expression of any or all of these genes. Deletion or loss of heterozygosity (LOH) on chromosome 2q was described in

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TABLE 1. Chromosomal localization of activin/inhibin subunits, receptors, and signaling proteins

Chromosome	Gene	Chromosomal location	Reference
2q	Activin-β_B	2qcen-q13	76
	ActRII	2q22.3→q23.2	85
	ActRI	2q23-q24	77
	Inhibin-α	2q33-36	76
3p	ActRIIB	3p22-21.3	85, 86
5q	Follistatin	5q11.2	85
7p	Activin β_A	7p15-p14	76
12q	Activin β_C	12q13.1	7
	Activin β_E	12q13.1[a]	9
	ActRIB	12q13	77
15q	Smad3	15q21-22	113
	Smad6	15q21-22	113
18q	Smad2 (MADH2)	18q21.1	88
	Smad4 (DPC4)	18q21.2	87
	Smad7 (MADH7)	18q21.1	89

[a] Possible localization as determined by close linkage with the activin β_C gene reported for the mouse genome (9).

many human tumors (Table 2). In ovarian carcinomas, 2q deletion was associated with aggressive, advanced-stage disease (78, 79). LOH at 2q21 was detected in 7.7 and 7.1% of adenomas and borderline tumors, respectively, whereas 33% of invasive ovarian carcinomas had LOH in this region (78). LOH at 2q33 occurred in 33% of ovarian carcinomas and in only 6% of granulosa cell tumors [GCTs (79)]. In prostate carcinoma, there was frequent loss of chromosome 2q (80), and genome-wide linkage analysis of siblings with prostate carcinoma identified 2q as a candidate region in hereditary forms of this disease (81). Down-regulation of inhibin α subunit gene expression occurred in human tumors, including prostate carcinoma (75) and a subset of ovarian GCTs (82, 83). Loss of expression was associated with LOH at the 2q33 locus, and hypermethylation of the inhibin α subunit gene promoter in prostate cancer (J. F. Schmitt and G. P. Risbridger, unpublished data). Hence, there is accumulating evidence that the inhibin α subunit is a tumor suppressor gene. As well as the inhibin α subunit, activin β_B subunit protein levels were down-regulated in prostate cancer (72). Over-representation of chromosome 2q was less commonly observed and was reported to occur in 19% of ovarian carcinomas (84).

In addition to loss of chromosome 2q, allelic loss on chromosome arms 3p and 18q was commonly observed. These regions house the genes for a number of activin signaling molecules, including ActRIIB at 3p22 (85, 86) and Smad2, -4, and -7 at 18q21 (87–89). Losses of these regions were reported for a number of endocrine tumors (Table 2). In breast cancer, loss or LOH involving chromosome 3p was often reported (90–104), although one group identified a gain involving this region (92, 105). Smad4 was identified as a tumor suppressor gene in pancreatic carcinomas, as it was mutated or deleted in more than 90% of pancreatic tumors (69, 87, 106–112). In ovarian cancer, LOH at 18q21 occurred in 21% of invasive carcinomas but not in ovarian adenomas or borderline tumors (78). Over-representation of the 18q locus was uncommon in endocrine tumors. Hence, losses on chromosomes 3p and/or 18q, which include the regions housing the ActRIIB gene and the Smad genes, would disrupt the activin signaling pathway and provide the tumor cells with resistance to the effects of activin.

Table 2 shows that both chromosomal loss and over-representation were observed in endocrine tumors for the chromosomal regions 5q, which harbors the follistatin gene [5q11.2 (85)]; 7p, which harbors the activin β_B subunit gene [7p15–14 (76)]; 12q, which harbors the activin β_C [12q13.1 (7)]; ActRIB [12q13 (77)] and possibly the activin β_E genes [12q13.1 (9)]; and 15q, which harbors the Smad3 and Smad6 genes [15q21-q22 (113)]. In ovarian adenomas, LOH at 5q21–22 occurred in 30% of invasive carcinomas, whereas LOH at a nearby region, 5q32, was seen in only 7.7% of invasive carcinomas (78). Over-representation of chromosome 5q occurred in adrenocortical tumors (114), breast cancer (91, 115, 116), and prostate cancer (117).

Deletions at chromosome 12q occurred in prostate (80) and breast carcinomas (118). In prostate carcinomas, the levels of expression of ActRIB mRNA was reduced with relation to nonmalignant prostate tissues (72), and in pancreatic carcinomas, mutations of ActRIB were described (73). Breast carcinoma and adrenocortical cancers demonstrated gain of chromosome 12q (92, 105, 114, 119).

The 15q chromosomal region was deleted in pancreatic cancer (120) and over-represented in breast cancer (91, 92, 105). In prostate cancer, both loss and gain of this region was reported (121).

The 7p locus was over-represented in tumors of the breast (122) and prostate (80, 123). In prostate cancer, Alers *et al.* (80, 124, 125) reported that increased chromosome copy number for chromosome 7 was associated with recurrent and metastatic prostate carcinoma. Losses or deletions involving 7p only occurred in breast cancer (122, 126).

The above summary details the chromosomal localization of the inhibin and activin subunit genes, the follistatin gene, and the genes that encode members of the activin signaling pathway. Many of these chromosomal regions are altered in endocrine tumors; losses on chromosomes 2q, 3p, and 18q are more common than gains of these chromosomal regions. Both loss and over-representation of chromosomal regions 5q, 7p, 15q, and 12q occur in endocrine tumors. Although these observations do not specifically implicate the inhibin and activin genes, they do provide impetus for further studies to identify genetic alterations in tumorigenesis of the inhibin/activin subunit genes and the genes encoding the downstream signaling effectors of activins.

IV. Endocrine and Other Tumors

A. Ovarian tumors

The mature ovary consists of two main regions, the outer cortex, containing the germinal epithelium and follicles, and the medulla, consisting of stroma. During the menstrual cycle, primordial follicles are recruited and either develop in the follicular phase to mature follicles destined to ovulate, or degenerate as a result of atresia. After ovulation, when the ovum is released, the remaining cells of the granulosa and theca interna undergo luteinization. The corpus luteum persists through the luteal phase, but in the absence of pregnancy, it undergoes degeneration and the cyclicity continues.

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TABLE 2. Genetic changes in chromosomal regions harboring activin related genes in endocrine cancer[a]

Chm	Losses or mutations	Ref.	Gains	Ref.
2q	Adrenocortical carcinoma 42%	114	Ovarian cancer, advanced grade 19%	84
	Breast carcinoma 40%	90, 91, 105, 118		
	Breast lymph node metastases	92, 105		
	Hepatocellular carcinoma >35%	127		
	Ovarian carcinoma 30–33%	78, 79		
	Ovarian granulosa cell tumors 6%	79		
	Prostate carcinoma 18–45%	80, 81, 121, 128		
	Testicular recurrent germ cell tumors >40%	129		
	Uterine endometrioid carcinoma 13%	130		
3p	Adrenocortical carcinoma 50%	114	Breast carcinoma	92, 105
	Breast carcinoma up to 77%	90–104, 131		
	Pancreatic carcinoma 23–60%	106–108, 132		
	Prostate carcinoma 89%	133		
	Renal cell carcinoma 84%	93		
	Testicular germ cell tumors >40%	129		
5q	Breast carcinoma 40–86%	90, 118, 134, 135	Adrenocortical cancer 42%	114
	Ovarian cancer <11%	78	Breast carcinoma	91, 115, 116
	Prostate carcinoma 10–39%	80, 117, 121, 136	Prostate 38%	137
7p	Breast carcinoma 7–36%	122, 126	Breast carcinoma 12%	122
			Prostate carcinoma	80, 124, 125
			Prostatic intraepithelial neoplasia 20%	123
12q	Breast carcinoma 40%	118	Adrenocortical cancers 42%	114
	Prostate carcinoma 20%	80	Breast carcinoma 20–50%	92, 105, 119
15q	Prostate cancer (39%)	121	Breast carcinoma	91, 92, 105
	Pancreatic carcinoma (60–70%)	120	Prostate carcinoma	138
18q	Adrenocortical cancer 33%	114		
	Breast carcinoma 11–42%	90, 96, 97, 139–145		
	Ovarian carcinoma 13–23%	78, 84, 139		
	Pancreatic carcinoma >90%	69, 87, 106–112		
	Prostate carcinoma 17–45%	117, 136, 146–149		
	Prostatic intraepithelial neoplasia 19%	123		
	Testicular germ cell tumors >40%	129		

[a] The table includes data published since 1995 and reports LOH and loss or gain of chromosomal regions limited to those regions on which the genes for the activin/inhibin subunits, receptors, and signaling proteins are found. Chm, Chromosome.

As the primordial follicle enters folliculogenesis to form preantral and then antral follicles, the follicles become sensitive to gonadotropins and synthesize inhibin α, β_A, and predominantly β_B subunit proteins in the granulosa cell layers surrounding the oocyte (150–152). In the late luteal and early follicular phases of the menstrual cycle, FSH levels increase. Under FSH stimulation, serum inhibin B is preferentially increased until negative feedback occurs, and FSH levels fall before ovulation. The dominant follicle, which is now responsive to LH, produces more inhibin A than B. After the LH surge, the corpus luteum produces inhibin α and β_A subunits, as reflected in elevated serum inhibin A in the luteal phase of the cycle. Thus, there is differential expression of the inhibins during the menstrual cycle.

The ovary has a finite number of follicles that can lead to ovulation, and the follicles are essentially depleted during the fourth decade of life. With the decline in developing follicles, serum inhibin B levels fall. This is followed by a decline in the levels of inhibin A and E2 in the serum. There is a corresponding increase in FSH and LH that can be elevated up to 100 times the levels found before menopause. After menopause, serum levels of inhibin A, B, and α subunit are very low or undetectable (4, 42, 153–155).

Tumors of the ovary arise from surface epithelium, germ cells, and sex-cord stroma. Malignant surface epithelial tumors of the ovary are the most common and include serous, endometrioid, mucinous, and clear-cell carcinomas, which represent 40, 20, 10, and 6% of ovarian cancers, respectively. Sex-cord stromal tumors, including GCTs, represent approximately 5% of ovarian cancers, and malignant germ cell tumors, including teratomas, represent approximately 1% of ovarian cancers.

1. Sex-cord stromal tumors. Inhibin was developed and successfully used as a serum and immunohistochemical marker of GCTs and for the early detection and monitoring of recurrence of GCTs (20, 156–167). The presence of inhibin α, β_A, and β_B subunit mRNA (168, 169) and protein by immunohistochemistry (168, 170–182) in GCT tissues was widely reported (Tables 3 , 4 , and 5). Other sex-cord tumors, including Sertoli cell and mixed Sertoli cell/Leydig cell tumors, were also positive for inhibin α and β_A subunit proteins (Ref. 176 and Table 3). Fibroma and thecoma (thecal cell tumors) were immunopositive for the α subunit but not for the β_A subunit. Immunohistochemical identification of inhibin has been clinically used to differentiate sex-cord stromal tumors and other tumors such as endometrioid carcinomas (176, 183).

All forms of inhibin (inhibin A and B, Pro-αC, and other inhibin α subunit-containing forms) were significantly elevated in serum in women with GCTs, and these assays were used for the early detection of the disease and monitoring its recurrence after surgery (see Table 5 for reference list). The clinical applications of inhibin are considered below.

The role of inhibin (and activin) in the formation and progression of ovarian GCTs is not known. The data from the inhibin-null mice suggested that loss of inhibin α subunit

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TABLE 3. Detection of inhibin α and β subunit mRNA in ovarian tumors[a]

Tumor	α subunit mRNA	β_A subunit mRNA	β_B subunit mRNA	ActRII mRNA	Follistatin mRNA
GCT	4/4	4/4	4/4	4/4	4/4
Serous					
Adenoma	5/6	6/6			
Borderline	5/9	7/9			
Carcinoma	9/24	19/24	4/4	4/4	4/4
Mucinous					
Adenoma	5/6	6/7	1/1	1/1	1/5
Borderline	4/8	7/8	1/1	1/1	1/5
Carcinoma	8/11	10/11	3/3	3/3	3/3
Endometrioid	2/5	4/5			

[a] These data show that inhibin α and β subunit expression is readily detected in GCTs, whereas in epithelial tumors, β subunit expression is higher than that of the inhibin α subunit. The presented data are the combination of several studies (168, 169) expressed as the proportion of tumors examined that show detectable expression levels.

TABLE 4. Detection of inhibin α and β subunits by immunohistochemistry in ovarian tumors[a]

Tumor Type	Antisera				
	α subunit[b]	α subunit[c]	β_A subunit[c,d]	β_B subunit[c]	Pro[e]
Sex-Cord Stromal Tumors					
GCT	275/277	14/14	17/21		6/6
Sertoli/Leydig	99/102		6/6		
Sclerosing stromal	36/45		1/3		
Fibroma/thecoma	69/108		0/5		
Epithelial Cell Tumors					
Serous					
Adenoma	0/14	0/4	10/10	4/4	
Borderline	0/12	0/1	8/9	1/1	
Carcinoma	1/98	0/13	55/77	10/10	0/2
Mucinous					
Adenoma	0/26	8/8	13/13	8/8	
Borderline	0/10	4/4	10/11	4/4	
Carcinoma	2/49	3/10	28/31	5/5	0/2
Endometrioid	5/69		17/29		0/2
Miscellaneous	23/182		9/17		1/3

[a] The inhibin α and β subunits were detected in the majority of sex-cord stromal tumors, whereas the β subunits were readily detected in the epithelial tumors. Controversy exists over the detection of the inhibin α subunit in mucinous tumors. The presented data is the combination of several studies expressed as the proportion of tumors examined that show detectable immunoreactivity. The following antibodies were used: [b], R1 antiserum (168, 170–182, 188); [c], Vale α, β_A, β_B subunit antiserum (176, 188); [d], β_A antiserum (E4) (168, 170, 171); and [e], Pro (INPRO) antiserum (5).

TABLE 5. Detection of serum inhibin and activin in women with ovarian granulosa and epithelial cell tumors[a]

Tumor	Total inhibin	Total inhibin	Pro-αC subunit	Inhibin A (Groome)	Inhibin A (Medgenix)	Inhibin B	Activin A	CA125
	RIA	IFMA	ELISA	ELISA	ELISA	ELISA	ELISA	
Granulosa cell	11/11	11/11	10/11	9/15		17/18	9/13	
Serous	21/65	19/65	10/66	3/66	12/15	1/66	4/8	
Mucinous-borderline	13/17	17/17	9/13	1/13		9/13		9/17
Mucinous-carcinoma	6/8	7/8	2/8	3/8		3/8		5/8
Mucinous-borderline and carcinoma	19/25	24/25	11/21	4/21	13/13	12/21	2/3	14/25
Endometrioid	4/11	5/11	3/10	2/11		1/11	0/6	8/11
Miscellaneous	15/35	20/35	10/35	3/35	10/11	4/35	0/6	20/34
Nonovarian	5/23	4/23	3/23	1/23	0/10	1/23		15/19

[a] The inhibin α subunit-directed (total) inhibin assays are more sensitive in detecting granulosa cell and mucinous tumors compared to the more specific inhibin and activin assays. The data are pooled from several studies and are presented as the proportion of women with ovarian cancers in whom the serum inhibin/activin levels are greater than those for normal postmenopausal controls (20, 158, 160, 161, 165, 167, 191, 192). IFMA, Immunofluoremetric assay.

expression, in combination with elevated gonadotropin and activin levels, was a key factor in GCT development. In contrast, women with GCTs have elevated serum inhibin levels and suppressed FSH levels, suggesting that alternate mechanisms of tumor formation apply in the mouse α subunit-knockout model and human GCTs. In two studies, loss or down-regulation of inhibin immunoreactivity in a subset of GCTs was reported; in one study, there was a correlation with reduced patient survival (82), whereas the other study failed to show a correlation with disease-free survival (83).

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2. Surface epithelial cell tumors. Epithelial cell carcinomas represent 90% of all ovarian tumors. These include serous, mucinous, and endometrioid tumors and are each subclassified as benign, borderline, or malignant. The origins of these tumors are unclear but are thought to involve invagination of the germinal or surface epithelium of the ovary with the formation of inclusion cysts as a consequence of the repeated trauma induced by the ovulation process. The progression of these tumors is believed to proceed either directly, or via a benign and borderline intermediate stage, to malignancy (184–187). The relative importance of the two pathways is still unclear (186). In the case of mucinous tumors, the close physical proximity of benign epithelium with an apparent intermediate transition stage suggested that this cancer originated from pre-existing mucinous adenomas. The much lower association of benign serous adenomas with serous carcinomas suggests that *de novo* formation may be the major cause (185). The role of activin and inhibin in the progression to malignancy is poorly understood because most of the studies to date center on defining the utility of inhibin in the diagnosis of ovarian tumors.

Mucinous carcinomas represent 15% of all malignant ovarian tumors and histologically resemble endocervical or enteric epithelium. Expression of the inhibin and activin α, β_A, and β_B subunits was demonstrated in benign, borderline, and malignant mucinous tumor tissues by RT-PCR (Table 3 and Refs. 168 and 169). Numerous immunohistochemical studies examined the activin β_A and β_B subunits in mucinous ovarian cancers and detected these proteins in more than 90% of the tissues (Table 4 and Refs. 170, 171, 174, 188, and 189). The specific localization of the inhibin α subunit to the malignant epithelium is controversial and variable and appears to be related to differences in antisera/fixation methods used by the various groups. Table 4 summarizes the data reporting differences in the detection of inhibin α subunit immunoreactivity in mucinous tumors using different antisera. Serum inhibin α subunit levels were consistently elevated in up to 90% of postmenopausal women with mucinous cancers, and this observation was of diagnostic value (20, 166, 167). Dimeric inhibin and activin serum levels were also increased (167, 190–192).

Serous carcinomas are the most common ovarian tumor (40%). These carcinomas resemble epithelial cells of the fallopian tube. The activin β_A and β_B subunit mRNAs, and to a lesser extent the inhibin α subunit mRNA, were detected by RT-PCR in serous carcinomas (Table 3 and Refs. 168 and 169). However, the cellular localization of these mRNAs by *in situ* hybridization methods is unknown. Activin β_A and β_B subunit immunoactivity were identified in more than 70% of serous carcinomas by immunohistochemistry (Table 4 and Refs. 165 and 168). In contrast, inhibin α subunit immunoreactivity was rarely detected (Table 4 and Refs. 168, 170–172, 174–182, and 188). Serum inhibin α subunit levels were elevated in a proportion of postmenopausal women with serous carcinomas (Table 5 and Refs. 20, 166, 167, and 190). The corresponding serum inhibin A and B levels showed limited increases (<5%), although activin A levels were elevated in a small study (Table 5 and Ref. 191).

In endometrioid carcinomas, inhibin α and activin β_A subunit mRNAs were detected by RT-PCR, and activin β_A protein was detected by immunohistochemistry (Tables 1–3 and Refs. 168, 170–172, 175, 176, 180, 182, 183, and 193). The inhibin α subunit was not readily immunodetectable in serum or tissues of patients with endometrioid carcinomas.

3. Germ cell tumors. Germ cell tumors, which make up the third major ovarian carcinoma grouping, were negative for inhibin and activin as assessed by immunohistochemistry (Table 4 and Ref. 176).

4. Association of inhibin with luteinized stromal tissue of the ovary. A number of immunohistochemistry studies (see Table 4) using the inhibin α subunit antibodies (in particular R1) noted that there was considerable immunoreactivity in the stromal area surrounding the ovarian tumor, even if the tumor itself was not apparently inhibin α subunit immunoreactive. These inhibin-positive cells resemble the theca-like cells around normal follicles (172, 173). Immunoreactivity was observed with all ovarian carcinomas including those metastasizing from other tissues to the ovary (168), for example, in Kruckenberg tumors that are colon carcinoma metastases to the ovary (172, 173). It is not clear whether these cells express the activin β_A subunit (168, 171). The corresponding localization of the inhibin α and activin β_A subunits in luteinized stromal cells in normal postmenopausal ovaries was limited (168) or not evident (172, 173). These observations suggest the expression of inhibin by the surrounding stromal tissue was an ovarian reaction to the presence of a tumor. Kommoss *et al.* (176) postulated that "neoplastic ovarian epithelium and germ cells stimulated stromal cells to differentiate into spindle-shaped or fully luteinized steroidogenic cells." In a study of tissue from ovaries that were removed because of a high risk of ovarian cancer, a preneoplastic phenotype was observed. Specifically, the presence of hyperactive stroma was noted in 15 of 20 ovaries from the high-risk group compared with 2 of 20 from a separate group of control ovaries (194). The authors queried whether it was the stroma in ovarian tumors that provided the abnormal growth stimulus to the epithelium.

5. Clinical application of inhibin assays to sex-cord stromal and epithelial tumors. Serum inhibin assays, particularly those that detect all inhibin forms, are clinically useful in the early detection of granulosa cell and mucinous tumors (20, 158, 160, 161, 163–167, 195). The assays detected benign, borderline, and malignant forms, and there was no differentiation between the various stages of disease, although the number of clinical cases observed in the earlier stages of the disease was limited. These assays appeared to fulfill a useful function for detecting mucinous carcinomas and were more effective at detecting this type of tumor than other cancer markers, including CA125 (20). Few studies examined serum activin A as a marker of ovarian cancers (192, 195). The role of activin B in ovarian carcinogenesis requires the development of a suitable human activin B assay.

The observation that CA125 was a marker for a range of epithelial carcinomas and inhibin was a marker for sex-cord stromal tumors and mucinous carcinomas suggested that a combination of the CA125 and inhibin α subunit assays would detect the majority of ovarian cancers. In fact, studies showed that the combined assays detected 90% of all ovarian

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cancers (20). However, the utility of the inhibin assays was compromised in some circumstances. In postmenopausal women, the assays were most appropriate for early detection and monitoring of recurrent cancer, as the serum inhibin levels were very low or nondetectable. In women of reproductive age, the inhibin assays were less useful because inhibin levels were elevated and fluctuated. In women with GCTs, serum levels of inhibin were high and readily measured and monitored; those with mucinous cancers had much lower levels of inhibin, requiring a low, stable background for reliable detection. In women of reproductive age, the use of inhibin to monitor the recurrence of disease after surgery to remove one ovary may be compromised because of the presence of the contralateral ovary.

Progress in understanding the role of activin and inhibin in ovarian cancer is hampered by the difficulties of culturing ovarian cell lines from normal and malignant tissue. In particular, ovarian cells proliferated poorly in culture and lost their ability to respond to gonadotropins. Studies with both primary ovarian cell cultures (196) and established cell lines (197) showed a loss of responsiveness to gonadotropins and a rapid decline in synthesis of the inhibin α subunit, although activin β_A subunit and activin A production was retained. A number of studies were undertaken to develop immortalized cell lines responsive to gonadotropins with some success (166, 198–202). Interestingly, a recent study reported that activin A inhibited growth and induced apoptosis in early neoplastic and tumorigenic ovarian surface epithelial cells (203). This is one of the few reports of a growth-inhibitory action of activin A on ovarian cells and contrasts with the mitogenic actions of activin in the normal ovary. Additional work is required to determine whether activin A has growth-inhibitory as well as stimulatory effects on ovarian tumor cells.

B. Prostate tumors

In the literature, the name "inhibin" was used to describe two unrelated proteins. Prostatic inhibin, isolated initially from seminal plasma, has numerous names including β-inhibin, prostatic inhibin peptide (204), β-microseminoprotein (205), Ig binding factor (206), and prostatic secretory protein of 94 amino acids (207). Prostatic inhibin is a 94-amino acid cysteine-rich, nonglycosylated protein of 10.7 kDa. Prostatic inhibin is not the same as dimeric inhibin and is not discussed further in this review.

Initial studies to determine expression and localization of inhibin and activin in normal prostate were performed using the rat prostate (208, 209). Normal rat prostate tissues expressed the inhibin and activin α, β_A, and β_B subunits, and immunoreactive activin and inhibin were detected and measured. Studies with human prostate biopsy tissue from men with benign prostatic hyperplasia demonstrated that the nonmalignant prostate had the capacity to make both inhibin and activin (210). The basal and secretory epithelial cells showed inhibin α subunit immunoreactivity as well as activin β_A subunit immunoreactivity. Expression of the activin β_B subunit differed from that of inhibin and activins α and β_A, as β_B expression localized predominantly to the basal epithelial cells with minimal expression observed in the secretory cells. In the stroma, smooth muscle cells were positive for the activin β_B subunit. The colocalization of inhibin α and activin β subunits to the nonmalignant prostate suggested that this tissue produced two forms of inhibin (inhibins A and B) and three forms of activin (activins A, B, and AB).

Prostate cancers are commonly adenocarcinomas (95%); neuroendocrine tumors are rarely detected. All studies that examined inhibin and activin expression in prostate cancer used adenocarcinomas or cancer cell lines such as LNCaP, DU145, and PC3. Inhibin and activin were implicated in prostate carcinogenesis after the observation that the pattern of expression of the subunits differed in malignant tissues relative to nonmalignant prostate epithelium. In high-grade prostate cancer biopsy tissues, the activin β_A and activin β_B subunits were expressed in both regions of nonmalignant epithelium and regions of carcinoma (211). In contrast, selective down-regulation of inhibin α subunit expression occurs in high-grade prostate cancer cells, whereas adjacent areas of nonmalignant epithelium retained inhibin α subunit expression (75). Similarly, the prostate cancer cell lines, LNCaP, DU145, and PC3, did not express the inhibin α subunit but expressed the activin β_A and β_B subunits (212, 213). Loss of inhibin α subunit in high-grade prostate cancer and the cancer cell lines is consistent with its role as a tumor suppressor. The mechanisms responsible for down-regulation of the inhibin α subunit gene may include gene deletion (LOH) at the 2q33–36 chromosomal regions, where the inhibin α subunit gene is found, and hypermethylation of the promoter of the inhibin α subunit gene (J. F. Schmitt and G. P. Risbridger, unpublished observations).

Despite frequent loss of inhibin α subunit expression in prostate cancer, the inhibin α subunit-null mice did not develop prostate cancer. There may be several reasons for this. Other than man, the dog is the only animal known to spontaneously develop prostate cancer, and carcinoma of the prostate has a long period of latency. The inhibin-deficient mice developed gonadal and adrenal tumors, and in the males, the tumors were lethal by 12 wk (49). This was probably insufficient time to make conclusions about the development of prostate tumors. A period of 10–20 wk was required for the development of prostate tumors in the TRAMP (transgenic adenocarcinoma of the mouse prostate) mouse, in which a region of the probasin promoter was fused to SV40 T-antigen; this is considered to be an aggressive model of prostate cancer (214). Furthermore, the inhibin-deficient mice initially developed testicular tumors, and adrenal tumors only emerged after castration. Because prostate carcinogenesis is androgen dependent, it is not possible to assess the development of androgen-regulated prostate cancer in this setting.

In prostate cancer, the loss of the inhibin α subunit expression, but not of the activin β subunit expression, implied that the synthesis and actions of activin were unopposed by inhibin. Hence, it is important to determine how activin contributes to the tumorigenic process. Studies with the LNCaP cell line demonstrated that activin A inhibited the proliferation of LNCaP cells, altered cell morphology, and induced apoptosis (212, 213, 215, 216). Primary human prostate epithelial cells were also growth inhibited by activin A (217). The specificity of the effects of activin on LNCaP cells,

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as well as on the primary prostate epithelial cells, was supported by the ability of follistatin to block these effects (213, 215).

The growth inhibitory effects of activin described in these studies were inconsistent with the rapid growth characteristics of tumor cells in malignancy. It was postulated that, like TGFβ, the tumor cells acquired resistance to activins (218). This could occur through mutation of the activin receptors or signaling molecules; whether such mutations occur in prostate cancer remains to be determined. A recent study, however, showed reduced expression levels of one of the activin receptors, ActRIB, in prostate cancer relative to nonmalignant prostate tissues (72).

The expression of follistatin provides another means by which prostate cancer cells could be protected from growth-inhibitory effects of activin. The effects of follistatin on prostate cancer cell sensitivity to activin were examined by comparing the LNCaP cell line, which was growth inhibited by activin, with the PC3 cell line, which was resistant to the antiproliferative and apoptotic effects of activin (212, 213). These cell lines showed differential expression of the follistatin isoforms (219); both cell lines expressed the secreted form of follistatin, FS315, but only the PC3 cell line expressed the membrane bound follistatin, FS288. Hence, the different sensitivities of the cell lines to the effects of exogenous activin may be related to the expression of FS288. Further support for this idea was gained from the studies by McPherson *et al.* (219), which showed that neutralization of FS288 protein in PC3 cells rendered them sensitive to exogenous activin A.

Using a specific antibody, the activin β_C subunit was detected in basal epithelial cells in nonmalignant prostate tissue and in the cancer cells and cell lines, as well as in liver (10). Because the activin β_C subunit forms heterodimers with the other activin β subunits ($\beta_C\beta_A$, $\beta_C\beta_B$), but not with the inhibin α subunit (10), a range of new activins, but not inhibins, may be present in these tissues. A change in the relative levels of the activin β subunits expressed during cancer progression could effect the proportion of homodimers and heterodimers produced in the cells and could result in a significant regulation of the levels of bioactive activin A. New, specific assays are required to measure and identify novel activins in prostate.

C. Testicular tumors

A wide range of histological types of testicular tumors are recognized and can be divided into two major groups: germ cell tumors, which account for 95% of cases, and nongerminal, stromal, or sex-cord tumors. Malignant transformation of germ cell tumors includes embryonal carcinomas, choriocarcinomas, teratomas in the form of squamous cell carcinomas, or adenocarcinomas and teratocarcinomas that contain both teratoma and embryonal carcinoma.

The testis is the primary site of inhibin production in the male (220), and the role of inhibin and activin in male reproductive processes was extensively studied (for reviews see (221–223). Inhibin B is the main form of inhibin found in the male circulation and seminal plasma (224, 225), and the α and β_β inhibin and activin subunits are expressed predominantly by Sertoli cells within the testis and also by Leydig cells (226–228). Activin A immunoreactivity was detected in seminal plasma, and expression of the activin β_A subunit was localized to the Sertoli and Leydig cells (228).

Although the testis is a major source of inhibin, few studies examined the inhibin and activin subunits in human testicular cancer. In the testis, inhibin appeared to be a marker of Sertoli cell tumors, and an elevation in the serum inhibin levels occurred in both humans and dogs with Sertoli cell tumors (229, 230) and in dogs with Leydig cell tumors (231, 232). Elevated serum inhibin levels in dogs with sex-cord testicular tumors were associated with increased mRNA levels for the inhibin α and activin β_B subunits within the testes (231). In a case study of a 12-yr-old boy with Sertoli cell tumors, elevated serum inhibin levels were associated with increased levels of mRNA for the inhibin and activin α, β_A, and β_β subunits within the diseased testes, as determined by Northern analysis (230). Removal of the testes resulted in a drop in serum inhibin levels. Similarly, a reduction in serum inhibin B to nondetectable levels was associated with eradication of testicular carcinoma *in situ* by radiotherapy (233).

Immunohistochemistry studies localized inhibin α subunit immunoreactivity to both malignant and nonmalignant Sertoli cells in sex-cord tumors (229) and to granulosa cells in a granulosa cell tumor of the testis (234). In a study of patients with unclassified sex-cord stromal tumors with incorporated germ cells, inhibin was present in the Sertoli cell tumors but not in the neoplastic germ cells within the tumors. However, in one sample, inhibin immunoreactivity was detected in germ cells with the morphological appearance of seminoma cells (235). Similar to the ovary, inhibin expression and secretion may provide a marker for determining the presence of Sertoli cell tumors of the testes.

D. Breast tumors

The early reports of changes to inhibin levels in breast cancer were related to the expression of the peptide hormone described by Sheth and colleagues (236). This protein is not the same protein as the inhibin discussed in this review (see *Section IV.B*). The α, β_A and β_B inhibin and activin subunits were immunolocalized to the epithelial cells of normal breast tissue (237). In this study, expression of all subunits was reduced in benign breast neoplasms, and inhibin α subunit expression was further reduced in breast carcinoma. Expression of the activin β_A and β_β subunits was not detected in breast carcinomas.

A limited study on MCF-7 cells showed that, as well as expressing the activin receptors, these cells produced the inhibin and activin subunit proteins (238). Indeed, activin A was found to be a potent inhibitor of MCF-7 cell growth (239), causing cell cycle arrest in G_1. Activin A also inhibited tubule formation by human mammary organoids *in vitro*, suggesting a role for activin A in regulating mammary cell growth and morphogenesis (239). The effect of inhibin in these systems was not determined, and it remains unknown as to whether inhibin can oppose the action of activin A.

Kalkhoven and colleagues (240) evaluated the effects of activin on a panel of breast cancer cell lines that were ER positive or negative. The ER-positive cell lines in the study were inhibited by activin A, whereas the ER-negative cell

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lines were not. In two of the ER-negative cell lines, resistance to the growth-inhibitory effects of activin A were explained by down-regulation of the activin receptors. In two other ER-negative cell lines, MDA-MB231 and MDA-MB468, activin insensitivity was not due to reduced activin receptor levels. Instead, the failure of the MDA-MB468 cell line to respond to activin was explained by loss of Smad4 expression in these cells. Transfection of Smad4 into these cells rendered them sensitive to inhibition by activin. The other activin resistance/ER-negative cell line, MDA-MB231, expressed both Smad4 and Smad2. In this case, additional studies revealed that these cells lacked a functional ActRI (240).

The limited data from the studies mentioned in the previous paragraph described the localization of activin and its effects in breast cancer cells and suggested that resistance to the growth-inhibitory effects of activin might involve changes to the activin signaling pathway. Additional studies are required to define the contribution of inhibin, activin, and the activin signaling pathway to tumorigenesis.

E. Adrenal tumors

The adrenal cortex is structurally and functionally distinct from the medulla and is a site of synthesis of glucocorticoids, mineralocorticoids, and sex steroids. Excessive production of glucocorticoids (Cushing's syndrome), aldosterone (Lonn's syndrome), or sex steroids may be due to primary adrenal neoplasms. Primary adrenal neoplasms, including adrenal cortical carcinomas, account for up to 25% of cases of endogenous Cushing's syndrome. Aldosterone-producing adenomas can lead to primary hyperaldosteronism and hypertension. Androgen secretion by cortical neoplasms may result in virilization in the female and precocious puberty in the male. Feminizing adrenal tumors associated with estrogen synthesis can also occur. The most significant adrenal medullary neoplasm is pheochromocytoma.

All zones of the human adult adrenal gland expressed both the activin β_A and β_B subunits, suggesting that activins were synthesized in this organ (241). Expression of the inhibin α subunit was investigated more widely because the earlier data from animal models suggested that it was an adrenal tumor suppressor (49, 51, 62). An early study detected inhibin α subunit immunoreactivity in hyperplastic tissues and adrenocortical carcinoma (159). This observation was supported by data from larger, subsequent studies of tissues from patients with adrenal cortical neoplasia (178, 241–244). In general, inhibin α subunit immunoreactivity was detected in adrenal cortical adenomas and carcinomas. Inhibin α subunit immunoreactivity provides a diagnostic marker that can be used to differentiate adrenal cortical tumors from histologically similar tumors, including phechromocytomas, hepatocellular, and renal cell carcinomas. The morphological distinction of adrenal cell carcinoma and renal cell carcinoma is not always feasible on the basis of cytology when fine-needle aspiration material is obtained from renal, adrenal, or metastatic tumors. In this context, positive staining with antibodies to the inhibin α subunit can be used by the cytopathologist to discriminate between adrenal and renal cell carcinomas.

As described in the previous paragraph, there is an apparent inconsistency between these observations in adrenal tumor tissues and the role of the inhibin α subunit gene as an adrenal tumor suppressor in the inhibin-null mice. However, a recent study (241) identified a subgroup of adrenal cortical carcinomas in which there was loss of inhibin α subunit immunoreactivity, and the authors suggested that this might indicate a role in tumor progression. It will be interesting to determine whether malignant progression correlates with loss of inhibin immunoreactivity in the adrenal gland.

F. Pituitary tumors

In the normal pituitary, the secretion of FSH and the stability of FSHβ subunit mRNA was reduced by inhibin. In contrast, activin increased FSHβ subunit expression and was a potent differentiation factor in the pituitary (245–247). Inhibin and activin α and β_A subunit immunoreactivities were localized within FSH- and LH-secreting gonadotropes, whereas immunoreactivities of the activin β_A subunit and the activin receptors (ActRIB and ActRII) were present throughout the anterior pituitary (248).

Pituitary carcinomas are rare and often originate in the adenohypophyseal cells, whereas adenomas are common and are present in up to 20% of normal pituitaries (249). Most of the studies on inhibin and activin expression and action in neoplastic pituitary examined tissue or cells from adenomas. In a range of pituitary adenomas, mRNAs for the inhibin α and activin β_B subunits (but not β_A subunit) and the activin receptors (ActRIA, ActRIIB, and splice variants of ActRIB) were detected (250). Follistatin expression was reduced in the gonadotrope adenomas compared with the normal pituitary (251).

Activin had an antiproliferative effect on cells cultured from a subset of pituitary adenomas, although cells cultured from other pituitary tumors were unresponsive to activin (252). The cells from tumors that were growth inhibited by activin expressed little or no follistatin, which implied that differential expression of follistatin affected activin-induced growth arrest (252). Interestingly, the human pituitary cell line hPit-1 expressed uniformly high levels of follistatin mRNA, and the cells were moderately tumorigenic in immune-deficient mice (253). To investigate the hypothesis that activin receptors acted as tumor suppressors in pituitary tumors, D'Abronzo *et al.* (254) performed mutational analysis of the intracellular kinase domains of the ActRI and ActRII genes and found that somatic mutations were rare. The effectors of downstream signaling events (*e.g.*, Smads) and their role in pituitary tumors remains to be studied.

G. Pancreatic tumors

There is evidence that the activin signaling pathway is tumor suppressive in pancreatic tumors. In pancreatic cancers, deletions were observed in ActRIB, as were mutations of the Smad4 gene (69, 73). The tumor-suppressive function of the TGFβ pathway in pancreatic cancers was confirmed by findings that 82% of pancreatic cancers had genetic inactivations of ALK-5 (TGFβRI), Smad4, or TGFβRII (255). The

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evidence to implicate specific activin ligands in pancreatic cancers was less obvious.

Expression of the inhibin α subunit was not detected in pancreatic carcinomas, whereas activin β_A subunit expression was detected (256). The effects of activin, like TGFβ, were growth inhibitory. Mice bearing a dominant-negative mutation of TGFβRII showed increased proliferation of pancreatic acinar cells and severely perturbed acinar differentiation (257) but remained responsive to activin A. These results suggested that either the inhibitory effects of activin and TGFβ are independent of one another, or the signaling pathways converge after receptor activation.

Carcinomas of the exocrine pancreas that arise from ductal epithelial cells are the most common type of pancreatic neoplasm. Cystic tumors are less common and represent about 5% of tumors. This group of cancers was reported to express ovarian-like stroma. Positive staining for the inhibin α subunit was one of the markers used to identify this type of stroma (258). In a study of 56 patients with mucinous cystic tumors of the pancreas, 66% had inhibin α subunit-positive stroma (259), and based on the similarities between pancreatic and ovarian mucinous cystic tumors, the authors suggested a common pathway of tumor development. As discussed in *Section IV.A*, the inhibin α subunit was used as a sensitive marker of primary and recurrent granulosa cell tumors of the ovary; it is not known whether inhibin α subunit can be used to detect/monitor cystic neoplasms of the pancreas.

H. Placental tumors

During pregnancy, serum inhibin A levels are higher than in the normal menstrual cycle, and the placenta is a source of inhibin. The cellular localization of inhibin in the placenta is controversial, and both the cytotrophoblasts and syncytiotrophoblasts were reported as positive for inhibin α subunit immunoreactivity (260–264). The conflicting results of such studies may be due to the use of different antibodies to the subunit protein, together with varying methods of detection including antigen retrieval or signal amplification.

Proliferation of trophoblastic tissue results in a range of tumors and tumor-like conditions that include hydatidiform mole, invasive mole, choriocarcinoma, and placental-site trophoblastic tumor. Several studies reported that immunohistochemical localization of the inhibin α subunit was useful in the differential diagnosis of gestational trophoblastic *vs.* nontrophoblastic lesions. At times, such distinctions can be difficult if an analysis is solely based on morphology.

Hydatidiform moles are characterized by cystic swellings of the chorionic villi accompanied by trophoblastic proliferation and are usually diagnosed by ultrasound examination and an elevation in serum levels of human (h)CG. In 10% of patients, invasive moles develop, and in 2.5% of patients, choriocarcinoma will occur. Serum inhibin may be a useful adjunct to hCG and human placental lactogen levels, which are widely used as markers for this condition (265).

Choriocarcinomas consist of abnormal proliferation of both cytotrophoblasts and syncytiotrophoblasts, and expression of the inhibin α subunit was observed in two patients examined (260). The authors concluded that, because choriocarcinoma within the uterus or in extrauterine sites can be confused with other malignant neoplasms, inhibin α may be a useful histochemical marker for diagnosis of the former lesion.

Placental-site trophoblastic tumors are rare tumors composed of proliferating intermediate trophoblasts, and levels of hCG are usually low in these tumors. Placental site nodules are described as being composed of intermediate trophoblasts and are usually benign lesions. In conjunction with cytokeratin 18, inhibin α subunit immunoreactivity was a useful marker to identify placental-site nodules and to distinguish them from squamous cell carcinoma of the cervix. In a study of 42 patients, all placental site nodules expressed the inhibin α subunit and cytokeratin 18, whereas there was no positive staining in squamous cell carcinoma of the cervix (266). Thus, the differential and correct diagnosis of these lesions may be improved with immunostaining for the inhibin α subunit.

Overall, the utility of immunohistochemical staining for the inhibin α subunit was based on the consistent expression of the protein in nearly all gestational trophoblastic lesions, whereas it was not expressed in other tissues that might be confused with gestational trophoblastic lesions (264). In contrast, there appeared to be no value in the measurement of activin A levels in serum from women with placental tumors (267), and the detection of activin in trophoblastic lesions has received little attention.

I. Endometrial tumors

The α, β_A, and β_B inhibin and activin subunits were detected in normal endometrium by immunohistochemistry and *in situ* hybridization (268). Inhibin A, inhibin B, and activin A production was detected in endometrial epithelial and stromal cells *in vitro* and in uterine flushings (195). Uterine fluid and serum from women with endometrial adenocarcinoma showed significantly elevated activin levels that were reduced after surgery (195).

J. Kidney tumors

Early studies by Shiozaki *et al.* (269) suggested that the relative levels of activin A and follistatin were important markers of chronic renal failure, and Sakamoto *et al.* (270) described the elevation of free follistatin levels in patients with chronic renal failure. There are no data to describe the changes to serum follistatin or activin in patients with renal cell carcinomas.

In contrast, the utility of inhibin α subunit staining for the diagnosis of renal cell carcinoma was examined. Adenocarcinomas of the kidney expressed the inhibin α subunit, whereas renal cell carcinomas were negative for the inhibin α subunit, facilitating the diagnosis by immunohistochemistry (159, 242, 244).

K. Liver tumors

Several studies showed that the inhibin α subunit can be used for immunohistochemical diagnosis of adrenal cortical neoplasms because these are inhibin α subunit positive, whereas renal cell carcinomas or hepatocellular carcinomas

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are mainly negative (242, 244, 271). Although one report suggested that hepatocellular carcinomas were positive for the inhibin α subunit (272), it was considered that this was a false positive result caused by the presence of endogenous biotin. In adult GCTs of the ovary, foci of hepatic cells were identified because they did not express the inhibin α subunit (273, 274). Therefore, inhibin immunostaining has diagnostic utility because hepatic carcinoma did not show inhibin α immunoreactivity.

Changes in the expression of activin A in the liver received little attention. Activin A inhibited the proliferation of the liver cell lines HepG2 and HLF (35, 275, 276). Inhibin had no activity of its own on HepG2 cells but antagonized the inhibition of liver cell growth by activin A (35). *In vivo,* the development of gonadal tumors in inhibin-deficient mice was rapidly followed by a cancer cachexia-like wasting syndrome (51). This syndrome was associated with hepatocellular necrosis around the central vein, consistent with previously reported effects of elevated activin A on rat hepatocytes (277). Subsequent studies supported the concept that the cancer cachexia-like symptoms were induced by elevated activin A levels (52).

V. Summary

A. The process of tumorigenesis

Tumorigenesis is a multistep process involving initiation, promotion, invasion, and metastasis. A carcinogen induces general or specific changes to DNA, but genomic damage alone seldom leads to tumor formation. In normal circumstances, the primary lesions are transient and are eliminated by the activation of cell death or DNA repair mechanisms. If these lesions are not repaired, they become mutations in the target cell population as it proliferates. Proliferation of cells containing this damage may often confer selected growth advantages and mark the promotion of tumorigenesis. The duration of tumor growth varies considerably, but at any stage progression can be accelerated by general or specific endogenous or exogenous factors. When this occurs, several sequential and parallel events are evident in the neoplasm; these may include acquisition of physiological properties that enhance invasiveness and cell motility, escape from immune surveillance, and the emergence of new growth-regulatory mechanisms in distant metastatic sites.

Tumors, including those of the breast and prostate, can be induced by the inappropriate expression or action of mitogenic or antiproliferative factors. Hormones and growth factors can exert proliferative and antiproliferative effects on a target cell directly or indirectly through activation of paracrine and autocrine regulatory loops. Inhibin and activin are growth factors that exert their effects via complex receptor-mediated signaling pathways. The data presented in this review support the hypothesis that the inappropriate activation or deactivation of these pathways could contribute to the tumorigenic process.

B. Role of inhibin in tumorigenesis

In this review, we considered the case that the inhibin α subunit is a tumor suppressor based on the results from transgenic mouse models in which deficiency of the inhibin α subunit gene was associated with tumorigenesis (49, 278). In contrast, the clinical data from women reported up-regulation of the inhibin α subunit and its use as a marker to detect and monitor the recurrence of some types of ovarian carcinomas, *e.g.*, GCTs. Other types of ovarian tumors, particularly serous carcinomas, demonstrate loss of inhibin α subunit immunoreactivity. These observations raise the following two issues: Does the inhibin α subunit have a different role in mice and women, and does the up-regulation or down-regulation of the inhibin α subunit contribute to tumorigenesis? It is difficult to reconcile the differences in the data from mice and humans with ovarian carcinomas. In other types of endocrine cancers, the inhibin expression is also diverse. Inhibin is elevated in testicular Sertoli and Leydig cell tumors, in adrenocortical adenocarcinomas, and in placental tumors. In contrast, negative staining for inhibin was reported in renal cell, hepatocellular, pancreatic, and prostate carcinomas.

In general, the current body of evidence suggests that the different patterns of inhibin α subunit expression are specific to the organ in which the tumor arises (Table 6A). One explanation may lie in the influence of gonadotropins on the different tissues. The gonads, adrenal, and placenta are gonadotropin responsive, and the data from the mouse models demonstrated that gonadotropins were modulators of inhibin α subunit gene activity. The prostate, breast, kidney, liver, and pancreas are not regarded as organs primarily regulated by gonadotropins, and therefore, the effects of inhibin may be different and modulated by other factors.

The importance of the changes to inhibin α subunit expression are unknown. Most studies employed inhibin as a diagnostic tool, and only a few studies related inhibin changes to patient outcome or survival. In particular, inhibin was a useful diagnostic marker, but its value as a prognostic marker for ovarian cancer survival was not thoroughly explored. In recent studies by Ala-Fossi *et al*. (82) and Gebhart *et al.* (83), down-regulation of inhibin α subunit immunostaining was associated with advanced ovarian GCTs. In one study, inhibin expression correlated with reduced patient survival (82), whereas in the other study, there was no correlation with disease-free survival (83). The identification of subsets of inhibin-negative patients needs further exploration in ovarian tumors, as well as in adrenal tumors, for which similar observations have been made.

This review highlighted the potential role of the inhibin α subunit gene in tumorigenesis of endocrine organs, in which altered expression may be of diagnostic or prognostic significance. However, the current data are limited. Many of the studies only examined a small number of tumors; *e.g.*, in the study of inhibin in choriocarcinoma, only two patient tissues were examined (260). In many of these studies, human control tissue was difficult to obtain, yet adequate analysis of appropriate control tissue is essential for comparison to malignant tissues. Furthermore, the range of different assay methods to detect inhibin α subunit gene expression contributed to variable results and affected the utility or application of serum inhibin peptide measurements for the detection and monitoring of endocrine cancers. For example, in the ovary, GCTs produced both monomeric inhibin α sub-

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TABLE 6. A summary of the evidence to support the hypothesis that inhibins and/or activins contribute to endocrine related tumorigenesis[a]

6A. Inhibin α subunit as a tumor suppressor

	Mouse models	Human tissues
Do models support a role for inhibin as a tumor suppressor?	Yes	Equivocal
What factors modify the action of the inhibin α subunit?	Gonadotropins (FSH, LH) Activin Follistatin Androgens Müllerian inhibitory substance	Gonadotropins (FSH, LH) Activin

[a] The role of the inhibin α subunit as a tumor suppressor. There is an apparent discrepancy between transgenic mouse models and human tissues, *e.g.*, in formation of ovarian tumors. In the mouse models, the functional evidence is consistent with a role for the inhibin α subunit gene as a tumor suppressor, but the data from the human studies are equivocal. Ovarian tumors overexpress inhibin, an observation that led to the use of serum inhibin levels to monitor recurrence of ovarian cancer. More recently, subsets of patients with ovarian tumors showed reduced inhibin α gene expression, as reported in prostate and breast carcinoma. However, the human data are limited; many of the studies used tissues derived from heterogeneous groups of patients for whom the outcomes were unknown, and thus, the results are inconclusive at present.

A further level of complexity was identified using the mouse models; *i.e.*, the ability of other hormones and growth factors to modify the actions of the inhibin α subunit. The gonadotropins (LH as well as FSH), activin, follistatin, androgens, and Müllerian inhibitory substance have a role in regulating the action of inhibin as a tumor suppressor. Similar effects of these "modifiers" of the inhibin α subunit gene in human tumorigenesis are unknown and may be an important consideration in compiling a unifying hypothesis for the role of inhibin α as a tumor suppressor in different endocrine tumor types.

6B. Activin signaling pathway in tumorigenesis

Effects of Activin	Antiproliferative	Proliferative
Effects of mutations	Loss of function confers resistance	Gain of function promotes proliferation
Examples of changes to activin signaling in endocrine tumors	Pancreas and breast: activin receptor and Smad mutations Prostate and pituitary: altered follistatin	Ovary[b] and testis: overexpression of activin subunits

[a] Evidence that the activin signaling pathway is involved in tumorigenesis. Activin had antiproliferative effects in many endocrine tissues, including pancreas, prostate, and breast. In these tissues, resistance to activin signaling occurred and was due, at least in part, to mutations in activin receptors and Smads (pancreas and breast) or altered follistatin expression (prostate and pituitary). In other tissues, such as the ovary and testis, in which activin promoted proliferation, it was postulated that activin expression was sustained or increased in tumorigenesis. However, the evidence to support sustained or over expression of activin in ovarian carcinoma is equivocal.

[b] A further level of complexity was evident from a more recent report that activin A was growth inhibitory in early neoplastic and tumorigenic ovarian surface epithelial cells. Hence, activin exhibited growth inhibitory and proliferative affects in the same endocrine tissue, *e.g.*, ovary (197, 203, 279). In summary, the results support a role for inhibin and activin in tumorigenesis of endocrine-related cancers. However, any unifying hypothesis must account for the differences between different tumor types, as well as the differences that emerge in comparing results from mouse models with human tumor tissues.

unit and dimeric inhibin forms detected by most types of inhibin assays, whereas mucinous tumors predominantly produced free inhibin α subunit. Thus, assay methods that detect both free inhibin α subunit and dimeric forms of inhibin appear to be more useful and specific in monitoring a range of ovarian cancers.

C. Role of activin in tumorigenesis

Activin, like TGFβ, can inhibit or stimulate cell growth. Accordingly, in tumors in which activin is growth inhibitory, the tumor cells must acquire resistance to activin to allow malignant progression. In tumors in which activin is growth stimulatory, sustained activin signaling would promote tumorigenesis (Table 6B).

Activins have growth-inhibitory effects on breast, liver, and prostate cancer cells, as well as on pituitary adenomas (see *Section IV*), yet there are limited data to show that the acquisition of resistance to activin is associated with tumor progression. In the prostate, the androgen-responsive LNCaP cells were growth inhibited by activin A. The PC3 tumor cells were androgen independent and resistant to the effects of activin A. *In vivo*, a switch from androgen-dependent to -independent tumor growth occurred in the progression of prostate cancer. In breast cancer cell lines, resistance to the growth-inhibitory effects of activin A was associated with the ER status. ER-positive cells lines were responsive to activin A, whereas receptor-negative cell lines were resistant to activin A.

Resistance to activin effect could be acquired through several mechanisms. Mutations of signaling molecules of the activin cascade would lead to activin resistance and were identified in pancreatic carcinomas in which ActRIB and Smad4 were mutated (69, 73). In breast and prostate cancer cell lines and tissues, resistance to activin was associated with low levels of activin receptor expression (72, 240), but inactivating mutations were not investigated.

In tumor tissues that are growth inhibited by activins, it is relevant to ask why the tumor cells should retain the capacity to synthesize these ligands. The answer may lie in the recognition that activins are found in numerous cells and tissues and have multiple actions such as suppression of the immune response, wound healing, tissue repair, and angiogenesis. Many of these features of the activin ligands would promote tumor progression.

In tissues in which activin has a proliferative effect, one would expect to observe changes consistent with sustained, or even enhanced, signaling of these ligands (Table 6B). Tissues that are growth promoted by activin include the testis

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and ovary. In the normal ovary, activin is mitogenic in combination with FSH (46, 47), and inhibin α subunit-null mice have elevated activin and FSH levels. A role for activin in promoting cachexia was suggested after the generation of mutant mice with loss of both the inhibin α subunit and the ActRII receptor. In these mice, cachexia was reduced, but tumors formed.

Thus, activin is growth stimulatory or inhibitory, and these actions may differ between organs or tissues or even in the same tissue type. For example, a recent study reported that ovarian surface epithelial cells were growth inhibited by activin A (203), which is in contrast to the effects of activin A in the normal ovary. Regardless of whether activin is proliferative or antiproliferative, the question arises as to the effect of activin in the absence and presence of the inhibin α subunit. Is the action of activin in tumorigenesis influenced by inhibin α subunit expression, and if so, how? Evaluation of this possibility requires investigation of the relative levels of expression of the subunits and their interaction; this needs to be considered at multiple stages of malignant progression.

D. Future directions

This review has identified a considerable body of evidence to support the role of inhibins and activins in endocrine-related cancers. However, additional work is required to define the exact mechanisms by which inhibins and activins contribute to the process of neoplastic transformation during the stages of initiation, promotion, or progression of endocrine-related cancers.

The current review emphasizes that future studies need to address a number of issues in determining the role and diagnostic/prognostic significance of these ligands in tumorigenesis. The expression and cellular localization of inhibin and activin subunits in normal tissues requires documentation. Changes to activin and inhibin subunit expression and localization that occur during malignant progression require characterization using a large number of patient tissues where the tumor pathology and disease outcome are clearly defined. To assess the contribution of activin and inhibin to tumorigenesis, the relative levels of these ligands needs to be determined. This will require specific assays to measure activin and inhibin, in bound and free forms, and to understand how subunit expression is regulated and directs production of the different dimeric proteins. In addition, the contribution of binding proteins and signaling molecules to the tumorigenic process requires evaluation and could provide targets for the development of novel therapeutics.

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