MINIREVIEW

Activin Signaling and Its Role in Regulation of Cell Proliferation, Apoptosis, and Carcinogenesis

YE-GUANG CHEN,* QIANG WANG,* SHI-LUNG LIN,† C. DONALD CHANG,† JODY CHUNG,‡ AND SHAO-YAO YING†,[1]

*State Key Laboratory of Biomembrane and Membrane Biotechnology, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, People's Republic of China; †Department of Cell and Neurobiology, Keck School of Medicine, University of Southern California, 1333 San Pablo Street, Los Angeles, California 90033; and ‡Department of Molecular Biology and Biochemistry, Keck School of Medicine, University of Southern California, 1441 Eastlake Avenue, Los Angeles, California 90089

Activins, cytokine members of the transforming growth factor-β superfamily, have various effects on many physiological processes, including cell proliferation, cell death, metabolism, homeostasis, differentiation, immune responses endocrine function, etc. Activins interact with two structurally related serine/threonine kinase receptors, type I and type II, and initiate downstream signaling *via* Smads to regulate gene expression. Understanding how activin signaling is controlled extracellularly and intracellularly would not only lead to more complete understanding of cell growth and apoptosis, but would also provide the basis for therapeutic strategies to treat cancer and other related diseases. This review focuses on the recent progress on activin-receptor interactions, regulations of activin signaling by ligand-binding proteins, receptor-binding proteins, and nucleocytoplasmic shuttling of Smad proteins. Exp Biol Med 231:534–544, 2006

Key words: activin; activin receptors; Smad; activin-binding protein; signaling pathway; cell proliferation; apoptosis; carcinogenesis; Cripto; betaglycan

Laboratory research by S.-Y.Y. was supported by the National Institutes of Health (CA 85772). Laboratory research by Y.-G.C. was supported by grants from the Bugher Foundation (New York), the National Science Foundation of China (30125021, 30270681, 30430360, 30500083), and the 973 Program (2004CB720002).

[1] To whom requests for reprints should be addressed at Department of Cell and Neurobiology, Keck School of Medicine, University of Southern California, 1333 San Pablo Street (BMT-403), Los Angeles, CA 90033. E-mail: sying@hsc.usc.edu

1535-3702/06/2315-0534$15.00

Introduction

Ever since activins were isolated and characterized based on the secretion of follicle stimulating hormone (FSH) by the anterior pituitary gland (1, 2), many other functions have been found to be exerted by activin, including their roles in cell proliferation, differentiation, apoptosis, metabolism, homeostasis, immune response, wound repair, and endocrine function (3, 4). This review is an update of our previous minireview entitled "Regulation of cell proliferation, apoptosis, and carcinogenesis by activin" (EBM 227:75–87, 2002) and focuses on the recent development in the roles activins play in cell growth and carcinogeneisis.

Activins are members of the transforming growth factor-β (TGF-β) superfamily, which includes activins, inhibins, TGF-βs, bone morphogenetic proteins (BMPs), growth and differentiation factor (GDF), Nodel, myostatin, Müllerian-inhibiting substance, lymphocyte inhibitory factor (LIF), and others. Members of the TGF-β family exert a wide range of biological effects on various cell types. Activins, similar to other members of the TGF-β superfamily, interact with two types of cell surface transmembrane receptors (types I and II) which have intrinsic serine/threonine kinase activities in their cytoplasmic domains (5). Activin binds to type II activin receptor (ActRII/IIB), leading to the recruitment, phosphorylation, and activation of type I activin receptor (ALK4, also known as ActRIB). The activated ALK4 transiently interacts with and then phosphorylates Smad 2 and Smad 3, two of the cytoplasmic Smad proteins. The phosphorylated Smad2 and

Smad3 form a heterocomplex with Co-Smad (Smad4), and the resulting Smad complex accumulates in the nucleus, binds to the promoter region of the target genes, and regulates their expression (6, 7). Increasing biochemical and developmental evidence also suggests non-Smad pathways in activin signal transduction. Examples include the RhoA-ROCK-MEKK1-JNK and MEKK1-p38 pathways, which lead to the control of actin cytoskeleton reorganization and epithelial cell migration and contribute to the physiologic and pathological effects of activins on epithelial morphogenesis (8).

Activin and Activin-Binding Proteins

Activin. The TGF-β family comprises at least 42 members in humans, which are all characterized by a distinct cysteine knot scaffold structure (9, 10). Activins and inhibins are structurally related, sharing common β subunits, with a nine-cysteine distribution pattern similar to TGF-ßs (7, 11, 12). Activins are dimeric proteins composed of two β subunits (activin-A [βAβA], activin-AB [βAβB], or activin-B [βBβB]), which are linked by a single covalent disulfide bond, while inhibins are heterodimers of a β subunit and the structurally related α subunit (inhibin-A [αβA] and inhibin-B [αβB]) (5, 13). In humans, genes encoding for four different activin/inhibin β subunits have been identified (βA, βB, βC, βE). The expression of activin C (βCβC), E (βEβE), AC (βAβC), AE (βAβE), CE (βCβE), and BC (βBβC) has been demonstrated, but little is known about their function and stability (14, 15). A recent study showed that activin C stimulated DNA synthesis and proliferation of AML12 cells in serum-free medium (16). Activin C also exerts its action independently of the activin receptor signaling and has activities opposite to those of activin A. The βC subunit appears to attenuate activin activities by forming a heterodimer with a βA subunit and subsequently preventing the formation of biologically active activins (16). Accordingly, overexpression of activin C or activin E in the mouse liver inhibits regenerative DNA synthesis of hepatic cells and induces apoptosis in human and rat hepatoma cells (17, 18). However, targeted deletion of the genes encoding βC and βE does not lead to abnormality in the liver (19).

Activin-Binding Proteins. Several activin-binding proteins, which play important roles in regulating the activin signaling, have been identified.

Follistatins. Follistatins are soluble extracellular proteins and function as mediators of cell growth, development, and differentiation in many tissues and organs. Follistatins bind activins with high affinity, block the interaction between activins and their cell-surface receptors, and consequently inhibit the signaling activity of activins (20). Follistatin consists of a 63-residue amino (N)-terminal segment, followed by three successive follistatin domains termed FSD1, FSD2, and FSD3, each of which contains ten cysteine residues. Alternative splicing generates three follistatin isoforms: FS288, FS303, and FS315. FS315 does not interact with cell-surface proteoglycans and is the predominant circulating form of the protein (20). In addition to its interaction with the cell surface through a basic heparin-binding sequence (HBS; residues 75–86), the FSD1 is also essential for suppressing activin biological activity (21). Follistatin has ten times higher binding affinity for activin A than for activin B, suggesting that the determinants for follistatin binding differ between the two isoforms of activin (22).

Domain mapping has identified the region containing amino acid residues 85–109 in activin A to be involved in follistatin binding (23). Binding of follistatin to this region would mask the residues that are important in the interaction of activin with both ActRII (Ser90, Leu92, and Lys102) and ALK4 (Met91, Ile105, and Met108) (24–26). In fact, activin interacts with follistatin through a much bigger region, as shown by recent crystal structures of the activin A-follistatin (FS288) complex, revealing that two follistatin molecules embrace one activin dimer and bury one-third of its residues and its receptor binding sites (27).

FLRG. FLRG (follistatin-related gene), also known as follistatin-like 3, is a recently characterized follistatin domain containing secreted glycoprotein. Similar to follistatin, FLRG also binds to activins, myostatins, and BMPs (28–31). It is possible that the residues, which are important for activin binding, are located in the conserved regions between the two proteins. FLRG differs from follistatin in lacking the third follistatin domain and a consensus heparin-binding sequence. Although both follistatin and FLRG have similar functions in binding members of the TGF-β superfamily and neutralizing their activities, different expression patterns and distinct regulations are observed. FLRG is highly expressed in the placenta, testes, skin, and cardiovascular tissues, whereas the expression of follistatin is high in the pituitary and ovaries (20). Interestingly, the expression of FLRG is induced by TGF-β in a Smad-dependent manner (32), suggesting cross-talking between the TGF-β family members.

Cripto. Human Cripto, *Mr* 36,000 molecule, is the prototypical member of the epidermal growth factor–Cripto-FRL-Criptic (EGF-CFC) protein family. Members of the EGF-CFC family share the six cysteine residues in the central region (amino acids 77–113 of Cripto); however, Cripto shows little resemblance to EGF and does not bind to EGF receptors (33, 34). Cripto does not bind directly to activin, and activin type II receptors seem to be required for the formation of a Cripto-activin complex (35). Cripto exists in two forms, a membrane-bound form and a soluble secreted form. The membrane-bound Cripto anchors to the lipid bilayer of cell surfaces by using a glycosyl-phosphatidylinositol molecule as a co-receptor for Nodel, an activin-like TGF-β family member (36). The soluble secreted Cripto acts as a growth factor–like signaling molecule (37, 38). A Cripto receptor has not been defined yet, but Cripto was found to interact with Glypican-1 (39) and indirectly with ErbB-4 (40) as well as fibroblast growth

factor receptor (41). Cripto plays an important role in embryonic development in zebrafish and Xenopus (42, 43).

It has been shown that Cripto binds Nodal *via* its EGF-like domain and interacts with ALK4 *via* its CFC domain to promote Nodal signaling (44). In contrast, Cripto inhibits activin signaling in many cell lines (35, 45) by inhibiting the recruitment of the signaling type I receptor, ALK4. The actions of Cripto to block activin signaling are dependent on the EGF-like domain of Cripto (35). The molecular mechanisms of the functions of Cripto remain to be elucidated.

Activin Receptors and the Regulation of Receptors Activity. *Activin Receptors.* Activins and other members of the TGF-β superfamily exert their biological effects by interacting with two types of transmembrane receptors that have intrinsic serine/threonine kinase activities. Type I receptors, referred to as ALKs (for activin receptor-like kinases), include ALK 1–7 (46). Functional studies have established that ALK4 is the *bona fide* type I receptor for activin. A recent study also suggests that ALK7 functions as a type I receptor for activin AB and activin B (47). The combination of ActRIIA and ALK7, preferred by activin AB and activin B but not by activin A, is responsible for the activin-mediated secretion of insulin from pancreatic β cell line, MIN6 (47).

Ligand-Receptor Binding. Recent structural analysis studies shed light on ligand-receptor interactions. Activin, similar to other members in the TGF-β superfamily, forms a butterfly-shaped dimer linked by an interchain disulfide bond, and each monomer of the dimer comprises one α-helix and nine β-sheets to form a curled "hand" structure that contains a wrist region, a fingertip region, and a knuckle region (25, 48, 49). Crystal structures of activin A bound to its type II receptor revealed that the ligand residues in the knuckle region are involved in type II receptor binding (24, 25). In the activin A-ActRIIB complex, activin adopts some very flexible conformations that may account for its binding affinity to the receptors, and the ligand-receptor binding may also be influenced by the membrane-restricted setting of the receptors (49, 50). The ActRIIB-activin A interface involves hydrophobic, charged, and polar residues in both ActRIIB and activin A. The residue Lys102 in the knuckle region of activin A appears to be highly critical, since when it is changed to Glu, the mutant completely loses its ability to bind ActRII (51). Based on the structure of BMP7 complexed with its type I receptor ALK3 (also called BMPRIA), it was proposed that activin associates with type I and type II receptors *via* different regions, contacting ALK4 through the residues in the wrist region (25, 50). Indeed, the replacement of the residue Met108 with Ala or Glu in this region of activin A led to significantly lower signaling activity, even though the mutant still retained wild-type-like binding affinity for ActRII (26). The defect of the M108A mutant in forming a cross-linked complex with ALK4 in the presence of ActRII indicates that the ability of M108A to bind ALK4 was disrupted. Mutagenesis studies have identified five hydrophobic amino acid residues in the ALK4 extracellular domain (Leu40, Ile70, Val73, Leu75, and Pro77) that likely constitute the binding surface for activin (52).

Specificity of Receptor-Smad Interaction. The receptor-regulated Smad proteins (R-Smad) can be divided into two groups. Group 1 contains Smad2 and Smad3, which bind to and are phosphorylated by the activated type I receptors of TGF-β and activin; Smad1, 5, and 8 make up the second group and are activated by BMP type I receptors. The specific receptor-Smad interaction is mainly determined by the discrete structural motifs in type I receptors and R-Smads: the L45 loop in the kinase domain of type I receptors and the L3 loop in the MH2 domain of R-Smads (53–55). The amino acid sequence of the L45 loop is conserved among the type I receptors that have similar substrate specificity; that is, ALK4 and ALK5 (also known as TβRI) have a similar L45 loop sequence, which is different from that of BMP type I receptors. Similarly, Smad2 and Smad3, substrates for ALK4 and ALK5, respectively, share the same L3 loop sequence, which is distinct from that of Smad 1, 5, and 8 (56).

Functional Difference in Smad2 and Smad3. Although Smad2 and Smad3 are structurally very similar, they have some functional differences in transducing signals for activin receptors. For example, Smad2 and Smad3 behave differently in regulating the transcription of a homeobox gene, *goosecoid*, through different interaction with Smad4 and a transcription factor FAST-2 (forkhead activin signal transducer-2) (57). The major structural difference between Smad2 and Smad3 is at the MH1 domain, and the second insertion at the MH1 domain of Smad2 may interfere with DNA binding (58). Therefore, the MH1 domain of Smad3 but not Smad2 is able to directly bind DNA (58–60). The absence of this insertion in the Smad2, as a product of alternative splicing with a deletion of exon 3 of the Smad2 gene, occurs naturally in mammalian cells, resulting in a gain of function in binding the activator protein-1 (AP-1) sites of the p3TP-lux promoter, which contains three repeats of AP-1 elements and the plasminogen activator inhibitor-1 (PAI-1) promoter (61).

In FAST-2–mediated transcriptional regulation using the activin-responsive element derived from *Xenopus* Mix.2 promoter as a reporter, Smad3 but not Smad2 alone was able to stimulate the transcription. In addition, Smad3 inhibits the transactivation of the promoter induced by co-expression of Smad2, Smad4, and an active activin type-I receptor. The Mad homology 1 (MH1) domain of Smad3 was found to be indispensable for the dual regulatory function of Smad3. However, this Smad3-specific function could not be manifested in Smad2 mutants that were devoid of the two amino acid insertions (at the MH1 domain) that comprise the major structural difference between Smad2 and Smad3. These findings indicate that other structural motifs are involved in determining the regulatory activity of Smad3 and the most N-terminal portion of Smad3 was

crucial for its function (62). The unique function of Smad3 in modulating the FAST-2–mediated transcription is contributed to by a subtle difference in the structural features at the MH1 domain. Indeed, studies using cells deficient in Smad2 for biochemical and cell biological assays and Smad3-deficient mice also demonstrate that there is a clear difference between Smad2 and Smad3 during development (63).

The Regulation of Receptor Activity. *Inhibin and Betaglycan.* Inhibins are heterodimers of αβ subunits and share the inhibin/activin βA or βB subunit with activins. They are potent antagonists of activins in a subset of activin-responsive cells, including pituitary gonadotropes (13, 64, 65). Although it has long been thought that inhibin antagonizes activin by competing for the binding to the activin signaling receptors, the molecular basis of the selective actions of inhibins was only recently elucidated. Betaglycan, a proteoglycan known as a type III TGF-β receptor, binds inhibin, and the binding affinity increases about 30-fold in cells co-expressing ActRII and betaglycan (66). ALK4 is excluded from this ternary inhibin-betaglycan-ActRII complex. Betaglycan also forms a complex with ActRIIB and BMPRII, thereby sequestering them away from activin or BMP (66, 67).

BAMBI. BAMBI is a transmembrane protein that lacks an intracellular kinase domain but has sequence similarity with the extracellular domain of the type I receptors, and thereby functions as a general negative modulator for BMP, activin, and TGF-β (68). BAMBI is highly conserved and has been identified in zebrafish, mice, rats, and human beings. The human homolog, also known as nma (non-metastatic gene A), might play an important role by providing cancer cells with an escape mechanism from TGF-β–mediated inhibition of cell growth. Knockdown of BAMBI expression with antisense oligonucleotides restored the inhibitory effect of TGF-β in several human gastric carcinoma cell lines (69). Furthermore, overexpression of BAMBI inhibits the response of tumor cells to TGF-β signaling, and the expression of BAMBI is activated by the Wnt/β-catenin signaling in colorectal tumor cells (70). The expression of BAMBI is also regulated in male germ cells, a finding correlating well with the previous observation that a decrease of activin activity is critical in gonadocyte differentiation (71).

ARIPs. Recent studies indicate that the cell surface level of ActRIIs, the primary receptors for activins and related ligands, is regulated by a group of proteins called ARIPs (activin receptor-interacting proteins) (72–74). ARIP1 contains several protein-protein interaction domains, including two WW domains and five or six PDZ domains. In addition to interacting with ActRIIs, ARIP1 can also bind to Smads, glutamate receptors, adhesion proteins, PTEN, and β-catenin, implying that it plays an important role in signal cross-talking (72, 73, 75, 76). In contrast, ARIP2, a small protein that has only one PDZ domain (74), decreases the expression levels of ActRIIs in the plasma membrane and reduces their response to ligands. Apparently, ARIP2 functions as an adaptor protein by recruiting RalBP1 (*via* the COOH-terminus) to ActRII (through the PDZ domain). Then, RalBP1 works with the small GTP-binding protein Ral to regulate the endocytosis of ActRIIs.

Dapper2. In the search for Dishevelled (Dvl)-binding proteins, Dapper was found to function as a general antagonist of Wnt signaling to inhibit both the canonical β-catenin pathway and the non-canonical c-Jun N-terminal kinase pathway (77, 78). Inhibition of maternal Dapper expression results in the loss of the notochord and head structures in Xenopus embryos, suggesting an important role of Dapper in modulating Wnt signaling for normal vertebrate development. Interestingly, Frodo, which has high similarity to Dapper and was also identified as a Dvl-interacting protein, has been shown to promote Wnt signaling (79). Recently, Frodo was proposed to mediate signaling directly from Dvl to TCF in a β-catenin-independent way (80). Dapper2, which is divergently related to Dapper, interferes with Nodal signals in mesoderm induction in zebrafish (81). Knockdown of Dapper2 expression by antisense oligonucleotides enhanced expression of mesoderm markers, whereas its overexpression resulted in eye fusion, a phenotype resembling that from mutation of Nodal coreceptor one-eye pinhead. Biochemical analysis studies demonstrated that Dapper2 specifically associates with ALK5 and Activin/Nodal type I receptor ALK4 and targets receptors for lysosomal degradation (81). Therefore, Dapper2 controls endocytosed receptor transport from late endosomes to lysosomes for degradation. By modulating the cell surface protein level of receptors, Dapper2 may function to fine-tune Nodal/activin signaling in the mesoderm formation. The function of Dapper proteins could be complex, as recent work suggests that both Dapper1 and Dapper2 are required for Wnt signaling in zebrafish embryogenesis (82). Further investigation is needed to establish the physiological role of Dappers in a spatial-temporal manner.

BMP-3. BMP-3, unlike other members of the TGF-β superfamily, inhibits biological activities of other BMPs and TGF-βs in a variety of mammalian systems (83). In Xenopus embryos, unlike BMP-2, BMP-4, and BMP-7, which are all potent ventralizing agents, BMP-3 induces dorsalization (83, 84). These effects of BMP-3 are similar to those of the BMP antagonists noggin, chordin, and follistatin. BMP-3β, a molecule 83% homologous to BMP-3, can also antagonize BMP-like ligands and Nodal-like proteins (84). BMP-3 also interferes with activin signaling (78) and binds to ActRIIB, a common type II receptor for activin-related proteins that is unable to activate R-Smads (78).

Small-Molecule Receptor Inhibitors. Specific small-molecule inhibitors of the signaling machinery have great potential therapeutic uses to target human diseases. Thus, there is considerable interest in two recently developed competitive inhibitors for the ATP binding site in the kinase

domain of ALK5 (86, 87). These inhibitors, SB-431542 and SB-505124, also inhibit ALK4 and ALK7, which share similar substrate specificity with ALK5. However, these small-molecule receptor inhibitors have no effect on ALK1-, ALK2-, ALK3-, or ALK6-induced Smad signaling, or on the activities of other protein kinases, suggesting that the inhibition of the receptors is remarkably specific (86, 87). SB-431542 has been shown to inhibit the TGF-β–induced proliferation of human osteosarcoma cells (88) and TGF-β–promoted cell proliferation, angiogenesis, and motility in malignant glioma (89, 113). The effect of these molecules in activin-dependent physiopathological systems remains to be determined.

The Downstream Smad Effectors and the Regulation of Their Activities. R-Smad and Co-Smad proteins consist of the sequence-conserved amino-terminal Mad homology 1 (MH1) domain, the carboxyl-terminal MH2 domain, and the sequence-divergent middle linker region. The MH1 domain is mainly involved in DNA binding and the interaction with other transcription factors. The MH2 domain contributes to transcriptional activation, Smad-Smad interaction, Smad-receptor interaction, and association with other binding proteins and transcription factors (5, 90, 91). The linker region provides a binding site for Smurfs, ubiquitin E3 ligases, and thus is involved in Smad ubiquitination and proteosome-mediated degradation (92–95). Inhibitory Smads (I-Smads: Smad6 and Smad7) form a distinct subclass of Smads that act in the opposite manner to R-Smads and antagonize signaling. Observations of recently identified Smad binding proteins, their roles in regulation of Smad activities, and our current understanding of the nucleocytoplasmic shuttling of Smad proteins are briefly summarized below.

Regulation of Smad Activity. The activity of Smad is regulated positively or negatively by a variety of signal inputs (6, 7, 56, 78, 96). The linker region of the Smad also contains the phosphorylation sites for ERK, CDK, and Ca^{2+}-calmodulin–dependent kinase II; phosphorylation of this region by these kinases negatively regulates Smad activity (97–99).

First, Smad proteins function in the nucleus as transcription factors. This transcriptional activity is dependent on the recruitment of other coactivators, such as CBP and p300 (100), or corepressors, such as c-Ski and SnoN (101, 102). The *DLX* genes constitute a subfamily of vertebrate homeobox genes that are structurally similar to the *Drosophila distal-less* gene, and they play a central role in appendage development, neurogenesis, and hematopoiesis (103, 104). Recent evidence shows that DLX1 is expressed in hematopoietic cells in a lineage-dependent manner. DLX1 interacts with Smad4 and blocks the differentiation of a hematopoietic cell lineage induced by activin A (105).

Second, at steady state, R-Smads are predominantly cytoplasmic, and Smad4 is distributed throughout the cytoplasm and nucleus; however, upon ligand stimulation both R-Smads and Smad4 accumulate in the nucleus (106). The Smads shuttle between the cytoplasm and the nucleus in both basal and activated states (107, 108). However, the R-Smads are continuously being dephosphorylated in the nucleus at a low rate by a yet unidentified phosphatase. This dephosphorylation allows R-Smads to dissociate from Smad4 and move to the cytoplasm (107, 108). If the receptors are still active, R-Smads are rephosphorylated, form complexes with Smad4, and relocalize to the nucleus. If the receptors are no longer active, then the Smad proteins accumulate in the cytoplasm over time (108).

Lastly, Smad4 contains a nuclear export signal (NES) and a nuclear localization signal (NLS) in the linker region. The nuclear exporter CRM1 binds to the NES of Smad4, and the nuclear importer importin-α binds to the NLS to facilitate Smad4 nucleocytoplasmic shuttling (106, 109, 110). Smad3 also contains a basic NLS in its MH1 domain that is thought to bind directly to importin-β; mutation of this NLS prevents Smad3 from accumulating in the nucleus, even when the cells are treated with TGF-β (111, 112). The Smad2 MH1 domain does not bind to importin-β (113, 155) due to the presence of a unique insert sequence in the exon 3 of Smad2 (112). Instead, there is an alternative pathway for Smad2 shuttling that does not involve classical signals (107, 113, 114). The Smad2 nucleocytoplasmic shuttling is independent of transport receptors. It is mediated by direct interactions of the MH2 domain with nucleoporins, particularly Nup214 and Nup153, which are present on the cytoplasmic and the nuclear side of the nuclear pore complex, respectively. The MH2 domain of Smad3, which is closely related to that of Smad2, also binds to components of the nuclear pore complex (114). The fact that Smad3 has a greater tendency to be in the nucleus as compared to Smad2 and that it can readily activate TGF-β transcriptional responses in the absence of signaling when overexpressed may be due to its ability to interact with both the nucleoporin complex and importin-β. CRM1 is not involved in the ATP-dependent export of Smad2 and Smad3 from the nucleus, suggesting that an alternative exporter is involved in their active exporting out of the nucleus (108).

Interestingly, Smad-binding proteins in the cytoplasm and the coactivators or corepressors in the nucleus may be involved in modulating the Smad nucleocytoplasmic shuttling through their interactions with Smads. For example, the purified Smad-binding domain (SBD) of the cytoplasmic Smad anchor for receptor activation protein (SARA) inhibits nuclear accumulation of the Smad2 MH2 domain (113), whereas FoxH1 overexpression leads to Smad2 nuclear accumulation in the absence of ligands (107, 115). Furthermore, the presence of Smad4 binding partners in the nucleus, such as the corepressor proteins SnoN and Ski, may also contribute to the nuclear retention of Smad4 in unstimulated cells (101, 102).

Smad-Independent Signal Pathways of Activin. Although the Smad pathway is the well-established central mediator for the TGF-β superfamily members from the

receptors to the nucleus, activation of Smad alone cannot account for all of the downstream signaling events of TGF-β/activin activation. Increasing biochemical and developmental evidence supports an alternative: non-Smad pathways in TGF-β signaling. TGF-β receptors can directly interact with or phosphorylate non-Smad proteins, initiating parallel signaling that cooperates with the Smad pathway in eliciting physiological responses (116). The results obtained from *in vitro* cell models imply that the small GTPase Ras and the mitogen-activated protein kinases (MAPKs) ERKs, p38, and c-Jun N-terminal kinases (JNKs) are involved in the TGF-β signaling (117).

Activins play a critical role in epidermis remodeling that involves epithelial cell movement. The MEKK1-JNK cascade is required for eyelid closure in mammals. It was demonstrated that the MEKK1-JNK cascade transmits TGF-β and activin signals to control epithelial cell movement in a Smad4-independent way (118). It was also revealed that activin A induced p38 phosphorylation and inhibited ERK1/2 phosphorylation in K562 cells (119). In keratinocytes, activins can activate MEKK1-dependent phosphorylation of JNK and c-Jun in a RhoA-dependent but Rac- and CDC42-independent way (8). Furthermore, activins stimulate p38 activity by a RhoA-independent mechanism in MEKK1-deficient keratinocytes. Interestingly, neither pathway is dependent on Smad activation, although the mechanism by which activins activate RhoA or MEKK 1 remains unclear. Dok-1, a Ras GAP-binding protein and a target of protein tyrosine kinases-like Src and Abl (121), has been suggested to mediate activin signaling to Smad proteins by binding to ActRIIA and ALK4 (120). Although Dok-1 cannot be tyrosine phosphorylated in response to activin signaling (120), it remains interesting to determine whether or not it functions as an adaptor mediating the signaling from receptor to GTPase in a non-Smad pathway. Indeed, TAK1, originally identified as a TGF-β activated kinase, has been suggested to mediate activin A signaling by the MKK3-p38 MAPK pathway to activate the expression of neurogenin3, a transcription factor essential for the differentiation of pancreatic endocrine cells (122).

Regulation of Cell Proliferation, Apoptosis, and Carcinogenesis by Activin

Activins regulate the growth and differentiation in numerous types of cells. The actions of activins on cell growth and differentiation are mainly through the Smad-dependent pathways. For instance, activin A induces growth inhibition of Hep3B cells by downregulating Bcl-xL (anti-apoptotic) expression *via* Smad2 or Smad3 (123). HepG2 cells treated with activin showed increased gene expression of the cyclin-dependent kinase inhibitor p15INK4B and induced cell cycle arrest (124). Activin is strongly expressed in wounded skin, and overexpression of activin in the epidermis of transgenic mice improves wound healing and enhances scar formation (127). Conversely, inhibition of activin activities in the skin delays wound healing but improves the quality of the healed wound. In addition to effecting cell growth, activin regulates the morphogenesis of branching organs such as the prostate, lung, and kidney (125), and it regulates the differentiation of human trophoblast tissues during early pregnancy (126).

TGF-β and BMP have been shown to be essential for the maintenance of stem cell renewal. Similarly, activin/Nodal signaling through Smad2/3 is also necessary to maintain the pluripotent status of human embryo stem cells (hESCs), and in order that WNT and TGFβ/activin/nodal signaling collaborate in maintaining pluripotency (128, 129). Inhibition of activin/Nodal signaling by follistatin, overexpression of Lefty or Cerberus-Short, or the activin receptor inhibitor SB431542 induces hESC differentiation (128). Furthermore, activin A is secreted by mouse embryonic feeder layers, and culture medium enriched with activin A is capable of maintaining hESCs in the undifferentiated state for over 20 passages in feeder-free cultures (130).

However, the functions of activin in embryonic stem cells are still dubious. In contrast to the effect of activin in maintaining pluripotency, activin A in combination with other factors has been used to induce embryonic stem cells to differentiate into pancreatic ß cells (131). Activin A induces mesoderm and endoderm in mouse embryoid bodies (132). Furthermore, Activin A has also been used to induce human embryonic stem cells to differentiate into definitive endoderm in culture (133). Mouse embryonic stem cells can also be directed to differentiate into $Rx^+/Pax6^+$ neural retinal precursors in the SFEB culture combined with Dkk1, LeftyA, FCS, and activin (134). Activin A has also been demonstrated to have an inhibitory effect on murine hematopoietic stem cells (135).

Activin also induces apoptosis in multiple cells and tissues. In hematopoietic cells, TGF-β family members regulate apoptosis *via* the inositol phosphatase SHIP (SH2 domain-containing 5′ inositol phosphatase), a central regulator of phospholipid metabolism. Activin/TGF-β–induced expression of SHIP results in the intracellular changes in the pool of phospholipids, as well as in the inhibition of both Akt/PKB phosphorylation and cell survival (136). Activin inhibits adrenal tumor growth by inducing x-zone apoptosis (137). In addition, activin/inhibin signaling can increase apoptosis in human adrenocortical cells and hematopoietic cells (136, 138). Activin C and E have also been shown to induce apoptosis in both human and rat hepatoma cells (139).

In addition to inducing apoptosis, activin may have an anti-tumorigenic effect. Activin inhibits proliferation of human tumor cells derived from gall bladder (140), prostate (141), and pituitary gland (142). The mRNAs encoding all three activin/inhibin subunits are expressed in breast carcinoma, fibroadenoma, and normal mammary tissue (143). Activin inhibits cellular proliferation of T47D breast cancer cells by enhancing the expression of p15 cyclin-

dependent kinase inhibitors, reducing cyclin A expression, and phosphorylating the retinoblastoma protein (144). In this regard, activin resembles the TGF-β effect on epithelial cells.

Activin A also exerts its tumor suppressor function as an inhibitor of angiogenesis. Neuroblastoma cells with restored activin A expression exhibit a diminished proliferation rate and form smaller xenograft tumors with reduced vascularity (145). Cells treated with activin A showed increased expression of cyclin-dependent kinase inhibitors $p15^{INK1b}$, $p21^{CIP1}$, and $p27^{KIP1}$, and decreased expression of vascular endothelial growth factor receptor-2, the receptor of a key angiogenic factor in cancer. Overexpression of the constitutively active ALK4 has the same effects, and these actions are mediated *via* the Smad2/Smad3 pathways. Consistent with the finding that decreased vascularity of the xenograft tumors, activin A inhibited several crucial angiogenic responses in cultured endothelial cells, such as the proteolytic activity, migration, and proliferation.

As with the many complicated functions of activin in cell proliferation and differentiation, the relationship between activin and carcinogenesis is not simple. Increased levels of activin A in human endometrial adenocarcinoma tissues have been proposed to be involved in carcinogenesis by reducing TGF-β–mediated signals that have inhibitory effects on these tissues (146). Activin A has been shown to mediate high N-cadherin expression on the carcinoma cell surface, and this is associated with tumor aggressiveness and a poor prognosis (147). Activin signaling has also been suggested to be active at the leading edge of ErbB2/Neu-induced tumors, even though the Smad-dependent TGF-β signaling is absent in these tumors. (148) Activin A can increase the proliferation of ovarian cancer cell lines SKOV3, OCC1, OVCAR3, and A2780-s, and this could be because activin A augmented invasion through Matrigel (149). This shows that activins can be tumorigenic or anti-tumorigenic, depending on the setting. It is far from clear whether their roles are causal or consequential; the molecular mechanisms for the actions of activin remain to be determined.

Conclusion

Activin A is pleiotropic and affects proliferation, differentiation, and apoptosis in a variety of cell types. Insight on the molecular basis for activin signal transduction has been gained; however, the actions of activin and the regulation and dysregulation of this molecule in the physiological and pathological conditions, respectively, remain to be elucidated.

1. Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R. Pituitary FSH is released by a heterodimer of the beta-subunits from the two forms of inhibin. Nature 321:779–782, 1986.
2. Vale W, Rivier J, Vaughan J, McClintock R, Corrigan A, Woo W, Karr D, Spiess J. Purification and characterization of an FSH releasing protein from porcine ovarian follicular fluid. Nature 321:776–779, 1986.
3. Woodruff TK, Mather JP. Inhibin, activin and the female reproductive axis. Annu Rev Physiol 57:219–244, 1995.
4. Risbridger GP, Schmitt JF, Robertson DM. Activins and inhibins in endocrine and other tumors. Endocr Rev 22:836–858, 2001.
5. Massague J. TGF-beta signal transduction. Annu Rev Biochem 67: 753–791, 1998.
6. Feng XH, Derynck R. Specificity and versatility in TGF-β signaling through Smads. Annu Rev Cell Dev Biol 21:659–693, 2005.
7. Chen YG, Lui HM, Lin SL, Lee JM, Ying SY. Regulation of cell proliferation, apoptosis, and carcinogenesis by activin. Exp Biol Med (Maywood) 227:75–87, 2002.
8. Zhang L, Deng M, Parthasarathy R, Wang L, Mongan M, Molkentin JD, Zheng Y, Xia Y. MEKK1 transduces activin signals in keratinocytes to induce actin stress fiber formation and migration. Mol Cell Biol 25:60–65, 2005.
9. Shi Y, Massague J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113:685–700, 2003.
10. Vitt UA, Hsu SY, Hsueh AJ. Evolution and classification of cystine knot-containing hormones and related extracellular signaling molecules. Mol Endocrinol 15:681–694, 2001.
11. Vale W, Hsueh A, Rivier C, Yu J. The inhibin/activin family of hormones and growth factors. In: Sporn MB, Roberts AB, Eds. Peptide Growth Factors and Their Receptors: Handbook of experimental pharmacology. Heidelberg: Springer-Verlag, pp211–248, 1990.
12. Massague J. The transforming growth factor-beta family. Annu Rev Cell Biol 6:597–641, 1990.
13. Ying SY. Inhibins, activins, and follistatins—gonadal proteins modulating the secretion of FSH. Endo Rev 9:267–304, 1988.
14. Vejda S, Cranfield M, Peter B, Mellor SL, Groome N, Schulte-Hermann R, Rossmanith W. Expression and dimerization of the rat activin subunits betaC and betaE: evidence for the formation of novel activin dimers. J Mol Endocrinol 28:137–148, 2002.
15. Mellor SL, Cranfield M, Ries R, Pedersen J, Cancilla B, de Kretser D, Groome NP, Mason AJ, Risbridger GP. Localization of activin beta(A)-, beta(B)-, and beta(C)-subunits in humanprostate and evidence for formation of new activin heterodimers of beta(C)-subunit. J Clin Endocrinol Metab 85:4851–4858, 2000.
16. Wada W, Maeshima A, Zhang YQ, Hasegawa Y, Kuwano H, Kojima I. Assessment of the function of the betaC-subunit of activin in cultured hepatocytes. Am J Physiol Endocrinol Metab 287:E247–254, 2004.
17. Chabicovsky M, Herkner K, Rossmanith W. Overexpression of activin beta(C) or activin beta(E) in the mouse liver inhibits regenerative deoxyribonucleic acid synthesis of hepatic cells. Endocrinology 144:3497–3504, 2003.
18. Vejda S, Erlach N, Peter B, Drucker C, Rossmanith W, Pohl J, Schulte-Hermann R, Grusch M. Expression of activins C and E induces apoptosis in human and rat hepatoma cells. Carcinogenesis 24:1801–1809, 2003.
19. Lau AL, Kumar TR, Nishimori K, Bonadio J, Matzuk MM. Activin betaC and betaE genes are not essential for mouse liver growth, differentiation, and regeneration. Mol Cell Biol 20:6127–6137, 2000.
20. Welt C, Sidis Y, Keutmann H, Schneyer A. Activins, inhibins, and follistatins: from endocrinology to signaling. A paradigm for the new millennium. Exp Biol Med (Maywood) 227:724–752, 2002.
21. Sidis Y, Schneyer AL, Keutmann HT. Heparin and activin-binding determinants in follistatin and FSTL3. Endocrinology 146:130–136, 2005.
22. Schneyer A, Schoen A, Quigg A, Sidis Y. Differential binding and neutralization of activins A and B by follistatin and follistatin like-3 (FSTL-3/FSRP/FLRG). Endocrinology 144:1671–1674, 2003.
23. Fischer WH, Park M, Donaldson C, Wiater E, Vaughan J, Bilezikjian

LM, Vale W. Residues in the C-terminal region of activin A determine specificity for follistatin and type II receptor binding. J Endocrinol 176:61–68, 2003.

24. Greenwald J, Groppe J, Gray P, Wiater E, Kwiatkowski W, Vale W, Choe S. The BMP7/ActRII extracellular domain complex provides new insights into the cooperative nature of receptor assembly. Mol Cell 11:605–617, 2003.
25. Thompson TB, Woodruff TK, Jardetzky TS. Structures of an ActRIIB:activin A complex reveal a novel binding mode for TGF-beta ligand:receptor interactions. Embo J 22:1555–1566, 2003.
26. Harrison CA, Gray PC, Fischer WH, Donaldson C, Choe S, Vale W. An activin mutant with disrupted ALK4 binding blocks signaling via type II receptors. J Biol Chem 279:28036–28044, 2004.
27. Thompson TB, Lerch TF, Cook RW, Woodruff TK, Jardetzky TS. The structure of the follistatin:activin complex reveals antagonism of both type I and type II receptor binding. Dev Cell 9:535–543, 2005.
28. Hayette S, Gadoux M, Martel S, Bertrand S, Tigaud I, Magaud JP, Rimokh R. FLRG (follistatin-related gene), a new target of chromosomal rearrangement in malignant blood disorders. Oncogene 16:2949–2954, 1998.
29. Tsuchida K, Arai KY, Kuramoto Y, Yamakawa N, Hasegawa Y, Sugino H. Identification and characterization of a novel follistatin-like protein as a binding protein for the TGF-beta family. J Biol Chem 275:40788–40796, 2000.
30. Tortoriello DV, Sidis Y, Holtzman DA, Holmes WE, Schneyer AL. Human follistatin-related protein: a structural homologue of follistatin with nuclear localization. Endocrinology 142:3426–3434, 2001.
31. Hill JJ, Davies MV, Pearson AA, Wang JH, Hewick RM, Wolfman NM, Qiu Y. The myostatin propeptide and the follistatin-related gene are inhibitory binding proteins of myostatin in normal serum. J Biol Chem 277:40735–40741, 2002.
32. Bartholin L, Maguer-Satta V, Hayette S, Martel S, Gadoux M, Bertrand S, Corbo L, Lamadon C, Morera AM, Magaud JP, Rimokh R. FLRG, an activin-binding protein, is a new target of TGFbeta transcription activation through Smad proteins. Oncogene 20:5409–5419, 2001.
33. Adamson ED, Minchiotti G, Salomon DS. Cripto: a tumor growth factor and more. J Cell Physiol 190:267–278, 2002.
34. Normanno N, Bianco C, De Luca A, Salomon DS. The role of EGF-related peptides in tumor growth. Front Biosci 6:D685–707, 2001.
35. Gray PC, Harrison CA, Vale W. Cripto forms a complex with activin and type II activin receptors and can block activin signaling. Proc Natl Acad Sci U S A 100:5193–5198, 2003.
36. Minchiotti G, Parisi S, Liguori G, Signore M, Lania G, Adamson ED, Lago CT, Persico MG. Membrane-anchorage of Cripto protein by glycosylphosphatidylinositol and its distribution during early mouse development. Mech Dev 90:133–142, 2000.
37. Yan YT, Liu JJ, Luo Y, Chaosu E, Haltiwanger RS, Abate-Shen C, Shen MM. Dual roles of Cripto as a ligand and coreceptor in the nodal signaling pathway. Mol Cell Biol 22:4439–4449, 2002.
38. Bianco C, Adkins HB, Wechselberger C, Seno M, Normanno N, De Luca A, Sun Y, Khan N, Kenney N, Ebert A, Williams KP, Sanicola M, Salomon DS. Cripto-1 activates nodal- and ALK4-dependent and -independent signaling pathways in mammary epithelial cells. Mol Cell Biol 22:2586–2597, 2002.
39. Bianco C, Strizzi L, Rehman A, Normanno N, Wechselberger C, Sun Y, Khan N, Hirota M, Adkins H, Williams K, Margolis RU, Sanicola M, Salomon DS. A Nodal- and ALK4-independent signaling pathway activated by Cripto-1 through Glypican-1 and c-Src. Cancer Res 63:1192–1197, 2003.
40. Bianco C, Kannan S, De Santis M, Seno M, Tang CK, Martinez-Lacaci I, Kim N, Wallace-Jones B, Lippman ME, Ebert AD, Wechselberger C, Salomon DS. Cripto-1 indirectly stimulates the tyrosine phosphorylation of erb B-4 through a novel receptor. J Biol Chem 274:8624–8629, 1999.
41. Saloman DS, Bianco C, Ebert AD, Khan NI, De Santis M, Normanno N, Wechselberger C, Seno M, Williams K, Sanicola M, Foley S, Gullick WJ, Persico G. The EGF-CFC family: novel epidermal growth factor-related proteins in development and cancer. Endocr Relat Cancer 7:199–226, 2000.
42. Minchiotti G, Parisi S, Liguori GL, D'Andrea D, Persico MG. Role of the EGF-CFC gene cripto in cell differentiation and embryo development. Gene 287:33–37, 2002.
43. Ding J, Yang L, Yan YT, Chen A, Desai N, Wynshaw-Boris A, Shen MM. Cripto is required for correct orientation of the anterior-posterior axis in the mouse embryo. Nature 395:702–707, 1998.
44. Yeo C, Whitman M. Nodal signals to Smads through Cripto-dependent and Cripto-independent mechanisms. Mol Cell 7:949–957, 2001.
45. Adkins HB, Bianco C, Schiffer SG, Rayhorn P, Zafari M, Cheung AE, Orozco O, Olson D, De Luca A, Chen LL, Miatkowski K, Benjamin C, Normanno N, Williams KP, Jarpe M, LePage D, Salomon D, Sanicola M. Antibody blockade of the Cripto CFC domain suppresses tumor cell growth in vivo. J Clin Invest 112:575–587, 2003.
46. Derynck R, Feng XH. TGF-beta receptor signaling. Biochim Biophys Acta 1333:F105–150, 1997.
47. Tsuchida K, Nakatani M, Yamakawa N, Hashimoto O, Hasegawa Y, Sugino H. Activin isoforms signal through type I receptor serine/threonine kinase ALK7. Mol Cell Endocrinol 220:59–65, 2004.
48. Daopin S, Piez KA, Ogawa Y, Davies DR. Crystal structure of transforming growth factor-beta 2: an unusual fold for the super-family. Science 257:369–373, 1992.
49. Greenwald J, Vega ME, Allendorph GP, Fischer WH, Vale W, Choe S. A flexible activin explains the membrane-dependent cooperative assembly of TGF-beta family receptors. Mol Cell 15:485–489, 2004.
50. Sebald W, Mueller TD. The interaction of BMP-7 and ActRII implicates a new mode of receptor assembly. Trends Biochem Sci 28:518–521, 2003.
51. Wuytens G, Verschueren K, de Winter JP, Gajendran N, Beek L, Devos K, Bosman F, de Waele P, Andries M, van den Eijnden-van Raaij AJ, Smith JC, Huylebroeck D. Identification of two amino acids in activin A that are important for biological activity and binding to the activin type II receptors. J Biol Chem 274:9821–9827, 1999.
52. Harrison CA, Gray PC, Koerber SC, Fischer W, Vale W. Identification of a functional binding site for activin on the type I receptor ALK4. J Biol Chem 278:21129–21135, 2003.
53. Chen YG, Hata A, Lo RS, Wotton D, Shi Y, Pavletich N, Massague J. Determinants of specificity in TGF-beta signal transduction. Genes Dev 12:2144–2152, 1998.
54. Chen YG, Massague J. Smad1 recognition and activation by the ALK1 group of transforming growth factor-beta family receptors. J Biol Chem 274:3672–3677, 1999.
55. Feng XH, Derynck R. A kinase subdomain of transforming growth factor-beta (TGF-beta) type I receptor determines the TGF-beta intracellular signaling specificity. Embo J 16:3912–3923, 1997.
56. Massague J, Chen YG. Controlling TGF-beta signaling. Genes Dev 14:627–644, 2000.
57. Labbe E, Silvestri C, Hoodless PA, Wrana JL, Attisano L. Smad2 and Smad3 positively and negatively regulate TGF beta-dependent transcription through the forkhead DNA-binding protein FAST2. Mol Cell 2:109–120, 1998.
58. Shi Y, Wang YF, Jayaraman L, Yang H, Massague J, Pavletich NP. Crystal structure of a Smad MH1 domain bound to DNA: insights on DNA binding in TGF-beta signaling. Cell 94:585–594, 1998.
59. Dennler S, Itoh S, Vivien D, ten Dijke P, Huet S, Gauthier JM. Direct binding of Smad3 and Smad4 to critical TGF beta-inducible elements in the promoter of human plasminogen activator inhibitor-type 1 gene. Embo J 17:3091–3100, 1998.
60. Zawel L, Dai JL, Buckhaults P, Zhou S, Kinzler KW, Vogelstein B,

Kern SE. Human Smad3 and Smad4 are sequence-specific transcription activators. Mol Cell 1:611–617, 1998.
61. Yagi K, Goto D, Hamamoto T, Takenoshita S, Kato M, Miyazono K. Alternatively spliced variant of Smad2 lacking exon 3. Comparison with wild-type Smad2 and Smad3. J Biol Chem 274:703–709, 1999.
62. Nagarajan RP, Chen Y. Structural basis for the functional difference between Smad2 and Smad3 in FAST-2 (forkhead activin signal transducer-2)-mediated transcription. Biochem J 350(Pt 1):253–259, 2000.
63. Goumans MJ, Mummery C. Functional analysis of the TGFbeta receptor/Smad pathway through gene ablation in mice. Int J Dev Biol 44:253–265, 2000.
64. Bilezikjian LM, Blount AL, Corrigan AZ, Leal A, Chen Y, Vale WW. Actions of activins, inhibins and follistatins: implications in anterior pituitary function. Clin Exp Pharmacol Physiol 28:244–248, 2001.
65. Bilezikjian LM VW. Local extragonadal roles of activins. Trends Endocrinol Metab 3:218–223, 1992.
66. Lewis KA, Gray PC, Blount AL, MacConell LA, Wiater E, Bilezikjian LM, Vale W. Betaglycan binds inhibin and can mediate functional antagonism of activin signalling. Nature 404:411–414, 2000.
67. Wiater E, Vale W. Inhibin is an antagonist of bone morphogenetic protein signaling. J Biol Chem 278:7934–7941, 2003.
68. Onichtchouk D, Chen YG, Dosch R, Gawantka V, Delius H, Massague J, Niehrs C. Silencing of TGF-beta signalling by the pseudoreceptor BAMBI. Nature 401:480–485, 1999.
69. Sasaki T, Sasahira T, Shimura H, Ikeda S, Kuniyasu H. Effect of Nma on growth inhibition by TGF-beta in human gastric carcinoma cell lines. Oncol Rep 11:1219–1223, 2004.
70. Sekiya T, Adachi S, Kohu K, Yamada T, Higuchi O, Furukawa Y, Nakamura Y, Nakamura T, Tashiro K, Kuhara S, Ohwada S, Akiyama T. Identification of BMP and activin membrane-bound inhibitor (BAMBI), an inhibitor of transforming growth factor-beta signaling, as a target of the beta-catenin pathway in colorectal tumor cells. J Biol Chem 279:6840–6846, 2004.
71. Loveland KL, Bakker M, Meehan T, Christy E, von Schonfeldt V, Drummond A, de Kretser D. Expression of Bambi is widespread in juvenile and adult rat tissues and is regulated in male germ cells. Endocrinology 144:4180–4186, 2003.
72. Shoji H, Tsuchida K, Kishi H, Yamakawa N, Matsuzaki T, Liu Z, Nakamura T, Sugino H. Identification and characterization of a PDZ protein that interacts with activin type II receptors. J Biol Chem 275: 5485–5492, 2000.
73. Tsuchida K, Matsuzaki T, Yamakawa N, Liu Z, Sugino H. Intracellular and extracellular control of activin function by novel regulatory molecules. Mol Cell Endocrinol 180:25–31, 2001.
74. Matsuzaki T, Hanai S, Kishi H, Liu Z, Bao Y, Kikuchi A, Tsuchida K, Sugino H. Regulation of endocytosis of activin type II receptors by a novel PDZ protein through Ral/Ral-binding protein 1-dependent pathway. J Biol Chem 277:19008–19018, 2002.
75. Hirao K, Hata Y, Ide N, Takeuchi M, Irie M, Yao I, Deguchi M, Toyoda A, Sudhof TC, Takai Y. A novel multiple PDZ domain-containing molecule interacting with N-methyl-D-aspartate receptors and neuronal cell adhesion proteins. J Biol Chem 273:21105–21110, 1998.
76. Tolkacheva T, Boddapati M, Sanfiz A, Tsuchida K, Kimmelman AC, Chan AM. Regulation of PTEN binding to MAGI-2 by two putative phosphorylation sites at threonine 382 and 383. Cancer Res 61:4985–4989, 2001.
77. Cheyette BN, Waxman JS, Miller JR, Takemaru K, Sheldahl LC, Khlebtsova N, Fox EP, Earnest T, Moon RT. Dapper, a Dishevelled-associated antagonist of beta-catenin and JNK signaling, is required for notochord formation. Dev Cell 2:449–461, 2002.
78. Chen YG, Meng AM. Negative regulation of TGF-beta signaling in development. Cell Res 14:441–449, 2004.
79. Gloy J, Hikasa H, Sokol SY. Frodo interacts with Dishevelled to transduce Wnt signals. Nat Cell Biol 4:351–357, 2002.
80. Hikasa H, Sokol SY. The involvement of Frodo in TCF-dependent signaling and neural tissue development. Development 131:4725–4734, 2004.
81. Zhang L, Zhou H, Su Y, Sun Z, Zhang H, Zhang Y, Ning Y, Chen YG, Meng A. Zebrafish Dpr2 inhibits mesoderm induction by promoting degradation of nodal receptors. Science 306:114–117, 2004.
82. Waxman JS, Hocking AM, Stoick CL, Moon RT. Zebrafish Dapper1 and Dapper2 play distinct roles in Wnt-mediated developmental processes. Development 131:5909–5921, 2004.
83. Daluiski A, Engstrand T, Bahamonde ME, Gamer LW, Agius E, Stevenson SL, Cox K, Rosen V, Lyons KM. Bone morphogenetic protein-3 is a negative regulator of bone density. Nat Genet 27:84–88, 2001.
84. Hino J, Nishimatsu S, Nagai T, Matsuo H, Kangawa K, Nohno T. Coordination of BMP-3b and cerberus is required for head formation of Xenopus embryos. Dev Biol 260:138–157, 2003.
85. Gamer LW, Nove J, Levin M, Rosen V. BMP-3 is a novel inhibitor of both activin and BMP-4 signaling in Xenopus embryos. Dev Biol 285: 156–168, 2005.
86. Inman GJ, Nicolas FJ, Callahan JF, Harling JD, Gaster LM, Reith AD, Laping NJ, Hill CS. SB-431542 is a potent and specific inhibitor of transforming growth factor-beta superfamily type I activin receptor-like kinase (ALK) receptors ALK4, ALK5, and ALK7. Mol Pharmacol 62:65–74, 2002.
87. DaCosta Byfield S, Major C, Laping NJ, Roberts AB. SB-505124 is a selective inhibitor of transforming growth factor-beta type I receptors ALK4, ALK5, and ALK7. Mol Pharmacol 65:744–752, 2004.
88. Matsuyama S, Iwadate M, Kondo M, Saitoh M, Hanyu A, Shimizu K, Aburatani H, Mishima HK, Imamura T, Miyazono K, Miyazawa K. SB-431542 and Gleevec inhibit transforming growth factor-beta-induced proliferation of human osteosarcoma cells. Cancer Res 63: 7791–7798, 2003.
89. Hjelmeland MD, Hjelmeland AB, Sathornsumetee S, Reese ED, Herbstreith MH, Laping NJ, Friedman HS, Bigner DD, Wang XF, Rich JN. SB-431542, a small molecule transforming growth factor-beta-receptor antagonist, inhibits human glioma cell line proliferation and motility. Mol Cancer Ther 3:737–745, 2004.
90. Heldin CH, Miyazono K, ten Dijke P. TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature 390:465–471, 1997.
91. Wrana JL. Regulation of Smad activity. Cell 100:189–192, 2000.
92. Zhu H, Kavsak P, Abdollah S, Wrana JL, Thomsen GH. A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation. Nature 400:687–693, 1999.
93. Kavsak P, Rasmussen RK, Causing CG, Bonni S, Zhu H, Thomsen GH, Wrana JL. Smad7 binds to Smurf2 to form an E3 ubiquitin ligase that targets the TGF beta receptor for degradation. Mol Cell 6:1365–1375, 2000.
94. Lin X, Liang M, Feng XH. Smurf2 is a ubiquitin E3 ligase mediating proteasome-dependent degradation of Smad2 in transforming growth factor-beta signaling. J Biol Chem 275:36818–36822, 2000.
95. Zhang Y, Chang C, Gehling DJ, Hemmati-Brivanlou A, Derynck R. Regulation of Smad degradation and activity by Smurf2, an E3 ubiquitin ligase. Proc Natl Acad Sci U S A 98:974–979, 2001.
96. Moustakas A, Souchelnytskyi S, Heldin CH. Smad regulation in TGF-beta signal transduction. J Cell Sci 114:4359–4369, 2001.
97. Kretzschmar M, Doody J, Timokhina I, Massague J. A mechanism of repression of TGFbeta/ Smad signaling by oncogenic Ras. Genes Dev 13:804–816, 1999.
98. Wicks SJ, Lui S, Abdel-Wahab N, Mason RM, Chantry A. Inactivation of Smad-transforming growth factor beta signaling by

Ca(2+)-calmodulin-dependent protein kinase II. Mol Cell Biol 20: 8103–8111, 2000.

99. Matsuura I, Denissova NG, Wang G, He D, Long J, Liu F. Cyclin-dependent kinases regulate the antiproliferative function of Smads. Nature 430:226–231, 2004.
100. Kawabata M, Imamura T, Inoue H, Hanai J, Nishihara A, Hanyu A, Takase M, Ishidou Y, Udagawa Y, Oeda E, Goto D, Yagi K, Kato M, Miyazono K. Intracellular signaling of the TGF-beta superfamily by Smad proteins. Ann N Y Acad Sci 886:73–82, 1999.
101. Stroschein SL, Wang W, Zhou S, Zhou Q, Luo K. Negative feedback regulation of TGF-beta signaling by the SnoN oncoprotein. Science 286:771–774, 1999.
102. Luo K, Stroschein SL, Wang W, Chen D, Martens E, Zhou S, Zhou Q. The Ski oncoprotein interacts with the Smad proteins to repress TGFbeta signaling. Genes Dev 13:2196–2206, 1999.
103. Stock DW, Ellies DL, Zhao Z, Ekker M, Ruddle FH, Weiss KM. The evolution of the vertebrate Dlx gene family. Proc Natl Acad Sci U S A 93:10858–10863, 1996.
104. Panganiban G, Rubenstein JL. Developmental functions of the Distal-less/Dlx homeobox genes. Development 129:4371–4386, 2002.
105. Chiba S, Takeshita K, Imai Y, Kumano K, Kurokawa M, Masuda S, Shimizu K, Nakamura S, Ruddle FH, Hirai H. Homeoprotein DLX-1 interacts with Smad4 and blocks a signaling pathway from activin A in hematopoietic cells. Proc Natl Acad Sci U S A 100:15577–15582, 2003.
106. Pierreux CE, Nicolas FJ, Hill CS. Transforming growth factor beta-independent shuttling of Smad4 between the cytoplasm and nucleus. Mol Cell Biol 20:9041–9054, 2000.
107. Xu L, Kang Y, Col S, Massague J. Smad2 nucleocytoplasmic shuttling by nucleoporins CAN/Nup214 and Nup153 feeds TGFbeta signaling complexes in the cytoplasm and nucleus. Mol Cell 10:271–282, 2002.
108. Inman GJ, Nicolas FJ, Hill CS. Nucleocytoplasmic shuttling of Smads 2, 3, and 4 permits sensing of TGF-beta receptor activity. Mol Cell 10: 283–294, 2002.
109. Watanabe M, Masuyama N, Fukuda M, Nishida E. Regulation of intracellular dynamics of Smad4 by its leucine-rich nuclear export signal. EMBO Rep 1:176–182, 2000.
110. Xiao Z, Latek R, Lodish HF. An extended bipartite nuclear localization signal in Smad4 is required for its nuclear import and transcriptional activity. Oncogene 22:1057–1069, 2003.
111. Xiao Z, Liu X, Henis YI, Lodish HF. A distinct nuclear localization signal in the N terminus of Smad 3 determines its ligand-induced nuclear translocation. Proc Natl Acad Sci U S A 97:7853–7858, 2000.
112. Kurisaki A, Kose S, Yoneda Y, Heldin CH, Moustakas A. Transforming growth factor-beta induces nuclear import of Smad3 in an importin-beta1 and Ran-dependent manner. Mol Biol Cell 12: 1079–1091, 2001.
113. Xu L, Chen YG, Massague J. The nuclear import function of Smad2 is masked by SARA and unmasked by TGFbeta-dependent phosphorylation. Nat Cell Biol 2:559–562, 2000.
114. Xu L, Alarcon C, Col S, Massague J. Distinct domain utilization by Smad3 and Smad4 for nucleoporin interaction and nuclear import. J Biol Chem 278:42569–42577, 2003.
115. Hoodless PA, Tsukazaki T, Nishimatsu S, Attisano L, Wrana JL, Thomsen GH. Dominant-negative Smad2 mutants inhibit activin/Vg1 signaling and disrupt axis formation in Xenopus. Dev Biol 207:364–379, 1999.
116. Moustakas A, Heldin CH. Non-Smad TGF-beta signals. J Cell Sci 118:3573–3584, 2005.
117. Yue J, Mulder KM. Activation of the mitogen-activated protein kinase pathway by transforming growth factor-beta. Methods Mol Biol 142: 125–131, 2000.
118. Zhang L, Wang W, Hayashi Y, Jester JV, Birk DE, Gao M, Liu CY, Kao WW, Karin M, Xia Y. A role for MEK kinase 1 in TGF-beta/activin-induced epithelium movement and embryonic eyelid closure. Embo J 22:4443–4454, 2003.
119. Huang HM, Chang TW, Liu JC. Basic fibroblast growth factor antagonizes activin A-mediated growth inhibition and hemoglobin synthesis in K562 cells by activating ERK1/2 and deactivating p38 MAP kinase. Biochem Biophys Res Commun 320:1247–1252, 2004.
120. Yamakawa N, Tsuchida K, Sugino H. The rasGAP-binding protein, Dok-1, mediates activin signaling via serine/threonine kinase receptors. Embo J 21:1684–1694, 2002.
121. Songyang Z, Yamanashi Y, Liu D, Baltimore D. Domain-dependent function of the rasGAP-binding protein p62Dok in cell signaling. J Biol Chem 276:2459–2465, 2001.
122. Ogihara T, Watada H, Kanno R, Ikeda F, Nomiyama T, Tanaka Y, Nakao A, German MS, Kojima I, Kawamori R. p38 MAPK is involved in activin A- and hepatocyte growth factor-mediated expression of pro-endocrine gene neurogenin 3 in AR42J-B13 cells. J Biol Chem 278:21693–21700, 2003.
123. Kanamaru C, Yasuda H, Fujita T. Involvement of Smad proteins in TGF-beta and activin A-induced apoptosis and growth inhibition of liver cells. Hepatol Res 23:211–219, 2002.
124. Ho J, de Guise C, Kim C, Lemay S, Wang XF, Lebrun JJ. Activin induces hepatocyte cell growth arrest through induction of the cyclin-dependent kinase inhibitor p15INK4B and Sp1. Cell Signal 16:693–701, 2004.
125. Ball EM, Risbridger GP. Activins as regulators of branching morphogenesis. Dev Biol 238:1–12, 2001.
126. Caniggia I, Lye SJ, Cross JC. Activin is a local regulator of human cytotrophoblast cell differentiation. Endocrinology 138:3976–3986, 1997.
127. Munz B, Smola H, Engelhardt F, Bleuel K, Brauchle M, Lein I, Evans LW, Huylebroeck D, Balling R, Werner S. Overexpression of activin A in the skin of transgenic mice reveals new activities of activin in epidermal morphogenesis, dermal fibrosis and wound repair. Embo J 18:5205–5215, 1999.
128. Vallier L, Alexander M, Pedersen RA. Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. J Cell Sci 118:4495–4509, 2005.
129. James D, Levine AJ, Besser D, Hemmati-Brivanlou A. TGFbeta/activin/nodal signaling is necessary for the maintenance of pluripotency in human embryonic stem cells. Development 132:1273–1282, 2005.
130. Beattie GM, Lopez AD, Bucay N, Hinton A, Firpo MT, King CC, Hayek A. Activin A maintains pluripotency of human embryonic stem cells in the absence of feeder layers. Stem Cells 23:489–495, 2005.
131. Shi Y, Hou L, Tang F, Jiang W, Wang P, Ding M, Deng H. Inducing embryonic stem cells to differentiate into pancreatic beta cells by a novel three-step approach with activin A and all-trans retinoic acid. Stem Cells 23:656–662, 2005.
132. Kubo A, Shinozaki K, Shannon JM, Kouskoff V, Kennedy M, Woo S, Fehling HJ, Keller G. Development of definitive endoderm from embryonic stem cells in culture. Development 131:1651–1662, 2004.
133. D'Amour KA, Agulnick AD, Eliazer S, Kelly OG, Kroon E, Baetge EE. Efficient differentiation of human embryonic stem cells to definitive endoderm. Nat Biotechnol 23:1534–1541, 2005.
134. Ikeda H, Osakada F, Watanabe K, Mizuseki K, Haraguchi T, Miyoshi H, Kamiya D, Honda Y, Sasai N, Yoshimura N, Takahashi M, Sasai Y. Generation of Rx+/Pax6+ neural retinal precursors from embryonic stem cells. Proc Natl Acad Sci U S A 102:11331–11336, 2005.
135. Utsugisawa T, Moody JL, Aspling M, Nilsson E, Carlsson L, Karlsson S. A road map towards defining the role of Smad signaling in hematopoietic stem cells. Stem Cells, First published online December 15, 2005, doi:10.1634/stemcells.2005-0263.
136. Valderrama-Carvajal H, Cocolakis E, Lacerte A, Lee EH, Krystal G, Ali S, Lebrun JJ. Activin/TGF-beta induce apoptosis through Smad-

dependent expression of the lipid phosphatase SHIP. Nat Cell Biol 4: 963–969, 2002.

137. Beuschlein F, Looyenga BD, Bleasdale SE, Mutch C, Bavers DL, Parlow AF, Nilson JH, Hammer GD. Activin induces x-zone apoptosis that inhibits luteinizing hormone-dependent adrenocortical tumor formation in inhibin-deficient mice. Mol Cell Biol 23:3951–3964, 2003.
138. Vanttinen T, Liu J, Kuulasmaa T, Kivinen P, Voutilainen R. Expression of activin/inhibin signaling components in the human adrenal gland and the effects of activins and inhibins on adrenocortical steroidogenesis and apoptosis. J Endocrinol 178:479–489, 2003.
139. Vejda S, Erlach N, Peter B, Drucker C, Rossmanith W, Pohl J, Schulte-Hermann R, Grusch M. Expression of activins C and E induces apoptosis in human and rat hepatoma cells. Carcinogenesis 24:1801–1809, 2003.
140. Yokomuro S, Tsuji H, Lunz JG 3rd, Sakamoto T, Ezure T, Murase N, Demetris AJ. Growth control of human biliary epithelial cells by interleukin 6, hepatocyte growth factor, transforming growth factor beta1, and activin A: comparison of a cholangiocarcinoma cell line with primary cultures of non-neoplastic biliary epithelial cells. Hepatology 32:26–35, 2000.
141. McPherson SJ, Mellor SL, Wang H, Evans LW, Groome NP, Risbridger GP. Expression of activin A and follistatin core proteins by human prostate tumor cell lines. Endocrinology 140:5303–5309, 1999.
142. Danila DC, Inder WJ, Zhang X, Alexander JM, Swearingen B, Hedley-Whyte ET, Klibanski A. Activin effects on neoplastic proliferation of human pituitary tumors. J Clin Endocrinol Metab 85:1009–1015, 2000.
143. Reis FM, Luisi S, Carneiro MM, Cobellis L, Federico M, Camargos AF, Petraglia F. Activin, inhibin and the human breast. Mol Cell Endocrinol 225:77–82, 2004.
144. Burdette JE, Jeruss JS, Kurley SJ, Lee EJ, Woodruff TK. Activin A mediates growth inhibition and cell cycle arrest through Smads in human breast cancer cells. Cancer Res 65:7968–7975, 2005.
145. Panopoulou E, Murphy C, Rasmussen H, Bagli E, Rofstad EK, Fotsis T. Activin A suppresses neuroblastoma xenograft tumor growth via antimitotic and antiangiogenic mechanisms. Cancer Res 65:1877–1886, 2005.
146. Tanaka T, Toujima S, Umesaki N. Activin A inhibits growth-inhibitory signals by TGF-beta1 in differentiated human endometrial adenocarcinoma cells. Oncol Rep 11:875–879, 2004.
147. Yoshinaga K, Inoue H, Utsunomiya T, Sonoda H, Masuda T, Mimori K, Tanaka Y, Mori M. N-cadherin is regulated by activin A and associated with tumor aggressiveness in esophageal carcinoma. Clin Cancer Res 10:5702–5707, 2004.
148. Landis MD, Seachrist DD, Montanez-Wiscovich ME, Danielpour D, Keri RA. Gene expression profiling of cancer progression reveals intrinsic regulation of transforming growth factor-beta signaling in ErbB2/Neu-induced tumors from transgenic mice. Oncogene 24: 5173–5190, 2005.
149. Steller MD, Shaw TJ, Vanderhyden BC, Ethier JF. Inhibin resistance is associated with aggressive tumorigenicity of ovarian cancer cells. Mol Cancer Res 3:50–61, 2005.