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Review

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The emerging role of follistatin under stresses and its implications in diseases

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ARTICLE INFO

ABSTRACT

Follistatin (FST), a single-chain glycosylated protein, is expressed in various tissues. The essential biological function of FST is binding and neutralizing transforming growth factor β (TGF-β) superfamily, including activin, myostatin, and bone morphogenetic protein (BMP). Emerging evidence indicates that FST also serves as a stress responsive protein, which plays a protective role under a variety of stresses. In most cases, FST performs the protective function through its neutralization of TGF-β superfamily. However, under certain circumstances, FST translocates into the nucleus to maintain cellular homeostasis independent of its extracellular antagonism activity. This review provides integrated insight into the most recent advances in understanding the role of FST under various stresses, and the clinical implications corresponding to these findings and discusses the mechanisms to be further studied.

1. The biological function of FST

Follistatin (FST) was first isolated from ovarian follicular fluid in 1987 as a specific inhibitor of follicle stimulating hormone (FSH) by Robertson and Ueno (Robertson et al., 1987; Ueno et al., 1987). FST gene has been localized on human chromosome 5 encoded by a relatively small region of ~6 kb. FST gene contains six exons separated by five introns, with an alternative splicing site where two major FST isoforms were produced, FST288, and FST315. A third isoform, FST303, appears to be derived from proteolytic cleavage of FST315. All the three FST isoforms embrace a residue N-terminal domain and three 10-cysteine FST domains (FDs), termed FSD1, FSD2 and FSD3 (Phillips and de Kretser, 1998; Keutmann et al., 2004).

As a multifunctional regulatory protein, the primary function of FST is the antagonism effect on transforming growth factor β (TGF-β) superfamily, including activin, myostatin, and bone morphogenetic protein (BMP). As secreted factors, TGF-β family members stimulate signal transduction through type I and II receptors. The canonical activin signaling pathway involves an activin dimer binding to type I and type II receptors, which ultimately leads to phosphorylation of the type I receptor. This receptor then phosphorylates the second messenger molecules Smad2 and Smad3 that, once activated, complex with a common Smad4. This complex then translocates to the nucleus where it activates gene transcription (Xia and Schneyer, 2009). Myostatin receptors also use Smad2/3 for its signaling transduction (Rodriguez et al., 2014). However, the activation of BMP receptor phosphorylates Smad1/5/8, which mediates Smad4 recruitment and nuclear translocation (McDowall et al., 2008). Mutation studies have shown that the N-terminal domain and the first two FDs are found to be critical for activin binding (Keutmann et al., 2004). The structure of the FST:activin complex revealed that two FST molecules encircle activin, covering a large percentage of the surface and completely blocking both type I and type II receptor binding sites (Thompson et al., 2005). Through neutralization of TGF-β superfamily, FST participates in various processes such as cell growth, development, and differentiation.

2. The role of FST under stresses

All living organisms undergo various ranges of environmental stresses, including oxidative stress, nutritional deficiency, hypoxia and so on. In response to stressors, cells conduct a multitude of molecular changes to protect cells against unfavorable environmental conditions, called cellular stress response. During cellular stress response, a series of stress responsive proteins are selectively expressed, which play essential roles in promoting the survival of cells. Evidence has shown that the expression of FST is up-regulated during a series of stresses

including oxidative stress and glucose deprivation. FST plays a protective role under most of these stresses, indicating that FST is a stress responsive protein. Based on the pivotal role of this protein, it is not surprising that dysregulation of FST was observed in the development of diseases.

2.1. The role of FST under oxidative stress

Oxidative stress is the consequence of an imbalance between production and elimination of reactive oxygenated species (ROS) which can induce oxidative injury, leading to cell damage subsequently. Oxidative stress is closely related with diseases such as preeclampsia and aging-related diseases (Lopez-Otin et al., 2013; Guerby et al., 2015). Studies have shown that FST reduces ROS production, and dysregulation of FST might induce oxidative stress and promote disease progression.

The case-control study of preeclampsia showed that the level of serum activin in women with preeclampsia is significantly higher than healthy controls (Yu et al., 2011). Inversely, FST serum level in women with preeclampsia is dramatically lower than healthy women (Garces et al., 2015). In cultured human umbilical vein endothelial cells, activin induces ROS production through activating Smad2/3 to increase NADPH oxidase 2 (NOX2) activities, which disturbs endothelial integrity in turn. However, FST could down-regulate NOX2 protein levels to attenuate the production of ROS, which protects endothelial integrity. These studies implicate that activin promotes ROS production and the pathogenesis of preeclampsia, while FST inhibits this process by neutralizing activin (Lim et al., 2015). Taking advantage of its secreting property, FST offers an opportunity for preeclampsia treatment.

Studies have reported an increase of ROS in skeletal muscle during aging (sarcopenia). The elevated ROS promotes cellular protein degradation through ubiquitin-proteasome pathway, which leads to loss of mass in the skeletal muscle (Powers et al., 2005). Myostatin, a negative regulator of muscle growth, is induced in aged skeletal muscle in humans and rodents (Baumann et al., 2003; Raue et al., 2006; Leger et al., 2008). However, FST expression is significantly decreased during aging. On the contrary, aerobic exercise training could up-regulate FST in aged skeletal muscle of male rats, which blocks the activity of myostatin to promote myogenesis (Ziaaldini et al., 2015). In cultured C2C12 myoblasts, myostatin induces the generation of ROS, which is mediated by TNF-α and NADPH oxidase. Higher levels of ROS further triggers muscle wasting (Sriram et al., 2011). This study indicated that myostatin is a pro-oxidant and signals the generation of ROS in muscle cells. As an inhibitor of myostatin, FST might inhibit ROS production and lead to reduced muscle wasting during sarcopenia.

Series of exogenous toxins such as nanoparticles, heavy metal, and H_2O_2 could induce cellular oxidative stress and cell damage (Lin et al., 2016). Recently, we have demonstrated that under silica nanoparticle-induced oxidative stress, FST transcription is induced. Down-regulation of FST promotes silica nanoparticle-induced apoptosis both in cultured cells and in mouse lung tissue. Furthermore, knockdown of FST increases while overexpression of FST decreases the expression level of NADPH oxidase 1 (NOX1) and NOX5 as well as the production of cellular ROS (Lin et al., 2016). These findings further consolidated the protective role of FST during oxidative stresses.

In contrast to the damaging effects of ROS, evidence has shown that ROS at lower, nontoxic levels is important for normal physiological process such as stem cell differentiation (Jang and Sharkis, 2007). In vitro and in vivo studies have shown that bone morphogenetic protein (BMP) activation triggers production of ROS and Nrf2 (nuclear factor erythroid 2-related factor 2) activation, which is required for esophageal basal cell differentiation. BMP antagonist FST inhibits BMP4-induced ROS production and basal cell differentiation. Not surprisingly, increased levels of FST and decreased BMP activation were observed in eosinophilic esophagitis (EoE) mouse model and human biopsies. The up-regulated FST neutralizes BMP and reduces Nrf2 signaling, leading to expansion of basal progenitor during the pathogenesis of EoE (Jiang et al., 2015).

2.2. The role of FST under energy deficiency

Solid tumors over a certain size are continuously exposed to glucose deficiency and hypoxia microenvironments because of the inadequate vascular supply, which will result in cellular energy deficiency. The cancer cells will limit energy expenditure to antagonize these energy-deprived conditions. An effective way is to reduce ribosome biogenesis, the most energy-consuming process in eukaryotic cells. As a matter of fact, cellular rRNA transcription is tightly regulated in response to cellular energy conditions (Moss et al., 2007). Our group has reported that glucose deprivation markedly enhances the expression and nucleolar localization of FST in HeLa cells. The nucleolar localization of FST relies on its nuclear localization signal (NLS) comprising the residues 64–87. FST in the nucleolus negatively regulates rRNA synthesis and ribosome biogenesis to keep cellular energy homeostasis. These functions depend on the presence of an intact NLS because the NLS-deleted mutant of FST lost the rRNA inhibition effect and the cell protective effect (Gao et al., 2010). In support of our findings, nuclear localization of FST was observed in epithelial cells of breast tissue, suggesting a nuclear function of FST (Bloise et al., 2009). Overall, our study identified a novel nucleolar function of FST, which is of importance in the modulation of cancer cell survival in response to glucose deprivation (Fig. 1).

Consistent with our in vitro study, mouse and human studies have also demonstrated that circulating FST level responds to energy metabolic challenges. In human physiology, plasma FST increases in conditions with increased energy demands such as prolonged fasting and acute exercise (Hansen and Plomgaard, 2016; Hansen et al., 2016). Hansen et al. (2011) has also reported that exercise increases mouse plasma FST level and it most likely originated from the liver. It is proposed that these conditions lead to increased glucagon level in combination with reduced insulin level. The increased glucagon-to-insulin ratio stimulates the liver to secrete FST into the circulation (Hansen and Plomgaard, 2016; Hansen et al., 2016).

Ischemic diseases, such as acute myocardial infarction, are also accompanied with acute glucose deprivation and hypoxia. It has been demonstrated that FST administration attenuates the myocardial, renal and hepatic ischemia-reperfusion injury (IRI) (Maeshima et al., 2001; Kanamoto et al., 2011; Chen et al., 2014). FST treatment, through binding and neutralizing the actions of activin B and subsequently activin A, reduced renal IRI by minimizing endothelial cell activation and dampening the systemic inflammatory response (Fang et al., 2016). Although the detailed mechanism is unknown, these studies implicate a protective function of FST in ischemia-reperfusion and may serve as a potential therapeutic target for these diseases.

2.3. The role of FST under other stresses

FST also responds to physical stresses, such as ionizing radiation (IR), and shear stress. IR, usually used for cancer treatment, could result in marked fibrotic response in a normal tissue of patients (Forrester et al., 2013). It was reported that the expressions of both activin A and FST are increased in fibroblasts originating from patients who developed severe radiation-induced fibrosis following radiotherapy and fibroblasts from patients who did not. However, the FST gene expression level in fibrosis patients is lower than controls, suggesting that FST might play a protective role in IR-induced fibrosis (Forrester et al., 2013).

Endothelial cells (ECs) in branched or curved arterial regions are exposed to disturbed flow conditions such as low mean and oscillatory shear stress (OS), which induce inflammation and atherosclerosis. It has been elucidated that OS could induce production of BMP4 in ECs, which increases monocyte adhesivity of ECs, a critical early atherogenic step.
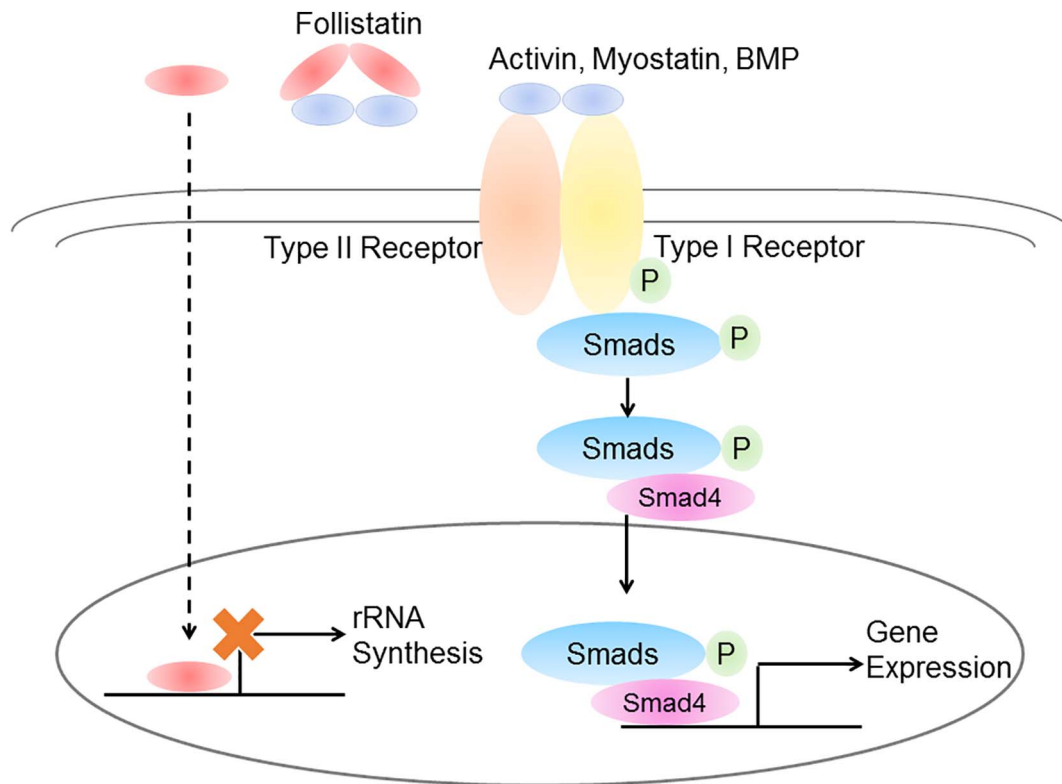
Fig. 1. The mechanisms of action of FST.
TGF-β family members, including activin, myostatin, and BMP, bind to type I and type II receptors and lead to type I receptor phosphorylation. This receptor then phosphorylates the second messenger molecule Smads, which complexes with a common Smad4. This complex then translocates to the nucleus where it activates gene transcription. Extracellular FST binds to TGF-β superfamily members and neutralizes their effects. On the other hand, FST itself can translocate into the nucleus where it down-regulates rRNA synthesis and ribosome biogenesis to maintain cellular energy homeostasis.
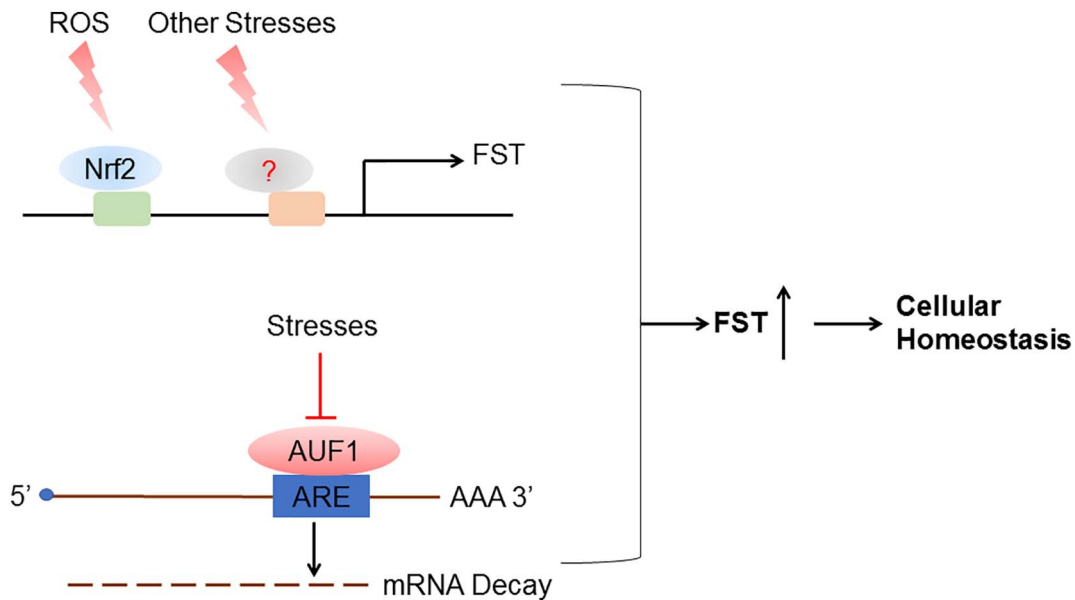

Fig. 2. The regulation of FST expression during stresses.
FST expression can be regulated at either transcriptional level or post-transcriptional level. FST promoter region contains a series of transcription factor binding sites. Stresses trigger the binding of a transcription factor such as Nrf2 to the FST promoter, and thereby activate FST transcription. On the other hand, FST mRNA can be degraded through the destabilizing element in its 3′UTR mediated by RNA-binding protein AUF1. Stresses such as glucose deprivation inhibit AUF1-promoted FST mRNA decay and thereby up-regulates FST expression.

OS also up-regulates FST expression, while the elevated FST acts as the antagonist of BMP4, as administration of FST could reduce monocyte adhesion. Co-expression of FST with BMP4 was detected in disturbed flow regions of mouse aortas and the atheromatous lesions in human coronary arterial endothelium. Hence, the expression of BMP4 and FST may be used as novel biomarkers of inflammation and atherosclerosis (Chang et al., 2007). However, the interplay between FST and BMP4 in OS remains largely unknown.

Activins can regulate either pro- or anti-inflammatory responses and play important roles in controlling the cytokine cascade. Production of

Table 1
The role of follistatin under various stresses and in diseases.

Stress	Disease	FST level	FST function	Reference
Oxidative stress	Preeclampsia	↓	Inhibition of the pathogenesis of preeclampsia	Garces et al. (2015); Lim et al. (2015)
	Sarcopenia	↓	Promotion of myogenesis	Ziaaldini et al. (2015); Sriram et al. (2011)
	SiO$_2$ NP exposure	↑	Protection of cells from apoptosis	Lin et al. (2016)
	Eosinophilic esophagitis (EoE)	↑	Inhibition of BMP4-induced ROS production and esophageal basal cell differentiation	Jiang et al. (2015)
Energy deficiency	Cancer	↑	Negatively regulation on rRNA synthesis and ribosome biogenesis to keep cellular energy homeostasis	Gao et al. (2010); Bloise et al. (2009)
	Ischemia-reperfusion injury (IRI):			
	Myocardium	Tissue ↑ serum ↓	Neutralization of activin, reduction of IRI	Chen et al. (2014)
	Kidney	↓	Neutralization of activin, reduction of IRI	Maeshima et al. (2001); Fang et al. (2016)
	Liver	–	Reduction of liver damage, with a resultant improvement of survival	Kanamoto et al. (2011)
Ionizing radiation (IR)	Fibrosis	↑	Blockage of activin action, attenuation fibrosis	Forrester et al. (2013)
Shear stress	Inflammation and atherosclerosis	↑	Reduction of monocyte adhesion	Chang et al. (2007)

Table 2
Therapeutic potentials of follistatin.

Disease	Reference
Muscle disease	
Sarcopenia	Dasarathy et al. (2011)
Duchenne muscular dystrophy (DMD)	Rodino-Klapac et al. (2013)
Declining muscle regeneration induced by Type 1 diabetes	Jeong et al. (2013)
Inflammatory-immune diseases	
Asthma	Hardy et al. (2006); Hardy et al. (2013)
Endotoxemia	Jones et al. (2007)
Cystic fibrosis-like lung inflammation	Hardy et al. (2015)
Cigarette smoke induced inflammation	Verhamme et al. (2014)
Experimental autoimmune orchitis	Nicolas et al. (2017)
Ischemia-reperfusion injury	
Myocardial ischemia-reperfusion injury	Chen et al. (2014)
Renal ischemia-reperfusion injury	Maeshima et al. (2001); Fang et al. (2016)
Hepatic ischemia-reperfusion injury	Kanamoto et al. (2011)

activin A during inflammatory responses stimulates fibrosis and tissue remodeling. FST can neutralize all of the actions of the activins, and can therefore modulate the inflammatory process and the resolution of ensuing tissue damage. The action of FST in inflammation and tissue repair is discussed in details by de Kretser et al. (2012).

3. The regulation of FST expression

A series of transcription factors have been reported to regulate FST transcription. Sequence analysis of the mouse FST promoter revealed several consensus binding sites for transcription factors including AP-1, Brachyury-T, CREB, Sp1, AP-2 and Tcf (de Groot et al., 2000). Many of them have been confirmed. For example, peroxisome proliferator-activated receptor γ down-regulates FST in intestinal epithelial cells through Sp1 (Necela et al., 2008). GnRH increases cAMP level in gonadotroph cells, which activates FST transcription through CREB factor (Winters et al., 2007). Activation of the WNT/β-catenin pathway in skeletal cells induces FST transcription through Tcf/LEF1 binding sites (Han et al., 2014). We have recently shown the induction of FST transcription by silica nanoparticle treatment. By analyzing the FST gene proximal promoter, we identified a putative antioxidant response element, which is a consensus binding sequence for the transcription factor Nrf2. Studies showed decreased FST expression in Nrf2-deficient

cells and the direct binding of Nrf2 to FST promoter region, indicating that Nrf2 mediates the transcription of FST (Lin et al., 2016).

The degradation of FST mRNA is also tightly regulated. We have shown that the induction of FST by glucose deprivation is due to an increase in the half-life of its mRNA. It is known that AU-rich element (ARE) sequences, which are present in the 3′ untranslated region (3′UTR) of certain mRNAs, function post-transcriptionally to regulate the mRNA stability (Chen and Shyu, 1995; Barreau et al., 2005). By analyzing the two transcripts of FST mRNA, we found that FST344 has a shorter 3′UTR, which is also a part of the longer 3′UTR of FST317. Further analysis showed that this shared region is AU-rich with 33% of adenine and 34% of uracil. Using the luciferase reporter assays, we identified an ARE (AUUUA) in this shared region that destabilizes FST mRNA. Our data further demonstrated that under normal condition, AUF1 (AU-rich element RNA-binding protein 1) bound to the ARE and promoted FST mRNA decay. During glucose deprivation, a majority of AUF1 shuttled from cytoplasm to nucleus, resulting in dissociation of AUF1 from FST mRNA and thus stabilization of FST mRNA (Gao et al., 2014).

In summary, FST expression can be regulated at either transcriptional level or post-transcriptional level. This might be due to the different signaling activated. Glucose deprivation mainly activates AMPK pathway, while AMPK activation mediates FST induction during glucose deprivation (Gao et al., 2014). On the contrary, silica nanoparticles mainly induce ROS generation and oxidative stress. Silica nanoparticle-induced ROS production activates transcription factor Nrf2, which induces FST transcription.

4. Perspectives

4.1. Elucidating the mechanisms of action of FST

Most of FST's effects are attributed to its TGF-β family neutralizing activity. However, we and others have reported nuclear localization of FST, indicating that this protein might play a role in the nucleus besides its extracellular function. However, a lot of questions still need to be addressed. For example, how is FST translocated to the nucleus? Is it through endocytosis or another mechanism? What proteins mediate FST nuclear translocation? Since protein-protein interactions are critical in every biological process, FST-interacting proteins should mediate or modulate a series of its biological activities. Therefore, identifying these mediators or modulators will help us elucidate new mechanisms of action of FST.

4.2. Clarifying the regulation of FST expression during stresses and diseases

FST expression is up-regulated in most of the stresses described above. We have demonstrated that its expression can be regulated by different mechanisms depending on the signaling pathways induced by certain stresses (Fig. 2). For example, energy deficiency mainly activates AMPK pathway, while AMPK activation promotes AUF1 nuclear translocation and mediates FST mRNA stabilization (Gao et al., 2014). However, oxidative stress induces FST transcription through the elevated ROS (Lin et al., 2016). Besides, WNT, MAPK, PKC, and PKA pathways are also involved in FST regulation (Winters et al., 2007; Mutiara et al., 2009; Han et al., 2014). Moreover, there are extensive cross-talks among these pathways, implying that an elaborate mechanism exists to precisely control the expression of FST. Therefore, the signaling pathway involved in FST expression regulation under specific stress needs to be elucidated. Besides, the specific transcription factors downstream of the signaling pathway mediating FST transcription should also be considered.

4.3. Therapeutic potentials of FST

FST is deregulated in a series of diseases, which might aggravate cellular stress and promote disease progression (Table 1). Animal studies have demonstrated the therapeutic potential of this protein (Table 2). FST administration has been proven to improve the symptoms of muscle diseases such as sarcopenia (Dasarathy et al., 2011), Duchenne muscular dystrophy (DMD) (Rodino-Klapac et al., 2013), and declining muscle regeneration induced by type 1 diabetes (Jeong et al., 2013). FST has also been used experimentally in the treatment of inflammatory diseases such as asthma (Hardy et al., 2006; Hardy et al., 2013), endotoxemia (Jones et al., 2007), cystic fibrosis-like lung inflammation (Hardy et al., 2015), cigarette smoke induced inflammation (Verhamme et al., 2014), and experimental autoimmune orchitis (Nicolas et al., 2017). Furthermore, FST treatment could attenuate the myocardial, renal and hepatic ischemia-reperfusion injury (IRI) (Maeshima et al., 2001; Kanamoto et al., 2011; Chen et al., 2014; Fang et al., 2016). These findings warrant further investigations in order to better assess the therapeutic role of FST in various diseases.

In summary, FST plays important roles in many stresses which are closely related with disease development. This protein is a secreting peptide, making it an ideal target for disease treatment. An ideal FST-oriented therapy will become possible after fully elucidating the mechanism of action of this protein in a disease-specific process.

Transparency Document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

References

Barreau, C., Paillard, L., Osborne, H.B., 2005. AU-rich elements and associated factors: are there unifying principles? Nucleic Acids Res. 33, 7138–7150.

Baumann, A.P., Ibebunjo, C., Grasser, W.A., Paralkar, V.M., 2003. Myostatin expression in age and denervation-induced skeletal muscle atrophy. J. Musculoskelet. Neuronal Interact. 3, 8–16.

Bloise, E., Couto, H.L., Massai, L., Ciarmela, P., Mencarelli, M., Borges, L.E., Muscettola, M., Grasso, G., Amaral, V.F., Cassali, G.D., Petraglia, F., Reis, F.M., 2009. Differential expression of follistatin and FLRG in human breast proliferative disorders. BMC Cancer 9, 320.

Chang, K., Weiss, D., Suo, J., Vega, J.D., Giddens, D., Taylor, W.R., Jo, H., 2007. Bone morphogenic protein antagonists are coexpressed with bone morphogenic protein 4 in endothelial cells exposed to unstable flow in vitro in mouse aortas and in human coronary arteries: role of bone morphogenic protein antagonists in inflammation and atherosclerosis. Circulation 116, 1258–1266.

Chen, C.Y., Shyu, A.B., 1995. AU-rich elements: characterization and importance in mRNA degradation. Trends Biochem. Sci. 20, 465–470.

Chen, Y., Rothnie, C., Spring, D., Verrier, E., Venardos, K., Kaye, D., Phillips, D.J., Hedger, M.P., Smith, J.A., 2014. Regulation and actions of activin A and follistatin in myocardial ischaemia-reperfusion injury. Cytokine 69, 255–262.

Dasarathy, S., McCullough, A.J., Muc, S., Schneyer, A., Bennett, C.D., Dodig, M., Kalhan, S.C., 2011. Sarcopenia associated with portosystemic shunting is reversed by follistatin. J. Hepatol. 54, 915–921.

Fang, D.Y., Lu, B., Hayward, S., de Kretser, D.M., Cowan, P.J., Dwyer, K.M., 2016. The role of activin A and B and the benefit of follistatin treatment in renal ischemia-reperfusion injury in mice. Transplant. Direct 2, e87.

Forrester, H.B., Ivashkevich, A., McKay, M.J., Leong, T., de Kretser, D.M., Sprung, C.N., 2013. Follistatin is induced by ionizing radiation and potentially predictive of radiosensitivity in radiation-induced fibrosis patient derived fibroblasts. PLoS One 8, e77119.

Gao, X., Wei, S., Lai, K., Sheng, J., Su, J., Zhu, J., Dong, H., Hu, H., Xu, Z., 2010. Nucleolar follistatin promotes cancer cell survival under glucose-deprived conditions through inhibiting cellular rRNA synthesis. J. Biol. Chem. 285, 36857–36864.

Gao, X., Dong, H., Lin, C., Sheng, J., Zhang, F., Su, J., Xu, Z., 2014. Reduction of AUF1-mediated follistatin mRNA decay during glucose starvation protects cells from apoptosis. Nucleic Acids Res. 42, 10720–10730.

Garces, M.F., Vallejo, S.A., Sanchez, E., Palomino-Palomino, M.A., Leal, L.G., Angel-Muller, E., Diaz-Cruz, L.A., Ruiz-Parra, A.I., Gonzalez-Clavijo, A.M., Castano, J.P., Abba, M., Lacunza, E., Dieguez, C., Nogueiras, R., Caminos, J.E., 2015. Longitudinal analysis of maternal serum Follistatin concentration in normal pregnancy and pre-eclampsia. Clin. Endocrinol. 83, 229–235.

de Groot, E., Veltmaat, J., Caricasole, A., Defize, L., van den Eijnden-van Raaij, A., 2000. Cloning and analysis of the mouse follistatin promoter. Mol. Biol. Rep. 27, 129–139.

Guerby, P., Vidal, F., Garoby-Salom, S., Vayssiere, C., Salvayre, R., Parant, O., Negre-Salvayre, A., 2015. Oxidative stress and preeclampsia: a review. Gynecol. Obstet. Fertil. 43, 751–756.

Han, X.H., Jin, Y.R., Tan, L., Kosciuk, T., Lee, J.S., Yoon, J.K., 2014. Regulation of the follistatin gene by RSPO-LGR4 signaling via activation of the WNT/beta-catenin pathway in skeletal myogenesis. Mol. Cell. Biol. 34, 752–764.

Hansen, J.S., Plomgaard, P., 2016. Circulating follistatin in relation to energy metabolism. Mol. Cell. Endocrinol. 433, 87–93.

Hansen, J., Brandt, C., Nielsen, A.R., Hojman, P., Whitham, M., Febbraio, M.A., Pedersen, B.K., Plomgaard, P., 2011. Exercise induces a marked increase in plasma follistatin: evidence that follistatin is a contraction-induced hepatokine. Endocrinology 152, 164–171.

Hansen, J.S., Rutti, S., Arous, C., Clemmesen, J.O., Secher, N.H., Drescher, A., Gonelle-Gispert, C., Halban, P.A., Pedersen, B.K., Weigert, C., Bouzakri, K., Plomgaard, P., 2016. Circulating follistatin is liver-derived and regulated by the glucagon-to-insulin ratio. J. Clin. Endocrinol. Metab. 101, 550–560.

Hardy, C.L., O'Connor, A.E., Yao, J., Sebire, K., de Kretser, D.M., Rolland, J.M., Anderson, G.P., Phillips, D.J., O'Hehir, R.E., 2006. Follistatin is a candidate endogenous negative regulator of activin A in experimental allergic asthma. Clin. Exp. Allergy 36, 941–950.

Hardy, C.L., Nguyen, H.A., Mohamud, R., Yao, J., Oh, D.Y., Plebanski, M., Loveland, K.L., Harrison, C.A., Rolland, J.M., O'Hehir, R.E., 2013. The activin A antagonist follistatin inhibits asthmatic airway remodelling. Thorax 68, 9–18.

Hardy, C.L., King, S.J., Mifsud, N.A., Hedger, M.P., Phillips, D.J., Mackay, F., de Kretser, D.M., Wilson, J.W., Rolland, J.M., O'Hehir, R.E., 2015. The activin A antagonist follistatin inhibits cystic fibrosis-like lung inflammation and pathology. Immunol. Cell Biol. 93, 567–574.

Jang, Y.Y., Sharkis, S.J., 2007. A low level of reactive oxygen species selects for primitive hematopoietic stem cells that may reside in the low-oxygenic niche. Blood 110, 3056–3063.

Jeong, J., Conboy, M.J., Conboy, I.M., 2013. Pharmacological inhibition of myostatin/TGF-beta receptor/pSmad3 signaling rescues muscle regenerative responses in mouse model of type 1 diabetes. Acta Pharm. Sin. 34, 1052–1060.

Jiang, M., Ku, W.Y., Zhou, Z., Dellon, E.S., Falk, G.W., Nakagawa, H., Wang, M.L., Liu, K., Wang, J., Katzka, D.A., Peters, J.H., Lan, X., Que, J., 2015. BMP-driven NRF2 activation in esophageal basal cell differentiation and eosinophilic esophagitis. J. Clin. Invest. 125, 1557–1568.

Jones, K.L., Mansell, A., Patella, S., Scott, B.J., Hedger, M.P., de Kretser, D.M., Phillips, D.J., 2007. Activin A is a critical component of the inflammatory response, and its binding protein, follistatin, reduces mortality in endotoxemia. Proc. Natl. Acad. Sci. U. S. A. 104, 16239–16244.

Kanamoto, M., Shimada, M., Morine, Y., Yoshizumi, T., Imura, S., Ikegami, T., Mori, H., Arakawa, Y., 2011. Beneficial effects of follistatin in hepatic ischemia-reperfusion injuries in rats. Dig. Dis. Sci. 56, 1075–1081.

Keutmann, H.T., Schneyer, A.L., Sidis, Y., 2004. The role of follistatin domains in follistatin biological action. Mol. Endocrinol. 18, 228–240.

de Kretser, D.M., O'Hehir, R.E., Hardy, C.L., Hedger, M.P., 2012. The roles of activin A and its binding protein, follistatin, in inflammation and tissue repair. Mol. Cell. Endocrinol. 359, 101–106.

Leger, B., Derave, W., De Bock, K., Hespel, P., Russell, A.P., 2008. Human sarcopenia reveals an increase in SOCS-3 and myostatin and a reduced efficiency of Akt phosphorylation. Rejuvenation Res. 11, 163–175B.

Lim, R., Acharya, R., Delpachitra, P., Hobson, S., Sobey, C.G., Drummond, G.R., Wallace, E.M., 2015. Activin and NADPH-oxidase in preeclampsia: insights from in vitro and

murine studies. Am. J. Obstet. Gynecol. 212 (86), e1–12.

Lin, C., Zhao, X., Sun, D., Zhang, L., Fang, W., Zhu, T., Wang, Q., Liu, B., Wei, S., Chen, G., Xu, Z., Gao, X., 2016. Transcriptional activation of follistatin by Nrf2 protects pulmonary epithelial cells against silica nanoparticle-induced oxidative stress. Sci Rep 6, 21133.

Lopez-Otin, C., Blasco, M.A., Partridge, L., Serrano, M., Kroemer, G., 2013. The hallmarks of aging. Cell 153, 1194–1217.

Maeshima, A., Zhang, Y.Q., Nojima, Y., Naruse, T., Kojima, I., 2001. Involvement of the activin-follistatin system in tubular regeneration after renal ischemia in rats. J. Am. Soc. Nephrol. 12, 1685–1695.

McDowall, M., Edwards, N.M., Jahoda, C.A., Hynd, P.I., 2008. The role of activins and follistatins in skin and hair follicle development and function. Cytokine Growth Factor Rev. 19, 415–426.

Moss, T., Langlois, F., Gagnon-Kugler, T., Stefanovsky, V., 2007. A housekeeper with power of attorney: the rRNA genes in ribosome biogenesis. Cell. Mol. Life Sci. 64, 29–49.

Mutiara, S., Kanasaki, H., Oride, A., Purwana, I.N., Shimasaki, S., Yamamoto, H., Miyazaki, K., 2009. Follistatin gene expression by gonadotropin-releasing hormone: a role for cyclic AMP and mitogen-activated protein kinase signaling pathways in clonal gonadotroph LbetaT2 cells. Mol. Cell. Endocrinol. 307, 125–132.

Necela, B.M., Su, W., Thompson, E.A., 2008. Peroxisome proliferator-activated receptor gamma down-regulates follistatin in intestinal epithelial cells through SP1. J. Biol. Chem. 283, 29784–29794.

Nicolas, N., Muir, J.A., Hayward, S., Chen, J.L., Stanton, P.G., Gregorevic, P., de Kretser, D.M., Loveland, K.L., Bhushan, S., Meinhardt, A., Fijak, M., Hedger, M.P., 2017. Induction of experimental autoimmune orchitis in mice: responses to elevated circulating levels of the activin-binding protein, follistatin. Reproduction 154, 193–205.

Phillips, D.J., de Kretser, D.M., 1998. Follistatin: a multifunctional regulatory protein. Front. Neuroendocrinol. 19, 287–322.

Powers, S.K., Kavazis, A.N., DeRuisseau, K.C., 2005. Mechanisms of disuse muscle atrophy: role of oxidative stress. Am. J. Phys. Regul. Integr. Comp. Phys. 288, R337–44.

Raue, U., Slivka, D., Jemiolo, B., Hollon, C., Trappe, S., 2006. Myogenic gene expression at rest and after a bout of resistance exercise in young (18–30 yr) and old (80–89 yr) women. J. Appl. Physiol. (1985) 101, 53–59.

Robertson, D.M., Klein, R., de Vos, F.L., McLachlan, R.I., Wettenhall, R.E., Hearn, M.T.,

Burger, H.G., de Kretser, D.M., 1987. 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.

Rodino-Klapac, L.R., Janssen, P.M., Shontz, K.M., Canan, B., Montgomery, C.L., Griffin, D., Heller, K., Schmelzer, L., Handy, C., Clark, K.R., Sahenk, Z., Mendell, J.R., Kaspar, B.K., 2013. Micro-dystrophin and follistatin co-delivery restores muscle function in aged DMD model. Hum. Mol. Genet. 22, 4929–4937.

Rodriguez, J., Vernus, B., Chelh, I., Cassar-Malek, I., Gabillard, J.C., Hadj Sassi, A., Seiliez, I., Picard, B., Bonnieu, A., 2014. Myostatin and the skeletal muscle atrophy and hypertrophy signaling pathways. Cell. Mol. Life Sci. 71, 4361–4371.

Sriram, S., Subramanian, S., Sathiakumar, D., Venkatesh, R., Salerno, M.S., McFarlane, C.D., Kambadur, R., Sharma, M., 2011. Modulation of reactive oxygen species in skeletal muscle by myostatin is mediated through NF-kappaB. Aging Cell 10, 931–948.

Thompson, T.B., Lerch, T.F., Cook, R.W., Woodruff, T.K., Jardetzky, T.S., 2005. The structure of the follistatin:activin complex reveals antagonism of both type I and type II receptor binding. Dev. Cell 9, 535–543.

Ueno, N., Ling, N., Ying, S.Y., Esch, F., Shimasaki, S., Guillemin, R., 1987. 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. U. S. A. 84, 8282–8286.

Verhamme, F.M., Bracke, K.R., Amatngalim, G.D., Verleden, G.M., Van Pottelberge, G.R., Hiemstra, P.S., Joos, G.F., Brusselle, G.G., 2014. Role of activin-A in cigarette smoke-induced inflammation and COPD. Eur. Respir. J. 43, 1028–1041.

Winters, S.J., Ghooray, D., Fujii, Y., Moore Jr., J.P., Nevitt, J.R., Kakar, S.S., 2007. 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.

Xia, Y., Schneyer, A.L., 2009. The biology of activin: recent advances in structure, regulation and function. J. Endocrinol. 202, 1–12.

Yu, J., Shixia, C.Z., Wu, Y., Duan, T., 2011. Inhibin A, activin A, placental growth factor and uterine artery Doppler pulsatility index in the prediction of pre-eclampsia. Ultrasound Obstet. Gynecol. 37, 528–533.

Ziaaldini, M.M., Koltai, E., Csende, Z., Goto, S., Boldogh, I., Taylor, A.W., Radak, Z., 2015. Exercise training increases anabolic and attenuates catabolic and apoptotic processes in aged skeletal muscle of male rats. Exp. Gerontol. 67, 9–14.