

Review

Clinical and Therapeutic Implications of Follistatin in Solid Tumours

LEI SHI^{1,2}, JEYNA RESAUL², SIONED OWEN², LIN YE² and WEN G. JIANG²

¹Urology Department, Yantai Yu Huang Ding Hospital, Yantai, Shandong Province, P.R. China;

²Cardiff China Medical Research Collaborative, Division of Cancer and Genetics, Cardiff University School of Medicine, Cardiff, U.K.

Abstract. Follistatin (FST), as a single-chain glycosylated protein, has two major isoforms, FST288 and FST315. The FST315 isoform is the predominant form whilst the FST288 variant accounts for less than 5% of the encoded mRNA. FST is differentially expressed in human tissues and aberrant expression has been observed in a variety of solid tumours, including gonadal, gastric and lung cancer, hepatocellular carcinoma, basal cell carcinoma and melanoma. Based on the current evidence, FST is an antagonist of transforming growth factor beta family members, such as activin and bone morphogenetic proteins (BMPs). FST plays a role in tumourigenesis, metastasis and angiogenesis of solid tumours through its interaction with activin and BMPs, thus resulting in pathophysiological function. In terms of diagnosis, prognosis and therapy, FST has shown strong promise. Through a better understanding of its biological functions, potential clinical applications may yet emerge.

Follistatin (FST), which was first isolated from porcine and bovine follicular fluid (1, 2), was initially described as a protein involved in the regulation of the secretion of follicle-stimulating hormone. This monomeric glycosylated polypeptide chain is encoded by a single gene located on the long arm chromosome 5q11.2 (3), which through alternative splicing may be transcribed into the mRNA precursors, FST317 and FST344. From these precursors, three major FST isoforms may be produced, namely FST288 (from pre-FST317), FST315 (from pre-FST344) and a third FST

isoform, FST303, produced from the post-translational truncation of the FST315 C-terminus. These three main FST isoforms can also be glycosylated to yield six further FST isoforms that were previously identified in bovine (4) and porcine (5, 6) follicular fluid. At the core, all FST isoforms contain a 63 residue N-terminal domain and three follistatin domains, termed FSD1, FSD2 and FSD3. These domains comprise 73-77 amino acid residues and are characterised by an arrangement of 10 conserved cysteine residues (7). Both FST288 and FST315 are differentially expressed in human tissues (6-9). FST315 is the predominant isoform, whilst the FST288 isoform accounts for less than 5% of the encoded mRNA (10, 11). Some molecules such as activin, transforming growth factor-beta (TGF β), forkhead domain transcription factor L2 (12, 13), gonadotropin-releasing hormone (14), zinc finger protein (GLI2) (15), dexamethasone (16), androgens (17), activators of wingless-related integration site (WNT) signalling (18, 19) and 1,25-dihydroxyvitamin D (20) have been shown to regulate the transcription of the FST gene. In addition, down-regulation of FST gene expression by peroxisome proliferator-activated receptor gamma (PPAR γ) or the transcription factor epiprofin (21, 22) has been shown.

As an antagonist of TGF β superfamily member activin, FST seems to primarily function in relation to the role that activin plays. Along with FST, activin acts as a pleiotropic growth factor system, which is involved in proliferation, differentiation, and apoptosis of a number of cell types (23-28). As such, the functions of the FST isoforms are therefore linked to their binding affinity for activin (6, 10, 11, 29-37). In fact, this critical binding and neutralisation of activin, either partial or complete, is based on the order of at least two of the FST cysteine domains and its N-terminal (38), conferring a difference in affinities for activin between the FST isoforms. The binding complex between activin and FST generally occurs in a 1:2 ratio, where the two FST molecules, connected C-terminus to N-

Correspondence to: Professor Wen G. Jiang, Cardiff China Medical Research Collaborative, Division of Cancer and Genetics, Cardiff University School of Medicine, Cardiff, CF14 4XN, U.K. Tel: +44 2920687865, e-mail: JiangW@cf.ac.uk

Key Words: Follistatin, activin, bone morphogenetic protein, cancer, review.

terminus, envelop the activin molecule, thus blocking access to both the type I and type II receptor binding sites (9). The binding complex handicaps the interaction of activin with its specific receptors, leading to the inhibition of most of its biological effects in various cells and tissues (5, 6, 11, 32, 35). An important contribution of the FSD1 region to FST localisation and hence, bioactivity, lies in its ability to bind to plasma membrane heparin sulfate proteoglycans. For example, FST288 binds to heparin sulfate proteoglycans with a high affinity and may function as a local regulator by potentially preventing the autocrine, endocrine and paracrine actions of activin (39). Additionally, FST288-activin binding complex can interact with cell-surface proteoglycans followed by the internalization and subsequent degradation of this complex with lysosomal enzymes (3, 5, 6). Conversely, FST315 is predominantly localised in the circulation, consistent by its low binding affinity for heparin, and its binding to activin appears to be irreversible (5).

Activin seems to be able to exert context-dependent and cell type-specific inhibiting and activating functions (40). There is evidence that activin can both have an inhibitory or proliferative effect on cell proliferation; dependent on the cell type (41-47). *In vivo* studies have shown that FST knockout mice died soon after birth due to a variety of skeletal and cutaneous defects amongst other defects seen in the activin-deficient mice (48). Lin *et al.* also demonstrated that mice with an FST315-expressing construct could overcome this neonatal lethality but still had severe biological defects. Overexpression of mouse FST using the mouse metallothionein I promoter in transgenic mice led to gonadal defects and eventual infertility, primarily due to local effects of FST in these tissues (50). Dermatological defects were present in both FST-knockout and -overexpressing mice, but there was no evidence of cancer (51). Some of these defects were similar to those that were seen in bone morphogenetic protein 5 (*Bmp5*)-mutant mice (52) and TGF β -overexpressing mice (53). These similarities suggested that FST might be a modulator of other TGF β -related proteins apart from activin or might function independently (48). Some other studies have demonstrated that FST may also bind to other molecules such as BMPs, especially BMP-2, -4, -5, -6, -7 and -11 (4, 54-58), myostatin (59) and TGF β 3 (60). Table I shows the expression profile of FST and its targeting ligands in various tissues and organs of the human body. In addition, FST may also have an interaction with other non-TGF β family proteins, such as angiogenin, known as a pro-angiogenic factor (61), and α 2-macroglobulin, known as a serum pan-protease inhibitor (62). However, further investigations are required to explain the functional value of the interplay between FST and these molecules.

Follistatin in the Development and Progression of Solid Tumours

A study on the molecular mechanisms underlying antral gastric tumours, which develop in gastrin-deficient mice, demonstrated elevated FST expression in the proliferative neck zone of hyperplastic antrum, a trend which has also been observed in human gastric cancer (63). These findings indicated that antral hyperplasia in gastrin-deficient mice involves amplification of mucous cell lineages, in which FST may play a role. In addition, strong FST expression was found in basal cell carcinoma and seemed to be mainly activated by transcription factor GLI2, which as a Hedgehog signalling mediator, has been shown to be up-regulated by the expression of FST (15, 64). This phenomenon is suggestive of the engagement of FST in the development of basal cell carcinoma, which has yet to be fully elucidated.

Stove *et al.* found that all melanoma cell lines expressed activin receptors. Treatment of these cell lines with activin also resulted in the phosphorylation of SMAD signal transduction molecules (65). Secretion of FST, either native or after retroviral transduction, efficiently prevented SMAD activation or activation of an activin-responsive luciferase reporter construct. In melanocytes, activin treatment led to growth inhibition and induction of apoptosis, processes which were counteracted by an addition of FST. Thus this study suggests that in melanoma, FST is involved in neutralising the effects of activin.

Activin also appears to be a major regulatory factor in liver tissue homeostasis, controlling cell proliferation and apoptosis. Both activin and TGF β itself may inhibit DNA synthesis and induce apoptosis *in vitro* as well as *in vivo* (66-72). It has been reported that experimentally administered FST acted as an inducer of hepatocellular DNA synthesis (73-76) through reduction in the local bioavailability of activin. Additionally, overexpression of FST was also found in rodent liver tumours (77), of which most were hepatocellular carcinomas, when compared to surrounding liver tissue; the level of overexpression of FST was different but independent of the carcinogen treatment that rodents had received and the histological stage of malignancy. Differential expression of FST and its interacting ligands in different malignancies is shown in Table II.

In addition, there is also some indirect proof regarding the growth-promoting effect of FST on prostate cancer cells. Rapamycin (78) and soy isoflavones (79), plant-derived polyphenolic compounds with estrogen-like properties, inhibit prostate cancer cell growth and are associated with a decrease in the expression of FST. Type III TGF β receptor has been reported to play an inhibitory role in the growth of prostate cancer cells (80). FST has been shown to directly bind to and completely block type III TGF β receptor-induced epithelial-mesenchymal transition of normal murine mammary gland

Table I. Expression profile of follistatin (FST) and its target molecules in different organs and tissues.

Tissue	FST	INH A	INH B A	INH B B	BMP2	BMP4	BMP5	BMP6	BMP7	BMP11	Myostatin	TGFβ3
Adipose tissue	0	0	0	0	0	77	0	0	0	0	0	77
Adrenal gland	30	0	0	30	0	0	0	0	0	30	0	0
Ascites	0	0	0	0	0	0	0	0	0	0	0	0
Bladder	0	0	0	0	0	0	0	0	0	0	0	0
Blood	0	0	24	0	0	0	0	98	0	16	0	0
Bone	83	0	55	0	27	97	0	0	13	55	0	41
Bone marrow	41	0	20	0	0	0	0	0	0	0	0	41
Brain	2	9	2	5	4	7	2	8	43	23	1	13
Cervix	0	0	0	0	247	0	0	61	0	0	0	0
Connective tissue	87	0	26	0	26	13	13	6	33	13	6	20
Ear	248	0	0	0	0	0	0	0	0	0	0	0
Embryonic tissue	42	0	42	0	14	108	37	9	32	9	0	14
Oesophagus	0	0	49	0	0	0	0	0	0	0	0	49
Eye	47	19	47	33	43	43	9	14	86	129	4	33
Heart	11	0	0	22	44	11	100	0	100	22	11	78
Intestine	12	0	38	8	17	219	4	21	30	25	0	8
Kidney	14	0	4	4	4	4	0	9	42	4	0	18
Larynx	127	0	85	0	426	0	0	42	0	0	0	0
Liver	24	9	4	9	9	63	9	0	0	14	0	9
Lung	41	2	23	20	29	14	11	20	5	11	11	23
Lymph	0	0	0	0	0	22	0	0	90	0	0	0
Lymph node	0	0	0	0	0	11	0	0	44	11	0	0
Mammary gland	26	0	19	26	6	26	72	6	39	33	0	26
Mouth	15	0	30	0	0	60	0	15	15	45	0	30
Muscle	0	0	18	0	0	0	28	0	9	18	9	0
Nerve	64	0	0	64	0	0	0	0	0	128	0	64
Ovary	325	49	9	0	0	29	9	78	39	0	0	29
Pancreas	32	0	37	4	14	4	32	0	4	14	0	51
Parathyroid	0	0	0	0	0	0	0	48	0	48	0	0
Pharynx	0	0	0	0	0	0	0	0	0	0	0	0
Pituitary gland	0	0	0	0	0	0	0	0	0	0	0	0
Placenta	3	49	88	0	21	63	74	35	67	7	3	52
Prostate	42	0	0	10	5	21	10	15	36	10	0	63
Salivary gland	0	0	0	0	0	49	0	0	0	0	0	0
Skin	4	0	61	9	0	4	0	0	9	37	0	0
Spleen	37	0	0	0	37	0	0	0	0	0	0	0
Stomach	0	0	62	0	31	125	0	0	10	0	0	10
Testis	4	22	0	20	4	6	2	6	34	6	0	4
Thymus	0	0	0	0	0	0	25	0	37	12	0	0
Thyroid	21	21	0	64	0	0	0	0	0	0	0	0
Tonsil	0	0	0	0	0	0	0	0	58	58	0	0
Trachea	0	0	0	0	0	0	57	0	19	0	0	0
Umbilical cord	0	0	0	0	0	0	0	72	0	72	0	0
Uterus	4	4	8	4	4	0	17	8	12	17	0	25
Vascular	0	0	290	0	0	19	0	135	0	0	0	0

INH A/BA/BB: Inhibin alpha/beta a/beta b subunit; BMP2/4/5/6/7/11: bone morphogenic protein 2/4/5/6/7/11; TGFβ3: transforming growth factor beta 3. Numbers of gene transcripts are transcripts per million that were collected from Unigene (<https://www.ncbi.nlm.nih.gov/unigene>).

epithelial cell line *in vitro* (60) and thus might promote prostate cell proliferation by targeting this receptor.

It was found that exogenously added activin A and B induced an inhibitory effect on the growth of two human prostate cancer cell lines, LNCaP and DU145, which was completely reversed by FST (24). Overexpression of FST was observed in human LNCaP cells (81), which is in line

with the theory of FST overproduction favouring the growth of prostate cancer by inhibiting signalling of activin (23). However, another human prostate cancer cell line, PC3, is resistant to the growth-inhibiting effects of activin A. Intriguingly, this phenomenon is further explained by the study of McPherson *et al.* which showed that only PC3 cells produced the FST288 isoform, while LNCaP and DU145

Table II. Expression of follistatin (FST) and its target genes in malignancies.

Tissue	FST	INHA	INHBA	INHBB	BMP2	BMP4	BMP5	BMP6	BMP7	BMP11	Myostatin	TGFβ3
Adrenal tumor	0↓↓	0	0	79↑↑	0	0	0	0	0	79↑↑	0	0
Bladder carcinoma	0	0	0	0	0	0	0	0	0	0	0	0
Breast tumor	42↑↑	0	21	32↑	0↓↓	42↑↑	118↑↑	0↓↓	42	53↑↑	0	42↑↑
Cervical tumor	0	0	0	0	347↑↑	0	0	86↑	0	0	0	0
Chondrosarcoma	132	0	48	0	24	48	0	12	12	72	0	48
Colorectal tumor	0↓↓	0	17	8	17	328	0	26	35	26	0	8
Esophageal tumor	0	0	57	0	0	0	0	0	0	0	0	57
Gastrointestinal tumor	25↑↑	0	25↓↓	0	16↓↓	202↑↑	0	0	8↓	0	0	8↓
Germ cell tumor	37	26	0	11	0	7	7	18	91	41	0	3
Glioma	9	0	0	0	46	9	0	0	74	46	0	0
Head and neck tumor	29	7	29	14	74	37	0	14	7	22	0	7
Kidney tumor	14	0	0↓↓	0↓↓	0↓↓	0↓↓	0	0↓↓	87↑↑	14↑↑	0	14↓
Leukemia	21↑	0	0	0	10	0	0	116	0	21	0	10
Liver tumor	20	10	10	10	10	104	0	0	0	31	0	0
Lung tumor	68↑↑	0↓↓	0↓↓	0↓↓	0↓↓	19↑	0↓↓	0↓↓	0↓↓	19↑	0↓↓	9↓↓
Lymphoma	0	0	0	0	0	27	0	0	207	0	0	0
Non-neoplasia	10	0	31	0	0	10	0	10	31	0	0	0
Normal	22	12	24	6	14	21	19	11	33	21	2	26
Ovarian tumor	393↑	13↓↓	0↓↓	0	0	39↑	13↑	91↑	52↑	0	0	13↓↓
Pancreatic tumor	66↑↑	0	76↑↑	9↑↑	0↓↓	9↑↑	19	0	9↑↑	9↓	0	47↓
Primitive neuro-ectodermal tumor	0	7	0	0	0	31	0	0	70	0	0	0
Prostate cancer	48↑	0	0	0↓↓	0↓↓	28↑	0	19↑	0↓↓	19↑↑	0	28↓↓
Retinoblastoma	0	0	0	43	0	0	0	0	0	64	0	0
Skin tumor	0	0	55	0	0	0	0	0	15	23	0	0
Soft tissue/muscle tissue tumor	31	0	7	0	7	0	39	0	39	23	15	0
Uterine tumor	11↑↑	0↓↓	22↑↑	11↑↑	0↓↓	0	0↓↓	0↓↓	33↑↑	33↑↑	0	22↓

INHA/BA/BB: Inhibin alpha/beta a/beta b subunit; BMP2/4/5/6/7/11: bone morphogenic protein 2/4/5/6/7/11; TGFβ3: transforming growth factor beta 3. Note: In comparison with corresponding normal control, ↑↑ represents an increase greater than 50%, while ↑ stands for a difference of less than 50%; ↓↓ represents a decrease greater than 50%, while ↓ stands for a reduction of less than 50%. Numbers of gene transcripts are transcripts per million which were collected from Unigene (<https://www.ncbi.nlm.nih.gov/unigene>).

cells predominantly produced the FST315 isoform. Blockade of FST288 activity with a neutralizing antibody rendered PC3 cells responsive to activin A, as measured by inhibition of proliferation (25). These results indicate that the resistance of PC3 cells to activin A is more likely due to predominantly expressed of FST288 in comparison with LNCaP and DU145 cells. Sidis *et al.* found that inhibition of endogenous activin bioactivity, irrespective of the FST isoforms being administered endogenously or exogenously, was correlated closely with its surface-binding activity (36). These findings suggest that the cell-surface dwelling FST288 isoform inhibits activin-mediated downstream signal transduction pathways more efficiently than the FST315 isoform. Further investigations will shed light on the interaction of the FST288 isoform with activin receptors and, consequently, the biological effects induced by these molecules.

In some cases, however, FST may also inhibit tumour growth. An *in vitro* study showed that the growth of inhibin-deficient gonadal tumour cells from mice could be stimulated

in culture *via* an autocrine mechanism of activin, while FST added to the culture medium slowed the growth rate of the tumour cells (82). Another *in vitro* study observed that for four ovarian cell lines not synthesizing FST, treatment with activin (1-100 ng/ml) resulted in an increase of proliferation, whereas FST treatment (1-100 ng/ml) inhibited their proliferation (83). This inhibitory function was further verified in an *in vivo* study, showing inhibin-deficient mice that carry the mouse metallothionein I follistatin transgene exhibited a less severe activin-mediated cancer cachexia-like wasting syndrome, lower serum activin levels, and a statistically significant prolonged survival in a number of cases compared with the mice deficient in inhibin alone (51).

Follistatin in Tumour-associated Angiogenesis

The effect of FST on tumour angiogenesis was found in a study by Ogino *et al.* (84), in which two human small cell lung cancer cell lines, SBC-3 and SBC-5, were transfected with the

FST gene and subsequently inoculated into the natural killer cell-depleted severe combined immunodeficient (SCID) mice. Histological analysis results revealed that the number of proliferating tumour cells and the tumour-associated microvessel density were significantly reduced in the lesions produced by FST transfectants, compared with the parental SBC-5 or mock control, which was consistent with the results seen in liver-metastatic lesions produced by FST transfectants. These observations in both lung- and liver-derived tumours is suggestive of FST inhibiting tumour angiogenesis. However, no obvious effect on cell proliferation, motility, invasion, or adhesion were observed for FST in endothelial cells *in vitro* nor on tumour growth and metastasis *in vivo* (84).

Unlike the findings by Ogino *et al.* (84), there is also some evidence indicating that FST may play a promotory role in the progression of tumour angiogenesis. Kozian *et al.* found that FST implantation in the cornea in combination with subcritical concentrations of fibroblast growth factor (bFGF) induced a strong angiogenic response (85), which demonstrates that FST by itself, but particularly in synergy with bFGF, induces angiogenesis. This may be primarily due to the action of FST antagonising activin, which inhibits bFGF-induced sprouting angiogenesis *in vitro* and *in vivo* (40). Other evidence that FST may have a promotory effect on tumour angiogenesis is the study by Krneta *et al.* in which SCID mice were inoculated with R30C mammary carcinoma cells transfected with FST288 or activin. The FST-expressing tumours had a denser network of small-diameter capillaries than activin-expressing tumours (40). FST288 may be required for the promotion of angiogenesis in a mouse model (49). Collectively, FST, especially the FST288 isoform, seems to be involved in tumour angiogenesis, at least partially through attenuating activin signalling.

Follistatin in Solid Tumour Metastasis

In the study by Ogino *et al.*, FST transfectants produced significantly fewer metastatic colonies in multiple organs, including lung, liver, and bone in natural killer cell-depleted SCID mice when compared with their parental cells or vector control clones (84), supporting the potential role of FST in controlling tumour metastasis.

BMP2, -4 and -7, together with their inhibitors, including FST, have been shown to be involved in osteoblastogenesis (86-88). The activin/FST system was reported to play a regulatory role in normal bone homeostasis (89-92). It has been speculated that FST might play a role in the pathogenesis of bone metastasis through interaction with BMPs and activin.

A study demonstrated that ribozyme transgene targeting BMP7 expression in the PC3 prostate cancer cell line resulted in increased invasiveness and motility, which appeared to be facilitated by changes in the level of the BMP antagonists noggin and FST (58). Although these findings

indicate the role of FST in solid tumours, more evidence is needed for greater clarification of its involvement in the metastases associated with solid tumours.

Value of Follistatin in Diagnosis, Prognosis and of Solid Tumours

FST is expressed in multiple tissues (10, 77, 93-97), and its expression levels are altered in tumour tissues (25, 90, 98-100) and in body fluids (101-104) of patients with solid tumours. These findings provoked studies investigating FST as a diagnostic or prognostic marker for solid tumours. One study showed that serum FST and ovarian carcinoma antigen CA125 levels were significantly increased in patients with ovarian cancer when compared with healthy individuals and patients with benign ovarian cysts, which suggests that the serum FST level has potential diagnostic value, especially when combined with CA125 detection in order to reduce the number of false-positive results (105). Serum levels of FST were also found to be elevated in patients with hepatocellular cancer (HCC) (21, 106). Survival of patients with HCC with high FST levels was significantly shorter than for those with low serum levels of FST (106). Multivariate analysis revealed that in addition to large tumour size and presence of portal vein thrombus, high FST levels were independently correlated with poor prognosis (106). Therefore, a promising potential is indicated for FST in HCC prognosis. A survival analysis of FST in breast, lung, ovarian and gastric cancer was also performed using an online Kaplan–Meier analysis (<http://kmplot.com/analysis/>) (Figure 1) (107). An increased expression of FST was associated with poorer overall survival of patients with ovarian, gastric and lung cancer. These data also correlate with the previously mentioned studies of serum level in ovarian cancer and HCC. In contrast, a reduced expression of FST was correlated with shorter relapse-free survival (Figure 1B), although no correlation was seen between FST expression and overall survival of patients with breast cancer (data not shown).

Blank *et al.* reported FST in serum was negatively correlated and was decreased in both distant and lymph node metastasis associated with gastric cancer compared to patients without metastasis. In contrast, they noted that tissue from the patients with metastasis exhibited higher FST levels. Additionally, neoadjuvantly treated patients with lymph node metastasis still exhibited lower FST levels, whilst those with distant metastasis and poorly differentiated tumours (G3/4) exhibited elevated FST serum levels. Prognostically, a tumour tissue level of FST below the median was associated with better survival. However, in primary resected and neoadjuvantly treated patients, FST levels in tissue had no impact (108). Therefore FST levels appear to have a further prognostic significance in gastric cancer.

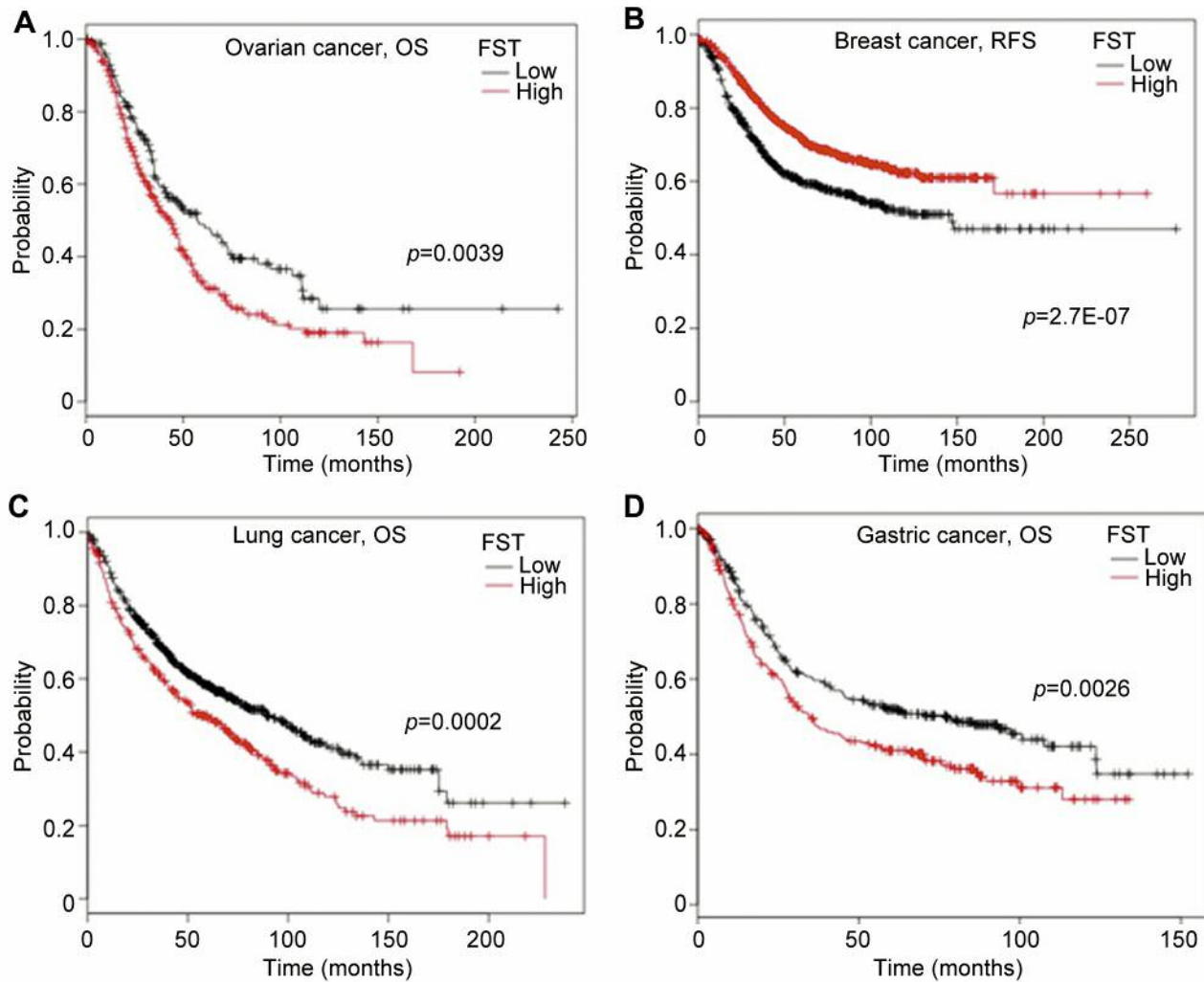


Figure 1. Expression of follistatin (FST) is associated with survival of patients with cancer. The association with survival of patients with ovarian, breast, lung and gastric cancer was analyzed in a combined cohort of these cancer types using an online Kaplan–Meier survival analysis tool (KMplot, <http://kmplot.com/analysis/>) (107). A: High expression of FST was found to be associated with shorter overall survival (OS) of patients with ovarian cancer. B: High FST expression was associated with relapse-free survival (RFS) of patients with breast cancer. C: High expression of FST was associated with shorter OS of patients with lung cancer. D: High FST expression was associated with shorter OS of patients with gastric cancer. Number of patients of each cohort are provided in Table III. The cut-off levels were auto-selected using the KMplot and number of patients in each group are listed in Table III.

Table III. Number of patients and cut-off levels of follistatin (FST) for the Kaplan–Meier survival analyses.

Cancer type (survival)	Low FST expression (no. of patients)	High FST expression (no. of patients)	Cut-off level (normalised FST expression level)
Ovarian (OS)	222	358	101
Breast (RFS)	792	868	238
Lung (OS)	829	316	456
Gastric (OS)	346	285	94

OS: Overall survival; RFS: relapse-free survival. The association with survival of patients with ovarian, breast, lung and gastric cancer was analysed in a combined cohort of these cancer types using an online Kaplan–Meier survival analysis tool (KMplot, <http://kmplot.com/analysis/>) (107). Numbers of patients of each cohort and the cut-off levels which were auto-selected using the KMplot are shown.

Other than the potential from diagnostic and prognostic perspectives, FST might also be considered as a therapeutic target. Grusch *et al.* reported that FST was overexpressed while both activin subunits were down-regulated in the majority of rat and human liver tumours studied (109). Treatment of normal and pre-neoplastic hepatocytes with FST stimulated DNA synthesis preferentially in pre-neoplastic rat hepatocytes, whereas activin A repressed it. This study suggests that the balanced expression of FST and activin becomes deregulated during hepatocarcinogenesis and the sensitivity of pre-neoplastic hepatocytes to activin signals suggests the activin/FST system might be a promising target for therapeutic intervention.

FST was indicated to have potential in clinical use (81, 104) in prostate cancer, but its diagnostic effectiveness was found not to be significantly superior to that of activin A or prostate-specific antigen (110), although it could still be useful as a biomarker for follow-up of patients with prostate cancer undergoing antimetastatic treatments. Together with another possibility that FST may contribute to fostering bone metastasis formation in patients with prostate cancer (58, 86-88, 111), FST may be regarded as a potential, molecular target in the treatment of metastatic bone disease.

Conclusion

Based on current findings, FST plays a role in tumorigenesis, progression, metastasis and angiogenesis of solid tumours. This molecule seems to exert its effect mainly by interaction with TGF β family members, such as activin and BMPs. As an antagonist of activin, FST seems primarily to function depending on the role that activin plays, exerting context-dependent and cell type-specific inhibition and activation (40), including a stimulating effect observed in activation of the FST-stimulating effect on gastric (63, 112) and prostate (23-25, 36, 81) cancer, while playing an inhibitory role in gonadal tumours (81, 82). FST288 has more inhibitory effects on activin-induced biological action than FST315, which has been supported by a series of studies (23-25, 36, 81). Future investigations can elucidate the exact role played by the FST288 isoform in solid tumours and also the corresponding application in diagnosis and treatment.

Acknowledgements

The Authors wish to thank Cancer Research Wales, Welsh Life Science Network (NRN, Ser Cymru) and the Wales Metastasis Platform for their support. Dr Shi was a recipient of the China Medical Scholarship from Cardiff University.

References

- Robertson DM, Klein R, de Vos FL, McLachlan RI, Wettenhall RE, Hearn MT, Burger HG and de Kretser DM: The isolation of polypeptides with FSH suppressing activity from bovine follicular fluid which are structurally different to inhibin. *Biochem Biophys Res Commun* 149: 744-749, 1987.
- Ueno N, Ling N, Ying SY, Esch F, Shimasaki S and 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* 84: 8282-8286, 1987.
- Sepporta MV, Tumminello FM, Flandina C, Crescimanno M, Giammanco M, La Guardia M, di Majo D and Leto G: Follistatin as potential therapeutic target in prostate cancer. *Target Oncol* 8: 215-223, 2013.
- Glister C, Kemp CF and 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* 127: 239-254, 2004.
- Sugino H, Sugino K, Hashimoto O, Shoji H and Nakamura T: Follistatin and its role as an activin-binding protein. *J Med Invest* 44: 1-14, 1997.
- Sugino K, Kurosawa N, Nakamura T, Takio K, Shimasaki S, Ling N, Titani K and Sugino H: Molecular heterogeneity of follistatin, an activin-binding protein. Higher affinity of the carboxyl-terminal truncated forms for heparan sulfate proteoglycans on the ovarian granulosa cell. *J Biol Chem* 268: 15579-15587, 1993.
- Phillips DJ and de Kretser DM: Follistatin: A multifunctional regulatory protein. *Front Neuroendocrinol* 19: 287-322, 1998.
- Schneyer AL, Wang Q, Sidis Y and Sluss PM: Differential distribution of follistatin isoforms: Application of a new FS315-specific immunoassay. *J Clin Endocrinol Metab* 89: 5067-5075, 2004.
- Thompson TB, Lerch TF, Cook RW, Woodruff TK and Jardtzyk 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.
- Michel U, Albiston A and Findlay JK: Rat follistatin: Gonadal and extragonadal expression and evidence for alternative splicing. *Biochem Biophys Res Commun* 173: 401-407, 1990.
- Schneyer AL, Hall HA, Lambert-Messerlian G, Wang QF, Sluss P and Crowley WF Jr.: Follistatin-activin complexes in human serum and follicular fluid differ immunologically and biochemically. *Endocrinology* 137: 240-247, 1996.
- Bartholin L, Maguer-Satta V, Hayette S, Martel S, Gadoux M, Corbo L, Magaud JP and 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* 21: 2227-2235, 2002.
- Blount AL, Schmidt K, Justice NJ, Vale WW, Fischer WH and Bilezikjian LM: Foxl2 and Smad3 coordinately regulate follistatin gene transcription. *J Biol Chem* 284: 7631-7645, 2009.
- Winters SJ, Ghooray D, Fujii Y, Moore JP Jr., Nevitt JR and Kakar SS: Transcriptional regulation of follistatin expression by GnRH in mouse gonadotroph cell lines: Evidence for a role for cAMP signaling. *Mol Cell Endocrinol* 271: 45-54, 2007.
- Eichberger T, Kaser A, Pixner C, Schmid C, Klingler S, Winklmayr M, Hauser-Kronberger C, Aberger F and Frischauf AM: GLI2-specific transcriptional activation of the bone morphogenetic protein/activin antagonist follistatin in human epidermal cells. *J Biol Chem* 283: 12426-12437, 2008.

- 16 Hayashi K, Yamaguchi T, Yano S, Kanazawa I, Yamauchi M, Yamamoto M and Sugimoto T: BMP/Wnt antagonists are up-regulated by dexamethasone in osteoblasts and reversed by alendronate and PTH: potential therapeutic targets for glucocorticoid-induced osteoporosis. *Biochem Biophys Res Commun* 379: 261-266, 2009.
- 17 Singh R, Bhasin S, Braga M, Artaza JN, Pervin S, Taylor WE, Krishnan V, Sinha SK, Rajavashisth TB and Jasuja R: Regulation of myogenic differentiation by androgens: Cross talk between androgen receptor/beta-catenin and follistatin/transforming growth factor-beta signaling pathways. *Endocrinology* 150: 1259-1268, 2009.
- 18 Willert J, Epping M, Pollack JR, Brown PO and Nusse R: A transcriptional response to Wnt protein in human embryonic carcinoma cells. *BMC Dev Biol* 2: 8, 2002.
- 19 Yao HH, Matzuk MM, Jorgez CJ, Menke DB, Page DC, Swain A and Capel B: Follistatin operates downstream of Wnt4 in mammalian ovary organogenesis. *Dev Dyn* 230: 210-215, 2004.
- 20 Necela BM, Su W and Thompson EA: Peroxisome proliferator-activated receptor gamma down-regulates follistatin in intestinal epithelial cells through SP1. *J Biol Chem* 283: 29784-29794, 2008.
- 21 Garcia LA, King KK, Ferrini MG, Norris KC and Artaza JN: 1,25(OH)₂ vitamin D₃ stimulates myogenic differentiation by inhibiting cell proliferation and modulating the expression of promyogenic growth factors and myostatin in C2C12 skeletal muscle cells. *Endocrinology* 152: 2976-2986, 2011.
- 22 Ruspita I, Miyoshi K, Muto T, Abe K, Horiguchi T and Noma T: Sp6 downregulation of follistatin gene expression in ameloblasts. *J Med Invest* 55: 87-98, 2008.
- 23 Thomas TZ, Wang H, Niclason P, O'Bryan MK, Evans LW, Groome NP, Pedersen J and Risbridger GP: Expression and localization of activin subunits and follistatins in tissues from men with high grade prostate cancer. *J Clin Endocrinol Metab* 82: 3851-3858, 1997.
- 24 van Schaik RH, Wierix CD, Timmerman MA, Oomen MH, van Weerden WM, van der Kwast TH, van Steenbrugge GJ and de Jong FH: Variations in activin receptor, inhibin/activin subunit and follistatin mRNAs in human prostate tumour tissues. *Br J Cancer* 82: 112-117, 2000.
- 25 McPherson SJ, Mellor SL, Wang H, Evans LW, Groome NP and Risbridger GP: Expression of activin A and follistatin core proteins by human prostate tumor cell lines. *Endocrinology* 140: 5303-5309, 1999.
- 26 Sulyok S, Wankell M, Alzheimer C and Werner S: Activin: An important regulator of wound repair, fibrosis, and neuroprotection. *Mol Cell Endocrinol* 225: 127-132, 2004.
- 27 Welt CK: The physiology and pathophysiology of inhibin, activin and follistatin in female reproduction. *Curr Opin Obstet Gynecol* 14: 317-323, 2002.
- 28 Abe Y, Minegishi T and Leung PC: Activin receptor signaling. *Growth Factors* 22: 105-110, 2004.
- 29 Knight PG: Roles of inhibins, activins, and follistatin in the female reproductive system. *Front Neuroendocrinol* 17: 476-509, 1996.
- 30 DePaolo LV: Inhibins, activins, and follistatins: The saga continues. *Proc Soc Exp Biol Med* 214: 328-339, 1997.
- 31 Nakamura T, Sugino K, Titani K and Sugino H: Follistatin, an activin-binding protein, associates with heparan sulfate chains of proteoglycans on follicular granulosa cells. *J Biol Chem* 266: 19432-19437, 1991.
- 32 Hashimoto O, Kawasaki N, Tsuchida K, Shimasaki S, Hayakawa T and Sugino H: Difference between follistatin isoforms in the inhibition of activin signalling: Activin neutralizing activity of follistatin isoforms is dependent on their affinity for activin. *Cell Signal* 12: 565-571, 2000.
- 33 Hashimoto O, Nakamura T, Shoji H, Shimasaki S, Hayashi Y and Sugino H: A novel role of follistatin, an activin-binding protein, in the inhibition of activin action in rat pituitary cells. Endocytotic degradation of activin and its acceleration by follistatin associated with cell-surface heparan sulfate. *J Biol Chem* 272: 13835-13842, 1997.
- 34 Harrington AE, Morris-Triggs SA, Ruotolo BT, Robinson CV, Ohnuma S and Hyvonen M: Structural basis for the inhibition of activin signalling by follistatin. *EMBO J* 25: 1035-1045, 2006.
- 35 Lerch TF, Shimasaki S, Woodruff TK and Jardetzky TS: Structural and biophysical coupling of heparin and activin binding to follistatin isoform functions. *J Biol Chem* 282: 15930-15939, 2007.
- 36 Sidis Y, Mukherjee A, Keutmann H, Delbaere A, Sadatsuki M and Schneyer A: Biological activity of follistatin isoforms and follistatin-like-3 is dependent on differential cell surface binding and specificity for activin, myostatin, and bone morphogenetic proteins. *Endocrinology* 147: 3586-3597, 2006.
- 37 Nakamura T, Takio K, Eto Y, Shibai H, Titani K and Sugino H: Activin-binding protein from rat ovary is follistatin. *Science* 247: 836-838, 1990.
- 38 Keutmann HT, Schneyer AL and Sidis Y: The role of follistatin domains in follistatin biological action. *Mol Endocrinol* 18: 228-240, 2004.
- 39 Innis CA and Hyvonen M: Crystal structures of the heparan sulfate-binding domain of follistatin. Insights into ligand binding. *J Biol Chem* 278: 39969-39977, 2003.
- 40 Krneta J, Kroll J, Alves F, Prahst C, Sananbenesi F, Dullin C, Kimmina S, Phillips DJ and Augustin HG: Dissociation of angiogenesis and tumorigenesis in follistatin- and activin-expressing tumors. *Cancer Res* 66: 5686-5695, 2006.
- 41 Erlebacher A, Price KA and Glimcher LH: Maintenance of mouse trophoblast stem cell proliferation by TGF-beta/activin. *Dev Biol* 275: 158-169, 2004.
- 42 Ota F, Maeshima A, Yamashita S, Ikeuchi H, Kaneko Y, Kuroiwa T, Hiromura K, Ueki K, Kojima I and Nojima Y: Activin A induces cell proliferation of fibroblast-like synoviocytes in rheumatoid arthritis. *Arthritis Rheum* 48: 2442-2449, 2003.
- 43 Cho SH, Yao Z, Wang SW, Alban RF, Barbers RG, French SW and Oh CK: Regulation of activin A expression in mast cells and asthma: Its effect on the proliferation of human airway smooth muscle cells. *J Immunol* 170: 4045-4052, 2003.
- 44 Hedger MP, Phillips DJ and de Kretser DM: Divergent cell-specific effects of activin-A on thymocyte proliferation stimulated by phytohemagglutinin, and interleukin 1beta or interleukin 6 *in vitro*. *Cytokine* 12: 595-602, 2000.
- 45 Martins da Silva SJ, Bayne RA, Cambay N, Hartley PS, McNeilly AS and Anderson RA: Expression of activin subunits and receptors in the developing human ovary: Activin A promotes germ cell survival and proliferation before primordial follicle formation. *Dev Biol* 266: 334-345, 2004.
- 46 Sakurai T, Abe Y, Kasuya Y, Takuwa N, Shiba R, Yamashita T, Endo T and Goto K: Activin A stimulates mitogenesis in Swiss

- 3T3 fibroblasts without activation of mitogen-activated protein kinases. *J Biol Chem* 269: 14118-14122, 1994.
- 47 Hashimoto M, Shoda A, Inoue S, Yamada R, Kondo T, Sakurai T, Ueno N and Muramatsu M: Functional regulation of osteoblastic cells by the interaction of activin-A with follistatin. *J Biol Chem* 267: 4999-5004, 1992.
- 48 Matzuk MM, Kumar TR, Vassalli A, Bickenbach JR, Roop DR, Jaenisch R and Bradley A: Functional analysis of activins during mammalian development. *Nature* 374: 354-356, 1995.
- 49 Lin SY, Craythorn RG, O'Connor AE, Matzuk MM, Girling JE, Morrison JR and de Kretser DM: Female infertility and disrupted angiogenesis are actions of specific follistatin isoforms. *Mol Endocrinol* 22: 415-429, 2008.
- 50 Guo Q, Kumar TR, Woodruff T, Hadsell LA, DeMayo FJ and Matzuk MM: Overexpression of mouse follistatin causes reproductive defects in transgenic mice. *Mol Endocrinol* 12: 96-106, 1998.
- 51 Cipriano SC, Chen L, Kumar TR and Matzuk MM: Follistatin is a modulator of gonadal tumor progression and the activin-induced wasting syndrome in inhibin-deficient mice. *Endocrinology* 141: 2319-2327, 2000.
- 52 Kingsley DM, Bland AE, Grubber JM, Marker PC, Russell LB, Copeland NG and Jenkins NA: The mouse short ear skeletal morphogenesis locus is associated with defects in a bone morphogenetic member of the TGF beta superfamily. *Cell* 71: 399-410, 1992.
- 53 Sellheyer K, Bickenbach JR, Rothnagel JA, Bundman D, Longley MA, Krieg T, Roche NS, Roberts AB and Roop DR: Inhibition of skin development by overexpression of transforming growth factor beta 1 in the epidermis of transgenic mice. *Proc Natl Acad Sci USA* 90: 5237-5241, 1993.
- 54 Iemura S, Yamamoto TS, Takagi C, Uchiyama H, Natsume T, Shimasaki S, Sugino H and 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 embryo. *Proc Natl Acad Sci USA* 95: 9337-9342, 1998.
- 55 Amthor H, Christ B, Rashid-Doubell F, Kemp CF, Lang E and Patel K: Follistatin regulates bone morphogenetic protein-7 (BMP-7) activity to stimulate embryonic muscle growth. *Dev Biol* 243: 115-127, 2002.
- 56 McConnell DS, Wang Q, Sluss PM, Bolf N, Khoury RH, Schneyer AL, Midgley AR Jr., Reame NE, Crowley WF, Jr. and Padmanabhan V: A two-site chemiluminescent assay for activin-free follistatin reveals that most follistatin circulating in men and normal cycling women is in an activin-bound state. *J Clin Endocrinol Metab* 83: 851-858, 1998.
- 57 Havard M, Dautry F and Tchenio T: A dormant state modulated by osmotic pressure controls clonogenicity of prostate cancer cells. *J Biol Chem* 286: 44177-44186, 2011.
- 58 Ye L, Lewis-Russell JM, Kynaston H and Jiang WG: Endogenous bone morphogenetic protein-7 controls the motility of prostate cancer cells through regulation of bone morphogenetic protein antagonists. *J Urol* 178: 1086-1091, 2007.
- 59 Amthor H, Nicholas G, McKinnell I, Kemp CF, Sharma M, Kambadur R and Patel K: Follistatin complexes myostatin and antagonises myostatin-mediated inhibition of myogenesis. *Dev Biol* 270: 19-30, 2004.
- 60 Nogai H, Rosowski M, Grun J, Rietz A, Debus N, Schmidt G, Lauster C, Janitz M, Vorkamp A and Lauster R: Follistatin antagonizes transforming growth factor-beta3-induced epithelial-mesenchymal transition *in vitro*: Implications for murine palatal development supported by microarray analysis. *Differentiation* 76: 404-416, 2008.
- 61 Gao X, Hu H, Zhu J and Xu Z: Identification and characterization of follistatin as a novel angiogenin-binding protein. *FEBS Lett* 581: 5505-5510, 2007.
- 62 Phillips DJ, McFarlane JR, Hearn MT and de Kretser DM: Inhibin, activin and follistatin bind preferentially to the transformed species of alpha 2-macroglobulin. *J Endocrinol* 155: 65-71, 1997.
- 63 Kang W, Saqui-Salces M, Zavros Y and Merchant JL: Induction of follistatin precedes gastric transformation in gastrin deficient mice. *Biochem Biophys Res Commun* 376: 573-577, 2008.
- 64 Eichberger T, Sander V, Schnidar H, Regl G, Kasper M, Schmid C, Plamberger S, Kaser A, Aberger F and Frischauf AM: Overlapping and distinct transcriptional regulator properties of the GLI1 and GLI2 oncogenes. *Genomics* 87: 616-632, 2006.
- 65 Stove C, Vanrobaeys F, Devreese B, Van Beeumen J, Mareel M and Bracke M: Melanoma cells secrete follistatin, an antagonist of activin-mediated growth inhibition. *Oncogene* 23: 5330-5339, 2004.
- 66 Nakamura T, Tomita Y, Hirai R, Yamaoka K, Kaji K and Ichihara A: Inhibitory effect of transforming growth factor-beta on DNA synthesis of adult rat hepatocytes in primary culture. *Biochem Biophys Res Commun* 133: 1042-1050, 1985.
- 67 Carr BI, Hayashi I, Branum EL and Moses HL: Inhibition of DNA synthesis in rat hepatocytes by platelet-derived type beta transforming growth factor. *Cancer Res* 46: 2330-2334, 1986.
- 68 Oberhammer F, Bursch W, Parzefall W, Breit P, Erber E, Stadler M and Schulte-Hermann R: Effect of transforming growth factor beta on cell death of cultured rat hepatocytes. *Cancer Res* 51: 2478-2485, 1991.
- 69 Oberhammer FA, Pavelka M, Sharma S, Tiefenbacher R, Purchio AF, Bursch W and Schulte-Hermann R: Induction of apoptosis in cultured hepatocytes and in regressing liver by transforming growth factor beta 1. *Proc Natl Acad Sci USA* 89: 5408-5412, 1992.
- 70 Schwall RH, Robbins K, Jardieu P, Chang L, Lai C and Terrell TG: Activin induces cell death in hepatocytes *in vivo* and *in vitro*. *Hepatology* 18: 347-356, 1993.
- 71 Yasuda H, Mine T, Shibata H, Eto Y, Hasegawa Y, Takeuchi T, Asano S and Kojima I: Activin A: An autocrine inhibitor of initiation of DNA synthesis in rat hepatocytes. *J Clin Invest* 92: 1491-1496, 1993.
- 72 Hully JR, Chang L, Schwall RH, Widmer HR, Terrell TG and Gillett NA: Induction of apoptosis in the murine liver with recombinant human activin a. *Hepatology* 20: 854-862, 1994.
- 73 Kogure K, Omata W, Kanzaki M, Zhang YQ, Yasuda H, Mine T and Kojima I: A single intraportal administration of follistatin accelerates liver regeneration in partially hepatectomized rats. *Gastroenterology* 108: 1136-1142, 1995.
- 74 Kogure K, Zhang YQ, Kanzaki M, Omata W, Mine T and Kojima I: Intravenous administration of follistatin: Delivery to the liver and effect on liver regeneration after partial hepatectomy. *Hepatology* 24: 361-366, 1996.
- 75 Kogure K, Zhang YQ, Shibata H and Kojima I: Immediate onset of DNA synthesis in remnant rat liver after 90% hepatectomy by an administration of follistatin. *J Hepatol* 29: 977-984, 1998.

- 76 Kogure K, Zhang YQ, Maeshima A, Suzuki K, Kuwano H and Kojima I: The role of activin and transforming growth factor-beta in the regulation of organ mass in the rat liver. *Hepatology* 31: 916-921, 2000.
- 77 Rossmannith W, Chabicovsky M, Grasl-Kraupp B, Peter B, Schausberger E and Schulte-Hermann R: Follistatin overexpression in rodent liver tumors: A possible mechanism to overcome activin growth control. *Mol Carcinog* 35: 1-5, 2002.
- 78 van der Poel HG, Hanrahan C, Zhong H and Simons JW: Rapamycin induces smad activity in prostate cancer cell lines. *Urol Res* 30: 380-386, 2003.
- 79 Handayani R, Rice L, Cui Y, Medrano TA, Samedi VG, Baker HV, Szabo NJ and Shiverick KT: Soy isoflavones alter expression of genes associated with cancer progression, including interleukin-8, in androgen-independent PC-3 human prostate cancer cells. *J Nutr* 136: 75-82, 2006.
- 80 Ajiboye S, Sissung TM, Sharifi N and Figg WD: More than an accessory: Implications of type III transforming growth factor-beta receptor loss in prostate cancer. *BJU Int* 105: 913-916, 2010.
- 81 Harkonen P, Torn S, Kurkela R, Porvari K, Pulkka A, Lindfors A, Isomaa V and Vihko P: Sex hormone metabolism in prostate cancer cells during transition to an androgen-independent state. *J Clin Endocrinol Metab* 88: 705-712, 2003.
- 82 Shikone T, Matzuk MM, Perlas E, Finegold MJ, Lewis KA, Vale W, Bradley A and Hsueh AJ: Characterization of gonadal sex cord-stromal tumor cell lines from inhibin-alpha and p53-deficient mice: The role of activin as an autocrine growth factor. *Mol Endocrinol* 8: 983-995, 1994.
- 83 Di Simone N, Crowley WF Jr., Wang QF, Sluss PM and Schneyer AL: Characterization of inhibin/activin subunit, follistatin, and activin type II receptors in human ovarian cancer cell lines: A potential role in autocrine growth regulation. *Endocrinology* 137: 486-494, 1996.
- 84 Ogino H, Yano S, Kakiuchi S, Muguruma H, Ikuta K, Hanibuchi M, Uehara H, Tsuchida K, Sugino H and Sone S: Follistatin suppresses the production of experimental multiple-organ metastasis by small cell lung cancer cells in natural killer cell-depleted SCID mice. *Clin Cancer Res* 14: 660-667, 2008.
- 85 Kozian DH, Ziche M and Augustin HG: The activin-binding protein follistatin regulates autocrine endothelial cell activity and induces angiogenesis. *Lab Invest* 76: 267-276, 1997.
- 86 Fajardo M, Liu CJ and Egol K: Levels of expression for BMP-7 and several BMP antagonists may play an integral role in a fracture nonunion: A pilot study. *Clin Orthop Relat Res* 467: 3071-3078, 2009.
- 87 Abe Y, Abe T, Aida Y, Hara Y and Maeda K: Follistatin restricts bone morphogenetic protein (BMP)-2 action on the differentiation of osteoblasts in fetal rat mandibular cells. *J Bone Miner Res* 19: 1302-1307, 2004.
- 88 Dudaric L, Cvek SZ, Cvijanovic O, Santic V, Maric I, Crncevic-Orlic Z and Bobinac D: Expression of the BMP-2, -4 and -7 and their antagonists gremlin, chordin, noggin and follistatin during ectopic osteogenesis. *Coll Antropol* 37: 1291-1298, 2013.
- 89 Kawabata N, Kamiya N, Suzuki N, Matsumoto M and Takagi M: Changes in extracellular activin A:Follistatin ratio during differentiation of a mesenchymal progenitor cell line, ROB-C26 into osteoblasts and adipocytes. *Life Sci* 81: 8-18, 2007.
- 90 Eijken M, Swagemakers S, Koedam M, Steenbergen C, Derkx P, Uitterlinden AG, van der Spek PJ, Visser JA, de Jong FH, Pols HA and van Leeuwen JP: The activin A-follistatin system: Potent regulator of human extracellular matrix mineralization. *FASEB J* 21: 2949-2960, 2007.
- 91 Gajos-Michniewicz A, Piastowska AW, Russell JA and Ochedalski T: Follistatin as a potent regulator of bone metabolism. *Biomarkers* 15: 563-574, 2010.
- 92 Gajos-Michniewicz A, Pawlowska E, Ochedalski T and Piastowska-Ciesielska A: The influence of follistatin on mechanical properties of bone tissue in growing mice with overexpression of follistatin. *J Bone Miner Metab* 30: 426-433, 2012.
- 93 Matzuk MM, Kumar TR, Shou W, Coerver KA, Lau AL, Behringer RR and Finegold MJ: Transgenic models to study the roles of inhibins and activins in reproduction, oncogenesis, and development. *Recent Prog Horm Res* 51: 123-154; discussion 155-127, 1996.
- 94 Shimonaka M, Inouye S, Shimasaki S and Ling N: Follistatin binds to both activin and inhibin through the common subunit. *Endocrinology* 128: 3313-3315, 1991.
- 95 Shimasaki S, Koga M, Buscaglia ML, Simmons DM, Bicsak TA and Ling N: Follistatin gene expression in the ovary and extragonadal tissues. *Mol Endocrinol* 3: 651-659, 1989.
- 96 Albano RM, Arkell R, Beddington RS and Smith JC: Expression of inhibin subunits and follistatin during postimplantation mouse development: Decidual expression of activin and expression of follistatin in primitive streak, somites and hindbrain. *Development* 120: 803-813, 1994.
- 97 Michel U, Rao A and Findlay JK: Rat follistatin: Ontogeny of steady-state mrna levels in different tissues predicts organ-specific functions. *Biochem Biophys Res Commun* 180: 223-230, 1991.
- 98 Gold E and Risbridger G: Activins and activin antagonists in the prostate and prostate cancer. *Mol Cell Endocrinol* 359: 107-112, 2012.
- 99 Thomas TZ, Chapman SM, Hong W, Gurusinghe C, Mellor SL, Fletcher R, Pedersen J and Risbridger GP: Inhibins, activins and follistatins: expression of mRNAs and cellular localization in tissues from men with benign prostatic hyperplasia. *Prostate* 34: 34-43, 1998.
- 100 Vaarala MH, Porvari K, Kyllonen A and Vihko P: Differentially expressed genes in two LNCaP prostate cancer cell lines reflecting changes during prostate cancer progression. *Lab Invest* 80: 1259-1268, 2000.
- 101 Buijs JT, Petersen M, van der Horst G and van der Pluijm G: Bone morphogenetic proteins and its receptors; therapeutic targets in cancer progression and bone metastasis? *Curr Pharm Des* 16: 1291-1300, 2010.
- 102 Simon DP, Vadakkadath Meethal S, Wilson AC, Gallego MJ, Weinecke SL, Bruce E, Lyons PF, Haasl RJ, Bowen RL and Atwood CS: Activin receptor signaling regulates prostatic epithelial cell adhesion and viability. *Neoplasia* 11: 365-376, 2009.
- 103 Kang Y, Siegel PM, Shu W, Drobnjak M, Kakonen SM, Cordon-Cardo C, Guise TA and Massague J: A multigenic program mediating breast cancer metastasis to bone. *Cancer Cell* 3: 537-549, 2003.
- 104 Sardana G, Jung K, Stephan C and Diamandis EP: Proteomic analysis of conditioned media from the PC3, LNCaP and 22Rv1 prostate cancer cell lines: Discovery and validation of candidate prostate cancer biomarkers. *J Proteome Res* 7: 3329-3338, 2008.

- 105 Ren P, Chen FF, Liu HY, Cui XL, Sun Y, Guan JL, Liu ZH, Liu JG and Wang YN: High serum levels of follistatin in patients with ovarian cancer. *J Int Med Res* 40: 877-886, 2012.
- 106 Tomoda T, Nouse K, Miyahara K, Kobayashi S, Kinugasa H, Toyosawa J, Hagihara H, Kuwaki K, Onishi H, Nakamura S, Ikeda F, Miyake Y, Shiraha H, Takaki A and Yamamoto K: Prognostic impact of serum follistatin in patients with hepatocellular carcinoma. *J Gastroenterol Hepatol* 28: 1391-1396, 2013.
- 107 Szasz AM, Lanczky A, Nagy A, Forster S, Hark K, Green JE, Boussioutas A, Busuttill R, Szabo A and Gyorffy B: Cross-validation of survival associated biomarkers in gastric cancer using transcriptomic data of 1,065 patients. *Oncotarget*, 2016.
- 108 Blank S, Deck C, Dreikhausen L, Weichert W, Giese N, Falk C, Schmidt T and Ott K: Angiogenic and growth factors in gastric cancer. *J Surg Res* 194: 420-429, 2015.
- 109 Grusch M, Drucker C, Peter-Vorosmarty B, Erlach N, Lackner A, Losert A, Macheiner D, Schneider WJ, Hermann M, Groome NP, Parzefall W, Berger W, Grasl-Kraupp B and Schulte-Hermann R: Deregulation of the activin/follistatin system in hepatocarcinogenesis. *J Hepatol* 45: 673-680, 2006.
- 110 Tumminello FM, Badalamenti G, Fulfaro F, Incorvaia L, Crescimanno M, Flandina C, Sepporta MV and Leto G: Serum follistatin in patients with prostate cancer metastatic to the bone. *Clin Exp Metastasis* 27: 549-555, 2010.
- 111 Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E and Zlotnik A: Involvement of chemokine receptors in breast cancer metastasis. *Nature* 410: 50-56, 2001.
- 112 Kim H, Eun JW, Lee H, Nam SW, Rhee H, Koh KH and Kim H: Gene expression changes in patient-matched gastric normal mucosa, adenomas, and carcinomas. *Exp Mol Pathol* 90: 201-209, 2011.

Received September 5, 2016

Revised October 1, 2016

Accepted October 5, 2016