Activins and follistatins: Emerging roles in liver physiology and cancer

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Abstract
Activins are secreted proteins belonging to the TGF-β family of signaling molecules. Activin signals are crucial for differentiation and regulation of cell proliferation and apoptosis in multiple tissues. Signal transduction by activins relies mainly on the Smad pathway, although the importance of crosstalk with additional pathways is increasingly being recognized. Activin signals are kept in balance by antagonists at multiple levels of the signaling cascade. Among these, follistatin and FLRG, two members of the emerging family of follistatin-like proteins, can bind secreted activins with high affinity, thereby blocking their access to cell surface-anchored activin receptors. In the liver, activin A is a major negative regulator of hepatocyte proliferation and can induce apoptosis. The functions of other activins expressed by hepatocytes have yet to be more clearly defined. Deregulated expression of activins and follistatin has been implicated in hepatic diseases including inflammation, fibrosis, liver failure and primary cancer. In particular, increased follistatin levels have been found in the circulation and in the tumor tissue of patients suffering from hepatocellular carcinoma as well as in animal models of liver cancer. It has been argued that up-regulation of follistatin protects neoplastic hepatocytes from activin-mediated growth inhibition and apoptosis. The use of follistatin as biomarker for liver tumor development is impeded, however, due to the presence of elevated follistatin levels already during preceding stages of liver disease. The current article summarizes our evolving understanding of the multi-faceted activities of activins and follistatins in liver physiology and cancer.

INTRODUCTION

The activin family
Activins are cytokines belonging to the TGF-β family of growth and differentiation factors[1] and were named according to their first identification as activators of follicle-stimulating hormone (FSH) release from pituitary cells[2]. Like TGF-β, activins are formed as the covalent dimerization of two subunits[3]. So far, five different subunits participating in the formation of activins have been identified. The subunits activin beta A, beta B, beta
C and beta E were found in humans as well as other mammalian species, while activin beta D has only been identified in Xenopus laevis. The four mammalian beta subunits are each encoded by a single gene, called INHBA, INHB, INHBC and INHBE respectively. INHBC and INHBE are closely linked in several species and are thought to have arisen from tandem duplication of an ancestral gene.

The different activin subunits can form homo- as well as heterodimers. A homodimer of two beta A subunits is called activin A, while a heterodimer of a beta A and a beta B subunit is called activin AB. The nomenclature for dimers of the other subunits follows the same scheme. While activins AB and AC have been described under physiological conditions in vitro, we and others have demonstrated the formation of activins AE, BC and CE after ectopic expression of the respective cDNAs in various cell lines. Activin subunits are synthesized as pro-proteins of 350 to 426 amino acids. The proteins are glycosylated in the pro-domain region, but addition of the carbohydrate group seems to be dispensable for secretion. This is in contrast to the related inhibin alpha subunit, a member of the TGF-family, activins AE, BC and CE after ectopic expression of the respective cDNAs in various cell lines. Activin subunits are synthesized as pro-proteins of 350 to 426 amino acids. The proteins are glycosylated in the pro-domain region, but addition of the carbohydrate group seems to be dispensable for secretion. This is in contrast to the related inhibin alpha subunit, a member of the TGF-beta family and dimerization partner of activin subunits (see below). Dimers are created by intermolecular disulfide bond formation between the sixth of nine conserved cysteines in the mature proteins. The other cysteines are involved in the formation of intramolecular disulfide bonds, creating the so-called cysteine knot, typical for members of the TGF-beta family and required for their biological activity.

Following dimerization, the protein is cleaved by pro-protein convertases of the subtilisin/kexin family in the ER and Golgi, producing a mature peptide chain of 115 amino acids. While the biologically active protein is secreted as a dimer of the mature peptides only, it has been suggested that the pro-region is required for correct folding, dimer formation and secretion. Unprocessed, dimeric activin A was found to be biologically inactive. Monomers have been reported to retain some affinity for the receptors of dimeric activin A but do not cause activation. In addition to dimerization with another beta subunit, activin beta A and activin beta B can form heterodimers with the inhibin alpha subunit, giving rise to activins A and B, both inhibiting FSH release. It remains uncertain if inhibin C exists, as there was no report of an ancestral gene.

**Activin signal transduction**

Like other members of the TGF-beta family, activins are believed to signal via single-pass transmembrane receptors with an intracellular Ser-Thr kinase domain. This has been proven for activins A, B and AB. Activin A first binds to dimers of the type II receptors ActR-II (aka ACVR2) or ActR-II B (aka ACVR2B), leading to the (preferential) recruitment and phosphorylation of dimers of the type I receptor ALK4 (aka ActR-IB/ACVR1B). While binding to the same type II receptors, activins B and AB preferentially recruit ALK7 (ACVR1C) as type I receptor. Upon ligand binding, receptors are typically internalized. It has been questioned however, if this internalization is generally necessary for signal transduction. As a consequence of activation, receptor-regulated Smads (R-Smads) are recruited to the receptor complex and phosphorylated by the type I receptor. This process is supported by accessory proteins like SARA and the motor protein kinesin-1. Depending on the identity of the receptor, either Smad 2 and Smad 3 (ALK4, ALK5, ALK7) or Smad 1, Smad 5 and Smad 8 (ALK1, ALK2, ALK3, ALK6) are recruited and activated. For TGF-beta it has been shown that the ligand can recruit different type I receptors, activating different subsets of Smads depending on the cell type. So far, activins have only been shown to signal through Smad 2 and Smad 3. R-Smads then form complexes with the common mediator Smad 4 and translocate to the nucleus where, together with cofactors, they are directly involved in regulation of gene expression.

In addition, recent evidence suggests Smad independent signaling of activin A via MAP kinases ERK 1/2 and p38 as well as the phosphatidylinositol 3'-kinase (PI3K)/Akt pathway. Rho and JNK were also found to be stimulated by activin A.

**ACTIVINS IN HEPATIC FUNCTION AND DYSFUNCTION**

**Beta A and beta B**

Activin A represents the most extensively investigated activin. Multiple biological functions of activin A in a variety of cells and tissues have been described, involving in mesoderm induction, stem cell biology, reproductive biology, erythroid differentiation, systemic inflammation, cell death induction, wound healing, and fibrosis. Knock-out mice for activin beta A show severe defects in craniofacial development and die shortly after birth. Activin A potently inhibits mitogen-induced DNA synthesis in the liver and induces hepatocyte apoptosis in vivo and in vitro. Activin beta A antisense oligonucleotides stimulated cell proliferation in the human hepatoma cell line HLF suggesting a growth inhibitory function of endogenous activin A. In regenerating liver, activin beta A gene expression was reduced at time points when hepatocyte replication took place and was increased at time points when liver regeneration terminated. Other studies, however, have described increased expression of beta A at earlier time points after partial hepatectomy.

Beside its effects on DNA synthesis and cell growth, activin A also regulates restoration of liver architecture after partial hepatectomy by stimulating collagen production in hepatic stellate cells (HSC) and tubulogenesis of sinusoidal endothelial cells. Stimulation of HSC may also...
Contribute to liver fibrosis and several investigations have found elevated levels of activin beta A in fibrotic and cirrhotic rat livers[47-50]. In hepatocytes, activin A was also demonstrated to stimulate the expression of connective tissue growth factor (CCTF/CCN2), an important regulator of liver fibrosis[51]. Elevated levels of circulating activin A were found in patients with acute liver failure, chronic viral hepatitis, alcohol induced liver cirrhosis and hepatocellular carcinoma (HCC)[52-57]. Elevated serum activin A was also reported in a study with patients suffering from non-alcoholic fatty liver disease (NAFLD), with particularly high levels in the subgroup with non-alcoholic steatohepatitis (NASH)[58]. These patients also had an increased activin beta A/follistatin mRNA ratio in liver tissue. In the same study activin A was shown in Huh7 hepatoma cells to promote collagen III and TGF-β1 expression, matrix metalloproteinase (MMP) activity, induce mitochondrial beta-oxidation and down-regulate fatty acid synthase (FAS) activity. Together these findings suggest an involvement of activin A not only in fibrosis but also in lipid accumulation. A study from our group in contrast, has found reduced expression of activin beta A transcripts in tumor tissue from chemically-induced rat liver tumors[59]. In addition to a pro-apoptotic and a pro-fibrotic effect, activin A has also been linked to hepatic neoangiogenesis via stimulation of VEGF expression in human hepatoma cells[60]. With respect to hepatic differentiation, it has been shown that a gradient of activin/TGF-β signaling controls differentiation of hepatoblasts into hepatocytes and biliary cells in the mouse, with high signaling activity required for development into biliary cells[61]. The contributing activin/TGF-β ligands, however, have not been fully identified. Several studies have used activin A as part of protocols to differentiate human embryonic stem cells (hESC) into hepatocyte-like cells[62-68].

Like activin beta A, the beta B subunit is expressed in multiple tissues and organs[69-71]. Knock-out mice for beta B are viable but show defects in eyelid development and female reproduction[72]. When the coding region of the mature peptide of the beta A subunit gene was replaced with the corresponding region of the beta B subunit, the developmental defects of the beta A knock-out mice were only partially rescued indicating differences in receptor activation or downstream signals[73]. In the liver, the function of the beta B subunit is not well characterized. One reason for this might be the low expression level in normal rat liver, where we observed the beta B subunit to be the only activin subunit undetectable by RNase protection assays[74]. By immunohistochemistry, however, weak staining of beta B was detected in hepatocytes of normal rat liver and in connective tissue septa in fibrotic livers[75]. Activin beta B mRNA was induced in stellate cells of CCl4 treated rat livers[76] and exposure to the peroxisome proliferator di-n-butyl phthalate led to a transient surge of beta B mRNA expression 6 h after treatment[77]. With respect to biological activities, recombinant activin B, in contrast to activins A and AB, did not inhibit EGF induced DNA synthesis in primary rat hepatocytes[78]. In contrast to the rat, beta A and beta B transcripts are expressed to similar levels in human liver (Rodgarkia-Dara, unpublished observation). Ectopic expression of ALK7, the preferred type two receptor for activins B and AB induced apoptosis in hepatoma cell lines in a Smad and MAPK-dependent manner[79]. Both activin B and ALK7 have been linked to obesity and diabetes, two well-known risk factors for HCC, via participation in regulatory circuits in adipose tissue and the pancreas[80-82].

**Beta C and beta E**

In contrast to beta A and beta B, whose expression level is the highest in reproductive organs, the liver is the organ where the beta C and the beta E subunit reach by far their highest expression levels. The activin beta C subunit was cloned from liver cDNA and demonstrated to be predominantly expressed in hepatocytes by Northern blot analysis and RNase protection assays[83-86]. By immunohistochemistry, significant activin beta C expression has been detected in cells from additional organs, including the prostate, ovary, testes, and pituitary gland[87-89]. After partial hepatectomy, a transient down-regulation of activin beta C expression was observed by several studies[84-86,89]. We have found reduced activin beta C expression in HepG2 and Hep3B hepatoma cells versus normal liver tissue[90] and a drop of beta C expression was also described in rat hepatocytes during primary culture with and without EGF treatment[79]. In contrast, increased activin beta C expression was reported in rat liver during the development of CCl4-induced cirrhosis[80,90] and in response to treatment with the peroxisome proliferator bi-n-butyl phthalate[91]. The functions of the activin beta C subunit are controversial. Activin beta C knock-out mice developed normally and liver regeneration after partial hepatectomy proceeded similar in knock-out animals and wild-type littermates[82]. Studies from our group showed that ectopic expression of activin beta C induced apoptosis in human (HepG2, Hep3B) and rat (H4IIEC3) hepatoma cells and delayed liver regeneration in mice[80,83]. In contrast, in AML12 cells, an immortalized mouse hepatocyte cell line, and in primary rat hepatocytes activin beta C increased DNA synthesis[92]. Adenovirus-mediated expression of activin beta C accelerated liver regeneration after partial hepatectomy in rats[85] and association of activin beta C immunoreactivity with mitotic hepatocytes was observed in regenerating liver after partial hepatectomy[93]. Activin C does not activate activin A-responsive promoters and it was suggested that the beta C subunit down-regulates the levels of bioactive activin A via the formation of signaling-incompetent activin AC heterodimers in PC3 human prostate cancer cells[94]. In a recent study from the same group, it was shown that homodimeric activin C inhibited activin A-induced Smad2 phosphorylation and growth inhibition, and that activin beta C transgenic mice develop prostate, testis and liver pathologies.
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Figure 1 Number and arrangement of follistatin/Kazal-like domains in follistatin-like proteins. SP: Signal peptide; FS: Follistatin/Kazal-like domain; EF: EF-hand domain; VWFC: von Willebrand factor type C repeat; IGFBP: IGF-binding protein N-terminal domain; Ig: Immunoglobulin-like domain. Text to the right shows the number of amino acids (aa), most common name(s) and Uniprot accession number.

344 aa  Follistatin  P19883
308 aa  Fstl1/FRP  Q12841
282 aa  IGFBP7(Fstl2)  Q16270
263 aa  Fstl3/FLRG  O95633
842 aa  Fstl4  Q6MZW2
847 aa  Fstl5  Q8N475

epithelial cells resulted in beta E downregulation \[96\]. Finally, in gene chip analysis, mRNA levels from INHBE were found to be altered in HepG2 in response to hypoxia \[87\]. One possible mode of action for activin beta E was described by Chow et al \[96\], who demonstrated that the expression of Inhibitor of DNA binding 2 (Id2) protein is down-regulated in response to overexpression of activin beta E. Id2 is a known target of TGF-β and a potential oncogene \[88\]. Large scale analysis identified mutations in the INHBE gene in breast cancer \[89\]. An evaluation of single nucleotide polymorphisms (SNPs) in genes coding for activins in testicular cancer showed a correlation for the risk of disease and mutations in INHBA but not in INHBB, INHBC or INHBE \[90\].

FOLLISTATINS AND THEIR ROLE IN ACTIVIN ANTAGONISM AND LIVER DISEASE

Follistatin was discovered as antagonist of activin activity with respect to FSH release from pituitary cells \[101\]. Sequence analysis of follistatin revealed no homology to the TGF-β family, but the presence of three domains with a similar architecture, namely 10 cysteines spaced in a conserved fashion resulting in a characteristic pattern of intramolecular disulphide bond formation \[102\]. Accordingly, this domain was termed follistatin domain. Follistatin domains have been identified in a number of additional extracellular proteins and some of these have been filed as follistatin-like proteins \[103-105\] (Figure 1). The connection of follistatin and follistatin-like proteins with activin signaling and their involvement in hepatic functions is discussed below. Additional regulation of activin signal transduction takes place at the receptor level by co-receptors, such as cripco, nodal, betaglycan, or BAMBI and intracellularly, for instance by the inhibitory Smad 6 and 7, and has been reviewed elsewhere \[106,107\].

suggestive of an activin A antagonistic effect \[87\]. In line with these observations, elevated beta C immuno-reactivity was found in human prostate, testis and liver cancers \[87\].

Like beta C, also the beta E subunit is highly expressed in the liver, but has been detected at lower levels in several other tissues as well \[7,11,88,89\]. In the liver of the developing mouse, activin beta E expression could not be detected until the very late stages of embryonic development and peaked at birth \[82\]. The biological functions and molecular interaction partners of activin beta E remain largely unknown. Like beta C, beta E knock-out (as well as beta C, beta E double knock-out) mice developed normally and showed no impairment of liver function or regeneration \[88\]. In vitro, overexpression of activin beta E in the human hepatoma cell lines HepG2 and Hep3B, as well as in the murine hepatocyte cell line AML12, caused decreased proliferation and induced apoptosis \[12,88\]. In vivo, transient overexpression of activin beta E inhibited regenerative DNA synthesis in mouse liver \[83\], while mice constitutively overexpressing beta E remain largely unknown. Like beta C, beta E functions and molecular interaction partners of activin \[83\] may be involved in hepatic development and peaked at birth \[88\]. In the liver \[82\] and a surge of beta E expression in response of activin beta E mRNA depending on food consumption in basal levels after 48 h \[82\]. We observed a diurnal variation \[82\]. In vitro, overexpression of activin beta E inhibited regenerative DNA synthesis in mouse liver \[83\], while mice constitutively overexpressing beta E remain largely unknown. Like beta C, beta E functions and molecular interaction partners of activin \[83\] may be involved in hepatic development and peaked at birth \[88\]. In the liver \[82\] and a surge of beta E expression in response of activin beta E mRNA depending on food consumption in basal levels after 48 h \[82\]. We observed a diurnal variation \[82\]. 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Follistatin-like proteins

Follistatin-like 1 (fst1l, also called follistatin-related protein, FRP or Tsc-36) contains only a single follistatin domain and no activin-binding activity has been reported. In fact, the interaction partners of fst1l on a molecular level have not been identified and its function is far from clear. Fst1l itself was identified as a TGF-β inducible gene and has been implicated in inflammation and cardioprotection. It has been suggested to act as a potential tumor suppressor in epithelial cancers, but is over-expressed in astrocytic brain tumors. Considering hepatoma cells, we recently demonstrated that the expression of fst1l is low in HepG2 cells, which show an epithelial morphology/proteome pattern and high in Hep3B cells with fibroblastoid characteristics. These observations suggest fst1l as potential indicator of epithelial-mesenchymal transition (EMT). The term follistatin-like 2 (fst2l) is only rarely used. It refers to a protein described to have IGF-1-like growth factor as well as activin-binding activity and sequence homology with follistatin. This protein was also termed mac25 and angiomodulin but is better known as IGFBP7 (IGF binding protein 7) or IGFBP-rP1 (IGF binding protein-related protein 1). It has been suggested to act as tumor suppressor, because its expression is reduced in neoplastic tissues of different cancer types including liver tumors from SV40T/t antigen transgenic mice. However, the biological relevance of IGFBP7 binding to activin is still unclear. In the course of evolution fish went through whole genome duplication and the term fst2l has also been used (synonymously with fst1lb) to denote the second zebrafish orthologue of mammalian fst1.

Among the follistatin like proteins follistatin-like 3 (fst3l), encoded by follistatin-related gene (FLRG), has the highest overall similarity with follistatin and shares its ability to bind TGF-β family proteins, but contains only two instead of three follistatin domains. The FLRG gene was originally identified as a target of chromosomal rearrangement in leukemia. The highest tissue expression of FLRG was found in placenta, whereas highest follistatin expression was found in ovary, testis, and pituitary. In HepG2 hepatoma cells, expression of both FLRG and follistatin was induced in response to activin A treatment suggesting that they participate in a feedback loop to restrict activin A signals in a feedback loop to restrict activin A signals. FLRG knock-out mice developed increased pancreatic islet number and size, beta cell hyperplasia, decreased visceral fat mass, and hepatic steatosis. This is in line with a physiological role of fst3 in antagonizing activin and myostatin activity in the pancreas, adipose tissue and liver. Elevated expression of FLRG has been linked to breast cancer and we have found increased FLRG transcript levels in chemically induced rat liver tumors but not in human liver tumor specimens. Recently follistatin-like 4 and 5 were identified as two additional follistatin-related proteins, but their expression pattern and function have yet to be worked out.
CONCLUSION

Despite the apparent gaps in our knowledge, it is becoming increasingly clear that tightly regulated activin signals are of fundamental importance for the maintenance of liver architecture and cellular homeostasis. While still much has to be learned, especially about the less explored members of the activin and follistatin families, the pace of progress has appreciably sped up in recent years. Deregulated expression of activin A and/or follistatin has been consistently observed in liver cancer in human patients and in a growing number of animal models, and was shown to causally contribute to the inflammatory and fibrotic conditions that promote carcogenesis (Figure 2). The picture that emerges is that inflammation-associated elevated activin A levels contribute to fibrotic tissue remodelling and cell death of normal hepatocytes, whereas preneoplastic and neoplastic hepatocytes become resistant to activin A-induced growth control, at least in part through overexpression of follistatin. Conditional and liver cell type-specific knock-out of activin beta A and follistatin in mouse hepatocarcinogenesis models could shed further light on the contribution of the activin-follistatin axis to liver cancer development. For the two activin subunits with predominant expression in hepatocytes, namely beta C and beta E, as well as for fsl1, 4 and 5 future efforts should be directed at elucidating their molecular interaction with cell surface receptors or secreted proteins as a prerequisite to better understand their biological activities. Although the complexity of the system may sometimes seem daunting, the hope is well founded that in the not-too-far future, the increasing knowledge on activins and follistatins will translate into improved diagnostic or therapeutic opportunities for patients suffering from chronic liver disease and HCC.

REFERENCES

1 Rodgarkia-Dara C, Vejda S, Erlach N, Losert A, Bursch W, Berger W, Schulte-Hermann R, Grusch M. The activin axis in liver biology and disease. Mutat Res 2006; 613: 123-137
2 Ling N, Ying SY, Ueno N, Shimasaki S, Esch F, Hotta M, Guillemin R. A homodimer of the beta-subunits of inhibin A stimulates the secretion of pituitary follicle stimulating hormone. Biochem Biophys Res Commun 1986; 138: 1129-1137
3 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 1986; 321: 779-782
4 Schmierer B, Hill CS. TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility. Nat Rev Mol Cell Biol 2007; 8: 970-982
5 Oda S, Nishimatsu S, Murakami K, Ueno N. Molecular cloning and functional analysis of a new activin beta subunit: a dorsal mesoderm-inducing activity in Xenopus. Biochem Biophys Res Commun 1995; 210: 581-588
6 Grusch M, Rodgarkia-Dara C, Bursch W, Schulte-Hermann R. Activins and the liver. In: Jakowlew S, editor. TGF-beta Superfamily Members in Normal and Tumor Biology. Totowa: Humana Press, 2008
7 Fang J, Wang SQ, Smiley E, Bonadio J. Genes coding for mouse activin beta C and beta E are closely linked and exhibit a liver-specific expression pattern in adult tissues. Biochem Biophys Res Commun 1997; 231: 655-661
8 Evans LW, Muttukrishna S, Knight PG, Groome NP. Development, validation and application of a two-site enzyme-linked immunosorbet assay for activin-AB. J Endocrinol 1997; 153: 221-230
9 Mellor SL, Ball EM, O’Connor AE, Ethier JF, Cranfield M, Schmitt JF, Phillips DJ, Groome NP, Risbridger GP. Activin beta subunit heterodimers provide a new mechanism of regulating activin levels in the prostate. Endocrinology 2003; 144: 4410-4419
10 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 2000; 85: 4851-4858
11 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 ornament of novel activin dimers. J Mol Endocrinol 2002; 28: 137-148
12 Wada W, Medina JJ, Kuwano H, Kojima I. Comparison of the function of the beta(C) and beta(E) subunits of activin in AML12 hepatocytes. Endocr J 2005; 52: 169-175
13 Antenos M, Steimer M, Boime I, Woodruff TK. N-linked oligosaccharides direct the differential assembly and secretion of inhibin alpha- and beta-subunit dimers. Mol Endocrinol 2007; 21: 1670-1684
14 Mason AJ. Functional analysis of the cysteine residues of activin A. Mol Endocrinol 1994; 8: 325-332
15 Gray AM, Mason AJ. Requirement for activin A and transforming growth factor--beta 1 pro-regions in homodimer assembly. Science 1990; 247: 1328-1330
16 Mason AJ, Farnworth PG, Sullivan J. Characterization and determination of the biological activities of noncleavable high molecular weight forms of inhibin A and activin A. Mol Endocrinol 1996; 10: 1055-1065
17 Husken-Hindi P, Tsuchida K, Park M, Corrigan AZ, Vaughan JM, Vale WW, Fischer WH. Monomeric activin A retains high receptor binding affinity but exhibits low biological activity. J Biol Chem 1994; 269: 19380-19384
18 Mason AJ, Hayflick JS, Ling N, Esch F, Ueno N, Ying SY,
reveals new activities of activin in epidemorphogenesis, dermal fibrosis and wound repair. EMBO J 1999; 18: 5205-5215

36 Werner S, Alzheimer C. Roles of activin in tissue repair, fibrosis, and inflammatory disease. Cytokine Growth Factor Rev 2006; 17: 157-171

37 Matzuk MM, Kumar TR, Vassalli A, Bickenbach JR, Roop DR, Jaenisch R, Bradley A. Functional analysis of activins during mammalian development. Nature 1995; 374: 354-356

38 Huly JR, Chang L, Schwall RH, Widmer HR, Terrell TG, Gillett NA. Induction of apoptosis in the murine liver with recombinant human activin A. Hepatology 1994; 20: 854-862

39 Schwall RH, Robbins K, Jardieu P, Chang L, Lai C, Terrell TG. Activin induces cell death in hepatocytes in vivo and in vitro. Hepatology 1993; 18: 347-356

40 Yasuda H, Mine T, Shibata H, Eto Y, Hasegawa Y, Takeuchi T, Asano S, Kojima I. Activin A: an autocrine inhibitor of initiation of DNA synthesis in rat hepatocytes. J Clin Invest 1993; 92: 1491-1496

41 Takabe K, Lebrun JN, Nagashima Y, Ichikawa Y, Mitsuhashi M, Momiyama N, Ishikawa T, Shimada H, Vale WW. Interruption of activin A autocrine regulation by antisenese oligodeoxynucleotides accelerates liver tumor cell proliferation. Endocrinology 1999; 140: 3125-3132

42 Gold EJ, Zhang X, Wheatley AM, Mellor SL, Cranfield M, Risbridger GP, Groome NP, Fleming JS. betaA- and betaC-activin, follistatin, activin receptor mRNA and betaC-activin peptide expression during rat liver regeneration. J Mol Endocrinol 2005; 34: 505-515

43 Date M, Matsuzaki K, Matushita M, Takashi Y, Sakitani K, Inoue K. Differential regulation of activin A for hepatocyte growth and fibronectin synthesis in rat liver injury. J Hepatol 2000; 32: 251-260

44 Zhang YQ, Shibata H, Schreve H, Kojima I. Reciprocal expression of mRNA for inhibin betaA and betaB subunits in hepatocytes. Endocr J 1997; 44: 759-764

45 Wada W, Kuvano H, Hasegawa Y, Kojima I. The dependence of transforming growth factor-beta-induced collagen production on autocrine factor activin A in hepatocyte stellate cells. Endocrinology 2004; 145: 2755-2769

46 Endo D, Kogure K, Hasegawa Y, Maku-uchi M, Kojima I. Activin A augments vascular endothelial growth factor activity in promoting branching tubulogenesis in hepatic sinusoidal endothelial cells. J Hepatol 2004; 40: 399-404

47 De Bleser PJ, Niki T, Xu G, Rogiers V, Geerts A. Localization and cellular sources of activins in normal and fibrotic rat liver. Hepatology 1997; 26: 905-912

48 Gold EJ, Francis RJJ, Zimmermann A, Mellor SL, Cranfield M, Risbridger GP, Groome NP, Wheatley AM, Fleming JS. Changes in activin and activin receptor subunit expression in rat liver during the development of CCl4-induced cirrhosis. Mol Cell Endocrinol 2003; 201: 143-153

49 Huang X, Li DG, Wang ZR, Wei HS, Cheng JL, Zhan YT, Zhou X, Xu QF, Li X, Lu HM. Expression changes of activin A in the development of hepatic fibrosis. World J Gastroenterol 2001; 7: 37-41

50 Sugiyama M, Ichida T, Sato T, Ishikawa T, Matsuda Y, Asakura H. Expression of activin A is increased in cirrhotic and fibrotic rat livers. Gastroenterology 1998; 114: 550-558

51 Gressner OA, Lahme B, Siluschek M, Rebbein K, Weiskirchen R, Gressner AM. Intracellular signalling of activin A in hepatocytes upregulates connective tissue growth factor (CTGF/CCN2) expression. Liver Int 2008; 28: 1207-1216

52 Patella S, Phillips DJ, de Kretser DM, Evans LW, Groome NP, Sievert W. Characterization of serum activin-A and follistatin and their relation to virological and histological determinants in chronic viral hepatitis. J Hepatol 2001; 34: 576-583
Yuen MF, Norris S, Evans LW, Langley PG, Hughes RD. Transforming growth factor-beta 1, activin and follistatin in patients with hepatocellular carcinoma and patients with alcoholic cirrhosis. *Scand J Gastroenterol* 2002; 37: 553-561.

Pirisi M, Fabris C, Luisi S, Santuz M, Toniotto P, Vitulli D, Federico E, Del Forno M, Mattiuzzo M, Branca B, Petraglia F. Evaluation of circulating activin-A as a serum marker of hepatocellular carcinoma. *Cancer Detect Prev* 2000; 24: 150-155.

Elsamak MY, Amin GM, Khalil GM, Ragab WS, Abaza MM. Possible contribution of serum activin A and IGF-1 in the development of hepatocellular carcinoma in Egyptian patients suffering from combined hepatitis C virus infection and hepatic schistosomiasis. *Clin Biochem* 2006; 39: 623-629.

Hughes RD, Evans LW. Activin A and follistatin in acute liver failure. *Eur J Gastroenterol Hepatol* 2003; 15: 127-131.

Lin SD, Kawakami T, Ushio A, Sato A, Sato S, Iwai M, Endo R, Takikawa Y, Suzuki K. Ratio of circulating follistatin and activin A reflects the severity of acute liver injury and prognosis in patients with acute liver failure. *J Gastroenterol Hepatol* 2006; 21: 374-380.

Yndestad A, Haukeland JW, Dahl TB, Bjoro K, Gladhaug IP, Berge C, Damas JK, Haaland T, Loberg EM, Linnestad P. A complex role of activin A in non-alcoholic fatty liver disease. *Am J Gastroenterol* 2009; 104: 2196-2205.

Grusch M, Drucker C, Peter-Vorosmarty B, Erlach N, Peter B, Drucker C, Rossmanith W, Pohl J, Schulte-Hermann R. Deregulation of the activin/follistatin system in hepatocarcinogenesis. *J Hepatol* 2006; 45: 673-680.

Wagner K, Peters M, Scholz A, Benecent K, Ruderisch HS, Wiedenmann B, Rosewicz S. Activin A stimulates vascular endothelial growth factor gene transcription in human hepatocellular carcinoma cells. *Gastroenterology* 2004; 126: 1828-1843.

Clotman F, Jacquemin P, Plumb-Rudewicz N, Pierreux CE, Van der Smissen P, Dietz HC, Courtoy PJ, Rousseau GG, Lemaigre FP. Control of liver cell fate decision by a gradient of TGF beta signaling modulated by Onecut transcription factors. *Genes Dev* 2005; 19: 1849-1854.

Basma H, Soto-Gutierrez A, Yannam GR, Liu L, Ito R, Yamamoto T, Ellis E, Carson SD, Sato S, Chen Y, Muirhead TJ, Carlsson B, Jenner P, Fox IJ. Differentiation and transplantation of human embryonic stem cell-derived hepatocytes. *Gastroenterology* 2009; 136: 990-999.

Cai J, Zhao Y, Liu Y, Ye F, Song Z, Qin H, Meng S, Chen Y, Zhou R, Song X, Guo Y, Ding M, Deng H. Directed differentiation of human embryonic stem cells into functional hepatic cells. *Hepatology* 2007; 45: 1229-1239.

Hay DC, Fletcher J, Payne C, Terrace JD, Gallagher RC, Snoeys J, Black JR, Wojtacha D, Samuel K, Hannoun Z, Pryde A, Filippi C, Currie IS, Forbes SJ, Ross JA, Newspaper PN, Iredale JP. Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. *Proc Natl Acad Sci USA* 2008; 105: 12301-1236.

Hay DC, Zhao D, Fletcher J, Hewitt ZA, McLean D, Urruticoechea-Uriguen A, Black JR, Elcombe C, Ross JA, Wolf R, Cui W. Efficient differentiation of hepatocytes from human embryonic stem cells exhibiting markers recapitulating liver development in vivo. *Stem Cells* 2008; 26: 894-902.

Tuuri T, Eramaa M, Hilden K, Ritvos O. The tissue distribution of activin beta A- and beta B-subunit and follistatin messenger ribonucleic acids suggests multiple sites of action for the activin-follistatin system during human development. *J Clin Endocrinol Metab* 1994; 78: 1521-1524.

Vassalli A, Matzuk MM, Gardner HA, Lee KE, Jaenisch R. Activin/inhibin beta B subunit gene disruption leads to defects in eyelid development and female reproduction. *Genes Dev* 1994; 8: 414-427.

Brown CW, Houston-Hawkins DE, Woodruff TK, Matzuk MM. Insertion of Inhbb into the Inhba locus rescues the Inhba-null phenotype and reveals new activin functions. *Nat Genet* 2000; 25: 453-457.

Kobayashi T, Niimi S, Fukuoka M, Hayakawa T. Regulation of inhibin beta chains and follistatin mRNA levels during rat hepatocyte growth induced by the peroxisome proliferator di-n-butyl phthalate. *Bio Pharm Bull* 2002; 25: 1214-1216.

Niimi S, Horikawa M, Seki T, Ariga T, Kobayashi T, Hayakawa T. Effect of activins AB and B on DNA synthesis stimulated by epidermal growth factor in primary cultured rat hepatocytes. *Bio Pharm Bull* 2002; 25: 437-440.

Kim BC, van Gelder H, Kim TA, Lee HJ, Baik KG, Chun HH, Lee DA, Choi KS, Kim SJ. Activin receptor-like kinase-7 induces apoptosis through activation of MAPKs in a Smad3-dependent mechanism in hepatoma cells. *J Biol Chem* 2004; 279: 28458-28465.

Bertolino P, Holmberg R, Reissmann E, Andersson O, Berggren PO, Ibanez CF. Activin B receptor ALK7 is a negative regulator of pancreatic beta-cell function. *Proc Natl Acad Sci USA* 2008; 105: 7246-7251.

Carlsson LM, Jacobson P, Walley A, Froguel P, Sjostrom L, Svensson PA, Sjoholm K. ALK7 expression is specific for adipose tissue, reduced in obesity and correlates to factors implicated in metabolic disease. *Biochem Biophys Res Commun* 2009; 382: 309-314.

Sjoholm K, Palming L, Jystig TC, Jennische E, Woodruff TK, Carlsson B, Carlsson LM. The expression of inhibin beta B is high in human adipocytes, reduced by weight loss, and correlates to factors implicated in metabolic disease. *Biochem Biophys Res Commun* 2006; 344: 1308-1314.

Hoetten G, Neidhardt H, Schneider C, Pohl J. Cloning of a new member of the TGF-beta family: a putative new activin beta C chain. *Biochem Biophys Res Commun* 1995; 206: 608-613.

Schmitt J, Hotten G, Jenkins NA, Gilbert DJ, Copeland NG, Pohl J, Schreve H. Structure, chromosomal localization, and expression analysis of the mouse inhibin/activin beta C (Inhbc) gene. *Genomics* 1996; 32: 358-366.

Gold EJ, O’Bryan MK, Mellor SL, Cranfield M, Risbridger GP, Groome NP, Fleming JS. Cell-specific expression of betaA-activin in the rat reproductive tract, adrenal and liver. *Mol Cell Endocrinol* 2004; 222: 61-69.

Esquela AF, Zimmer TA, Koniaris LG, Sitzmann JV, Lee SJ. Transient down-regulation of inhibin-betaC expression following partial hepatectomy. *Biochem Biophys Res Commun* 1997; 235: 553-556.

Takamura K, Tsuchida K, Miyake H, Tashiro S, Sugino H. Activin and activin receptor expression changes in liver regeneration in rat. *J Surg Res* 2005; 126: 3-11.

Veija S, Erlach N, Peter B, Drucker C, Rossmanith W, Pohl J, Schulte-Hermann R, Drucker C. Expression of activins C and E induces apoptosis in human and rat hepatoma cells. *Carcinogenesis* 2003; 24: 1801-1809.

Huang X, Li D, Lu H, Wang Z, Wei H, Wang Y, Zhang J, Xu Q. Expression of activins, follistatin mRNA in the development of hepatic fibrosis. *Zhonghua Ganzangbing Zazhi* 2002; 10: 85-88.

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* 2000; 20: 6127-6137.
**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* 2003; 144: 3497-3504

**Wada W**, Maeshima A, Zhang YQ, Hasegawa Y, Kuwano H, Kojima I. Assessment of the function of the beta-C-subunit of activin in cultured hepatocytes. *Am J Physiol Endocrinol Metab* 2004; 287: E247-E54

**Wada W**, Medina J, Hasegawa Y, Kuwano H, Kojima I. Adenovirus-mediated overexpression of the activin beta(C) subunit accelerates liver regeneration in partially hepatectomized rats. *J Hepatol* 2005; 43: 823-828

**Butler CM**, Gold EJ, Risbridger GP. Should activin beta C be more than a fading snapshot in the activin/TGFbeta family album? *Cytokine Growth Factor Rev* 2005; 16: 377-385

**Gold E**, Jetyl N, O'Bryan MK, Meachem S, Sinivasan D, Behuria S, Sanchez-Partida LG, Woodruff T, Hedwards S, Wang H, McDougall H, Casey V, Niranjan B, Patella S, Risbridger G. Activin C antagonizes activin A in vitro and overexpression leads to pathologies in vivo. *Am J Pathol* 2009; 174: 184-195

**Hashimoto O**, Tsuchida K, Ushiro Y, Hosoi Y, Hoshi N, Sugino H, Hasegawa Y. cDNA cloning and expression of human activin betaE subunit. *Mol Cell Endocrinol* 2002; 194: 117-122

**O'Bryan MK**, Sebire KL, Gerdprasert O, Hedger MP, Heam MT, de Kretser DM. Cloning and regulation of the rat activin beta subunit. *J Mol Endocrinol* 2000; 24: 409-418

**Hashimoto O**, Ushiro Y, Sekiyama K, Yamaguchi O, Yoshioka K, Mutoh K, Hasegawa Y. Impaired growth of pancreatic exocrine cells in transgenic mice expressing human activin betaE subunit. *Biochem Biophys Res Commun* 2006; 341: 416-424

**Atienza F**, Gerets H, Dufrane S, Tilmant K, Corret M, Dhalluin S, Ruty B, Rose G, Canning M. Determination of phospholipidosis potential based on gene expression analysis in HepG2 cells. *Toxicol Sci* 2007; 96: 101-114

**Sawada H**, Takami K, Asahi S. A toxicogenomic approach to drug-induced phospholipidosis: analysis of its induction mechanism and establishment of a novel in vitro screening system. *Toxicol Sci* 2005; 83: 282-292

**Rosendahl A**, Checchin D, Fehniger TE, ten Dijke P, Heldin CH, Siders P. Activation of the TGF-beta/activin-Smad2 pathway during allergic airway inflammation. *Am J Respir Cell Mol Biol* 2001; 25: 60-68

**Nishino Y**, Ooishi K, Uchikawa S, Fujino K, Murakami M, Madarame H, Hashimoto O, Sugiyama K, Funaba M. Gene expression of the TGF-beta family in rat brain infected with Borna disease virus. *Microbes Infect* 2009; 11: 737-743

**Sekiyama K**, Hashimoto O, Ushiro Y, Adachi C, Kikusui T, Tanemura K, Hasegawa Y. Abnormalities in aggression and anxiety in transgenic mice overexpressing activin E. *Biochem Biophys Res Commun* 2009; 385: 319-323

**Chow LS**, Lam CW, Chan SY, Tsao SW, To KF, Tong SF, Hung WK, Dammann R, Huang DP, Lo KW. Identification of RASSFIA mutated genes in nasopharyngeal carcinoma. *Oncogene* 2006; 25: 310-316

**Fisher TS**, Etages SD, Hayes L, Crimin K, Li B. Analysis of ARDI function in hypoxia response using retroviral RNA interference. *J Biol Chem* 2005; 280: 17749-17757

**Lasorella A**, Uo T, Iavarone A. Id proteins at the crossroad of development and cancer. *Oncogene* 2001; 20: 8326-8333

**Sjoblom T**, Jones S, Wood LD, Parsons DW, Lin J, Barber TD, Mandelker D, Leary RJ, Ptak J, Silliman N, Szabo S, Buckhaults P, Farrell C, Meeh P, Markowitz SD, Dawson D, Willson JK, Gazdar AF, Hartigan J, Wu L, Liu C, Parmigiani G, Park BH, Biclan KF, Papadopoulos N, Vogelstein B, Kinzler KW, Velculescu VE. The consensus coding sequences of human breast and colorectal cancers. *Science* 2006; 314: 268-274

**Purdue MP**, Graubard BI, Chanock SJ, Rubertone MV, Erickson RL, McGlynn KA. Genetic variation in the inhibition pathway and risk of testicular germ cell tumors. *Cancer Res* 2008; 68: 3043-3048

**Ueno N**, Ling N, Ying SY, Esch F, Shimasaki S, Guillemin R. Isolation and partial characterization of follistatin: a single-chain Mr 35,000 monomeric protein that inhibits the release of follicle-stimulating hormone. *Proc Natl Acad Sci USA* 1987; 84: 8282-8286

**Esch FS**, Shimasaki S, Mercado M, Cooksey K, Ling N, Ying S, Ueno N, Guillemin R. Structural characterization of follistatin: a novel follicle-stimulating hormone release-inhibiting polypeptide from the gonad. *Mol Endocrinol* 1987; 1: 849-855

**Phillips DJ**, de Kretser DM. Follistatin: a multifunctional regulatory protein. *Front Neuroendocrinol* 1998; 19: 287-322

**Ullman CG**, Perkins SJ. The Factor I and follistatin domain families: the return of a prodigal son. *Biochem J* 1997; 326 (Pt 3): 939-941

**Pathy L**, Nikolics K. Functions of agrin and agrin-related proteins. *Trends Neurosci* 1993; 16: 76-81

**Deli A**, Kreidl E, Santifaller S, Trotter B, Seir K, Berger W, Schulte-Hermann R, Rodgarkia-Dara C, Grusch M. Activins and activin antagonists in hepatocellular carcinoma. *World J Gastroenterol* 2008; 14: 1699-1709

**Harrison CA**, Gray PC, Vale WW, Robertson DM. Antagonists of activin signaling: mechanisms and potential biological applications. *Trends Endocrinol Metab* 2005; 16: 73-78

**Michel U**, Rao A, Findlay JK. Rat follistatin: ontogeny of steady-state mRNA levels in different tissues predicts organ-specific functions. *Biochem Biophys Res Commun* 1991; 180: 223-230

**Sugino K**, Kurosawa N, Nakamura T, Takio K, Shimasaki S, Ling N, Titani K, Sugino H. Molecular heterogeneity of follistatin, an ovarian-binding protein. Higher affinity of the carboxyl-terminal truncated forms for heparan sulfate proteoglycans on the ovarian granulosa cell. *J Biol Chem* 1993; 268: 15579-15587

**Schneyer AL**, Rzucidlo DA, Sluss PM, Crowley WF Jr. Characterization of unique binding kinetics of follistatin and activin or inhibitor in serum. *Endocrinology* 1994; 135: 667-674

**Harrington AE**, Morris-Triggs SA, Ruotolo BT, Robinson CV, Ohnuma S, Hyvenon M. Structural basis for the inhibition of activin signalling by follistatin. *EMBO J* 2006; 25: 1035-1045

**de Winter JP**, ten Dijke P, de Vries CJ, van Achterberg TA, Sugino H, de Waal H, Huylebroek D, Verschueren K, van den Eijnden-van Raaij AJ. Follistatinis neutralize activin bioactivity by inhibition of activin binding to its type II receptors. *Mol Cell Endocrinol* 1996; 116: 105-114

**Thompson TB**, Lech TF, Cook RW, Woodruff TK, Jardetzky TS. The structure of the follistatin:activin complex reveals antagonism of both type I and type II receptor interaction. *Dev Cell* 2009; 8: 535-543

**Shimasaki S**, Koga M, Esch F, Cooksey K, Mercado M, Koba A, Ueno N, Ying SY, Ling N, Guillemin R. Primary structure of the human follistatin precursor and its genomic organization. *Proc Natl Acad Sci USA* 1988; 85: 4218-4222

**Keutmann HT**, Schneyer AL, Sidis Y. The role of follistatin domain in follistatin biological action. *Mol Cell Endocrinol* 2004; 18: 228-240

**Schneyer A**, Schoen A, Quigg A, Sidos Y. Differential binding and neutralization of activins A and B by follistatin and follistatin-like-3 (FSTL-3/FRSP/FLRC). *Endocrinology* 2003; 144: 1671-1674
Kreibl E et al. Activins and Follistatins in HCC

117 Iemura S, Yamamoto TS, Takagi C, Uchiyama H, Natsume T, Shimasaki S, Sugino H, Ueno N. Direct binding of follistatin to a complex of bone-morphogenetic protein and its receptor inhibits ventral and epidermal cell fates in early Xenopus embryos. Proc Natl Acad Sci USA. 1998; 95: 9337-9342

118 Amthor H, Nichols G, McKinnell I, Kemp CF, Sharma M, Kambadur R, Patel K. Follistatin complexes Myostatin and antagonises Myostatin-mediated inhibition of myogenesis. Dev Biol 2004; 270: 19-30

119 GliSter C, Kemp CF, Knight PG. Bone morphogenetic protein (BMP) ligands and receptors in bovine ovarian follicle cells: actions of BMP-4, -6 and -7 on granulosa cells and differential modulation of Smad-1 phosphorylation by follistatin. Reproduction 2014; 127: 239-254

120 Kogure K, Zhang YQ, Maeshima A, Suzuki K, Kuwano H, Kojima I. The role of activin and transforming growth factor-beta in the regulation of organ mass in the rat liver. Hepatology 2000; 31: 916-921

121 Takabe K, Wang L, Leal AM, Macconell LA, Wiater E, Tomiya T, Ohno A, Verma IM, Vale W. Adenovirus-mediated overexpression of follistatin enlarges intact liver of adult rats. Hepatology 2003; 38: 1107-1115

122 Kogure K, Ohara W, Kanzaki M, Zhang YQ, Yasuda H, Mine T, Kojima I. A single intraperitoneal administration of follistatin accelerates liver regeneration in partially hepatectomized rats. Gastroenterology 1995; 108: 1136-1142

123 Kogure K, Zhang YQ, Shibata H, Kojima I. Immediate onset of DNA synthesis in remnant rat liver after 90% hepatectomy by an administration of follistatin. J Hepatol 1998; 29: 977-984

124 Endo D, Maku-Uchi M, Kojima I. Activin or follistatin: which is more beneficial to support liver regeneration after massive hepatectomy? Endocr J 2006; 53: 73-78

125 Patella S, Phillips DJ, Tchjongue J, de Kretser DM, Sievert W. Follistatin attenuates early liver fibrosis: effects on hepatic stellate cell activation and hepatocyte apoptosis. Am J Pathol (Gastrointestinal Liver Pathology) 2006; 290: G137-G144

126 Rossmanith W, Chabicovsky M, Grasli-Kraupp B, Peter B, Schausberger E, Schulte-Hermann R. Follistatin overexpression in rodent liver tumors: a possible mechanism to overcome activin growth control. Mol Carcinog 2002; 35: 1-5

127 Fujwi M, Ishikawa M, Iuchi M, Tashiro S. Effect of follistatin on rat liver regeneration and tumor growth after portal occlusion. Hepatogastroenterology 2005; 52: 833-838

128 Mashima H, Kanzaki M, Nobusawa R, Zhang YQ, Suzuki M, Mine T, Kojima I. Derangements in the activin-follistatin system in hepatoma cells. Gastroenterology 1995; 108: 834-840

129 Wang F, Denison S, Lai JP, Phillips LA, Montoya D, Keck N, Schule B, Klein C, Shridhar V, Roberts LR, Smith DI, Parkin gene alterations in hepatocellular carcinoma. Genes Chromosomes Cancer 2004; 40: 85-96

130 Fujiwara M, Marusawa H, Wang HQ, Iwai A, Ikeuchi K, Imai Y, Kataoka A, Nukina N, Takahashi R, Chiba T. Parkin as a tumor suppressor gene for hepatocellular carcinoma. Oncogene 2008; 27: 6002-6011

131 Beale G, Chattopadhyay D, Gray J, Stewart S, Hudson M, Day C, Trerotoli P, Giannelli G, Manas D, Reeves H, AFP, PIVKAI, GP3, SCCA-1 and follistatin as surveillance biomarkers for hepatocellular cancer in non-alcoholic and alcoholic fatty liver disease. BMC Cancer 2008; 8: 200

132 Gao X, Hu H, Zhu J, Xu Z. Identification and characterization of follistatin as a novel angioenin-binding protein. FEBS Lett 2007; 581: 5505-5510

133 Shibanuma M, Mashimo J, Mita A, Kuroki T, Nose K. Cloning from a mouse osteoblastic cell line of a set of transforming-growth-factor-beta 1-regulated genes, one of which seems to encode a follistatin-related polypeptide. Eur J Biochem 1993; 217: 13-19

134 Kawabata D, Tanaka M, Fujii T, Umehara H, Fujita Y, Yoshii Fuji H, Mimori T, Ozaki S. Amelioriative effects of follistatin-related protein/TSC-36/FSTL1 on joint inflammation in a mouse model of arthritis. Arthritis Rheum 2004; 50: 660-669

135 Miyamae T, Marinov AD, Sowards D, Wilson DC, Devlin J, Boudreau R, Robbins P, Hirsch R. Follistatin-like protein-1 is a novel proinflammatory molecule. J Immunol 2006; 177: 4758-4762

136 Oshima Y, Ouchi N, Sato K, Izumiya Y, Pimentel DR, Walsh K. Follistatin-like-1 is an Akt-regulated cardioprotective factor that is secreted by the heart. Circulation 2008; 117: 3099-3108

137 Mashimo J, Maniwa R, Sugino H, Nose K. Decrease in the expression of a novel TGF-beta-inducible and ras-recision gene, TSC-36, in human cancer cells. Cancer Lett 1997; 113: 213-219

138 Sumitomo K, Kurisaki A, Yamakawa N, Tsuchida K, Shimizu E, Sone S, Sugino H. Expression of a TGF-beta-inducible gene, TSC-36, causes growth inhibition in human lung cancer cell lines. Cancer Lett 2000; 155: 37-46

139 Hodgson G, Hager JH, Volik S, Hariono S, Wernick M, Moore D, Nowak N, Albertson DG, Pinkel D, Collins C, Hanahan D, Gray JW. Genome scanning with array CGH delineates regional alterations in mouse islet carcinomas. Nat Genet 2001; 29: 459-464

140 Chan QK, Ngan HY, Ip PP, Liu VW, Xue WC, Cheung AN. Tumor suppressor effect of follistatin-like-1 in ovarian and endometrial carcinogenesis: a differential expression and functional analysis. Carcinogenesis 2009; 30: 114-121

141 Reddy SP, Britto R, Vinnakota K, Aparna H, Sreepathi S, Gokulnath MS, Seir K, Rodgarkia-Dara C, Hellerbrand C, Gerner C. Cell Characterization by Proteome Profiling Applied to Primary Hepatocytes and Hepatocyte Cell Lines Hep-G2 and Hep-3B. J Proteome Res 2009; Epub ahead of print

142 Kato MV, Sato H, Tsukada T, Ikawa Y, Aizawa S, Nagayoshi M. A follistatin-like gene, mac25, may act as a growth suppressor of osteosarcoma cells. Oncogene 1996; 12: 1361-1364

143 Kato MV. A secreted tumor-suppressor, mac25, with activin-binding activity. Mol Cell 2000; 6: 126-135

144 Hwa Y, Oh Y, Rosenfeld RC. The insulin-like growth factor-binding protein (IGFBP) superfamily. Endocr Rev 1999; 20: 761-787

145 Komatsu S, Okazaki Y, Tateno M, Kawai J, Konno H, Kusakabe M, Yoshiki A, Muramatsu M, Held WA, Hayashizaki Y. Methylation and downregulated expression of mac25/insulin-like growth factor binding protein-7 is associated with liver tumorigenesis in SV40T/t antigen transgenic mice, screened by restriction landmark genomic scanning for methylation (RLGS-M). Biochem Biophys Res Commun 2000; 267: 109-117

146 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 2000; 275: 40788-40796

147 Hayette S, Gadoux M, Martel S, Bertrand S, Tiegad I, Magaud JP, Rimokh R. FLRG (follistatin-related gene), a new target of chromosomal rearrangement in malignant blood disorders. Oncogene 1998; 16: 2949-2954

148 Tortoriello DV, Sidis Y, Holtzman DA, Holmes WE,
Schneyer AL. Human follistatin-related protein: a structural homologue of follistatin with nuclear localization. *Endocrinology* 2001; 142: 3426-3434

Bartholin L, Maguer-Satta V, Hayette S, Martel S, Gadoux M, Corbo L, Magaud JP, Rimokh R. Transcription activation of FLRG and follistatin by activin A, through Smad proteins, participates in a negative feedback loop to modulate activin A function. *Oncogene* 2002; 21: 2227-2235

Mukherjee A, Sidis Y, Mahan A, Raher MJ, Xia Y, Rosen ED, Bloch KD, Thomas MK, Schneyer AL. FSTL3 deletion reveals roles for TGF-beta family ligands in glucose and fat homeostasis in adults. *Proc Natl Acad Sci USA* 2007; 104: 1348-1353

Razanajaona D, Joguet S, Ay AS, Treilleux I, Goddard-Leon S, Bartholin L, Rimokh R. Silencing of FLRG, an antagonist of activin, inhibits human breast tumor cell growth. *Cancer Res* 2007; 67: 7223-7229

Glusman G, Kaur A, Hood L, Rowen L. An enigmatic fourth runt domain gene in the fugu genome: ancestral gene loss versus accelerated evolution. *BMC Evol Biol* 2004; 4: 43