On the connective tissue regulator Follistatin-like 1
Sylva, M.

Citation for published version (APA):
Sylva, M. (2014). On the connective tissue regulator Follistatin-like 1
Follistatin-like 1 in vertebrate development

M. Sylva, A.F.M. Moorman, M.J.B. van den Hoff

Abstract
Follistatin-like 1 (FSTL1) is a member of the secreted protein acidic rich in cysteins (SPARC) family and has been implicated in many different signaling pathways, including bone morphogenetic protein (BMP) signaling. In many different developmental processes like, dorso-ventral axis establishment, skeletal, lung and ureter development, loss of function experiments have unveiled an important role for FSTL1. FSTL1 largely functions through inhibiting interactions with the BMP signaling pathway, although, in various disease models, different signaling pathways, like activation of pAKT, pAMPK, Na/K-ATPase or innate immune responses, are linked to FSTL1. How FSTL1 inhibits BMP signaling remains unclear, although it is known that FSTL1 does not function through a scavenging mechanism, like the other known extracellular BMP inhibitors such as Noggin. It has been proposed that FSTL1 interferes with BMP receptor complex formation and as such inhibits propagation of the BMP signal into the cell. Future challenges will encompass the identification of the factors that determine the mechanisms that underlie the fact that FSTL1 acts by interfering with BMP signaling during development, but through other signaling pathways during disease.
Aim of the review

Intercellular communication is vital both in development and homeostasis of multicellular organisms. Follistatin-like 1 (FSTL1) is a signaling molecule that has been implicated in many different signaling pathways, including bone morphogenetic protein (BMP) signaling. Disruption of FSTL1 results in a variety of developmental defects. In the last 15 years more than 100 papers have been written on this gene. Although important progress has been made on the elucidation of its roles in development, FSTL1 remains to be an enigmatic molecule, witnessed by the fact that many studies have reported conflicting results. Here, we summarize the literature on FSTL1 and aim to provide a unifying view on its function in development, albeit many uncertainties still have to be resolved.

Fstl1 is a unique member of the SPARC family

In Human, FSTL1 is a secreted, 308 amino acid long protein, which is highly conserved among species (Figure 1). Although species-specific differences between mouse and human FSTL1 are described, 91% overlap exists between the amino acid sequences [106 Murakami, K (2012)]. FSTL1 is post-translationally modified by the addition of sugar residues and based on this modification of the protein, two isoforms are known [107 Hambrock, HO (2004)], albeit with no known functional differences between the isoforms. In vitro FSTL1 has a half-life of 3 hours [108 Wu, Y (2010)] and is a target for degradation of matrix metallo protease 2 (MMP2) [109 Dean, RA (2007)] (Table1).

Figure 1: Protein domains of Fstl1 and phylogenetic tree

A) Schematic representation of the protein domains of Fstl1. The numbers represent the amino acid numbers in the human Fstl1 protein. The signal (Sign) domain is required for the secretion of Fstl1. The follistatin domain (FS) is subdivided in a follistatin/osteonectin-like EGF domain (FOLN) and a Kazal domain (Kazal), because not all SPARC family members share the FOLN domain. The extracellular calcium binding (EC) domain contains a region involved in the Na/K-ATPase interaction, which is depicted in gray.
and three glycosylation sites marked by triangles. The von Willebrand C (VWC) domain is the most C terminal domain of Fstl1 and is not shared in other SPARC family members.

B) A phylogenetic tree of the human SPARC family including FSTL members -1, -4 and -5. The tree has been produced by comparing the EC domains of the different SPARC family members. Similar results were obtained by comparing the Kazal domains.

Will the real Fstl1 please stand up

Fstl1 was initially identified as TGFβ stimulated Clone-36 (TSC-36), similarities to the follistatin gene product were observed and it was therefore named follistatin-related polypeptide [110 Shibanuma, M (1993)]. After its identification in frog, rat, chicken, human and several other organisms, different aliases have been used, such as follistatin-related protein, (FRP) [111 Zwijsen, A (1994)], [112 Ohashi, T (1997)] or follistatin-like (Flik) [113 Amthor, H (1996)], [114 Patel, K (1996)]. In brains of macaques FSTL1 was identified as a gene expressed in the neurons of the visual neocortex and named OCC1 [115 Tochitani, S (2001)]. From a hematologic tumor line, a different gene was characterized, also bearing similarities to follistatin, which initially was termed follistatin-related gene (FLRG) and later follistatin-like 3 (FSTL3) [116 Hayette, S (1998)]. In zebrafish the fstl1 gene is duplicated resulting in a fstl1a and fstl1b gene [117 Esterberg, R (2008)], the latter was also called fstl2 [118 Dal-Pra, S (2006)]. Until recently, in mice the name Fstl2 was used for a completely different gene: Insulin-like growth factor binding protein 7, and thus is not a paralogue for zebrafish fstl2.

In subsequent publications all these different names have been used for FSTL1 and, more confusingly, some of the above-mentioned names have been used for both FSTL3 as well as FSTL1. Confusion is further added to the field because studies on FSTL3 have been incorrectly cited as if they dealt with FSTL1.

To identify all papers about FSTL1 we searched “pub med” using the following search terms: “Follistatin like”[All Fields] OR “Follistatin related”[All Fields] OR Fstl-1[All Fields] OR “Fstl 1” OR Fstl1[All Fields] OR OCC1[All Fields] OR OCC-1[All Fields] OR “OCC 1”[All Fields] OR TSC-36[All Fields] OR “TSC 36”[All Fields] OR TSC36[All Fields]. This resulted in 300 hits. In cases where it was unclear from the title or abstract whether FSTL1 or another protein, such as FSTL3, was discussed, primer sequences or other features of the study involved were used to identify the protein described. From those papers, 104 were dealing with FSTL1 and written in English (see Supplementary Table 1).

To which family does FSTL1 belong?

FSTL1 is a member of the secreted protein acidic rich in cysteins (SPARC) family, based on the presence of a follistatin domain and an extracellular calcium-binding domain, which binds calcium with its two EF-hand motifs. In the SPARC family trees described, however, FSTL1 is a unique member with no other proteins in its sub-branch [122 Bradshaw, AD (2012)], [209 Vannahme, C (2003)]. Moreover, its extracellular calcium-binding domain does not bind calcium, unlike the other members of the SPARC family [107 Hambrock, HO (2004)]. When FSTL1 is compared to the other follistatin-like proteins, FSTL1 shows more similarity to FSTL4 and -5 than to follistatin, or any other member of the SPARC family [138 Glusman, G (2004)]. FSTL4 and -5, in turn, are more similar to each other than to FSTL1 [119 Adams, D (2007)], both contain a 400 amino acids-long C-terminus not shared with FSTL1. The function of FSTL4 and -5 is not known. Interestingly, unlike FSTL3 and follistatin, both FSTL4 and FSTL5 have an extracellular calcium-binding domain in addition to their follistatin domain. Nonetheless, they are not considered to be a part of the SPARC family described in recent reviews [122 Bradshaw, AD (2012)], [123 Brekken, RA (2001)]. Based on the criteria of having both a follistatin and an extracellular calcium-binding domain, however, the SPARC family would encompass FSTL4 and -5, it would then consist of the following branches: I) SPARC and Hevin; II) SPARC related modular calcium binding (SMOC)1,-2; III) Testican1,-2,-3; IV) FSTL1,-4,-5 (see Figure 1 phylogenetic tree). However, since the calcium-binding properties of the FSTL1 extracellular calcium-binding domain seem lacking [107 Hambrock, HO (2004)] it can be disputed of whether the follistatin-like proteins share any functional similarities to the other members of the SPARC protein family.

Regulators of FSTL1

FSTL1 originally was identified as the gene up-regulated in response to transforming growth factor beta (TGFβ) stimulation of an osteoblastic cell line [110 Shibanuma, M (1993)]. Since then several factors have been shown to up-regulate FSTL1 such as interleukin 1, beta (IL1β), tumor necrosis factor alfa (TNFα), FBJ murine osteosarcoma viral oncogene homolog (FOS), extracellular signal-regulated kinases (ERK)1/2 or v-akt murine thymoma
viral oncogene homolog (AKT). Interestingly, some of these factors are also regulated by FSTL1 itself (Table1), suggestive of the presence of auto-regulatory feedback loops. In some cases the inducing action on FSTL1 can be attributed to induced TGFβ signaling. For instance, a state of induced TGFβ signaling was demonstrated upon transfection of cells with the oncogenic Adenovirus 12 [193 Vertegaal, AC (2000)]. Also estrogen-mediated induction of FSTL1 can be attributed to TGFβ signaling, as in the estrogen treated cells TGFβ was also reported to be upregulated [112 Ohashi, T (1997)]. DNA regulatory elements for FSTL1 have not been studied extensively and are limited to the in vitro characterization of the promoter of FSTL1 which was bound and inhibited by the transcription factor Kruppel-like factor 15 (KLF15) in a model of in vitro adipocyte differentiation [108 Wu, Y (2010)].

**FSTL1 in disease models**

In several human diseases FSTL1 is implicated. In serum of rheumatic arthritis patients antibodies against FSTL1 were found [186 Tanaka, M (1998)], [185 Tanaka, M (2003)]. Also FSTL1 itself is elevated in serum of rheumatic arthritis patients compared to control human serum [201 Wilson, DC (2010)], [148 Kim, HA (2012)], [153 Li, D (2011)], [195 Wang, Y (2011)]. The function of FSTL1 in immunologic conditions is not clear however, with opposing results of FSTL1 being either a pro [161 Miyamae, T (2006)], [124 Chaly, Y (2012)], [127 Clutter, SD (2009)], [106 Murakami, K (2012)] or anti-inflammatory [120 Adams, DC (2010)], [146 Kawabata, D (2004)], [151 Le Luduec, JB (2008)], [185 Tanaka, M (2003)] molecule. It has been shown to interact with CD14 and toll-like receptor 4 (TLR4) (Figure 2) [184 Tanaka, M (2010)], [106 Murakami, K (2012)] and it has been suggested that a dual role in inflammatory processes exists for FSTL1, one as a pro-inflammatory molecule via CD14 and TLR4 and, on the other hand, as an inhibitor of tissue destruction via the downregulation of Mmps, which is thought to be regulated via a different pathway involving DIP2 disco-interacting protein 2 homolog A (DIP2A) (see below), pAKT and up regulation of FOS [106 Murakami, K (2012)].

In cardiac diseases FSTL1 seems to have a protective effect. In trans-aortic constricted hearts FSTL1 protects cardiomyocytes from apoptosis and hypertrophy via phosphorylated S’-prime-AMP-activated protein kinase (AMPK) signaling [174 Shimano, M (2011)], [165 Ogura, Y (2012)] and, in infarcted hearts, FSTL1 protects from apoptosis and induces angiogenesis via phosphorylated AKT signaling [167 Oshima, Y (2008)], [157 Liu, S (2010)], [156 Liu, S (2006)], [168 Ouchi, N (2010)]. In the latter case the phosphorylated AKT signaling was dependent on the interaction of FSTL1 with the transmembrane protein DIP2A functioning as a receptor for FSTL1.

In cancer development loss of FSTL1 expression is usually associated with progression to a more proliferative and metastatic state of the cancer, although for proliferation and apoptosis opposing results are also reported [135 Geng, Y (2011)], [203 Xu, J (2012)], [126

In the nervous system FSTL1 is expressed specifically in the visual cortex of primates, in an activation dependent manner, but not in mice (reviewed in [196 Watakabe, A (2006)]. In mice FSTL1 was shown to be important for sensory neuron functioning. It was demonstrated that FSTL1 directly binds to the ATPase, Na+/K+ transporting, alpha 1 polypeptide (ATP1A1) subunit of the Na/K ATPase, activating it, which in turn resulted in an inhibition of synaptic transmission; mice deficient for FSTL1 in the sensory neurons exhibited higher pain sensitivity [155 Li, KC (2011)].

Figure 2: Schematic representation of the known signaling events effected by Fstl1
The corresponding references are listed in the supplementary table 1.
FSTL1 loss of function defects in development

**Dorso-ventral axis establishment.**
In chicken, zebrafish and frogs, FSTL1 is expressed from early gastrulation onwards [114 Patel, K (1996)], [4 van den Berg, G (2007)], [118 Dal-Pra, S (2006)], [166 Okabayashi, K (1999)], primarily in the organizer, primitive streak, notochord and floor plate of the neural groove. In chicken embryos, where an explanted Hensen’s node induces a second axis, FSTL1 expression was detected in the ectoderm of the newly induced neural groove [114 Patel, K (1996)]. Later in development FSTL1 is expressed in somites, preferentially at the dorso-medial side, facing the axial structures [113 Amthor, H (1996)]. When the developing somites are experimentally separated from the axial structures, like the neural tube and notochord, FSTL1 expression is lost. Experimental deletion or addition of the notochord did not affect the expression of FSTL1, whereas removal of the neural tube did, suggesting that FSTL1 expression in somites is regulated by signals derived from the neural tube [113 Amthor, H (1996)].
In antisense oligonucleotide-treated chicken embryos, early loss (Hamburger Hamilton stage 4) of FSTL1 was associated with defects in both establishment of the dorso-ventral body axis and decreased neurulation [190 Towers, P (1999)].
Establishment of the dorso-ventral axis greatly depends on BMP signaling, which imposes ventral identity on the gastrulating embryo, whereas active inhibition of BMP signaling at the other side of the embryo is required for its dorsal identity [210 Plouhinec, JL (2011)]. Deficiency or knock-down of the BMP antagonist chordin in zebrafish results in partial ventralisation [118 Dal-Pra, S (2006)]. In zebrafish fstl1b is expressed in the gastrula stage embryos, whereas fstl1a is expressed at later stages [118 Dal-Pra, S (2006)], [117 Esterberg, R (2008)]. Knockdown of only fstl1b did not result in a phenotype, however, in addition to chordin knock down, fstl1b morpholinos aggravated the ventralisation phenotype to a degree comparable to combined noggin and chordin knockdown, demonstrating a role for fstl1b in the BMP mediated dorso-ventral axis establishment [118 Dal-Pra, S (2006)].

**Skeletal development**
In mice, homozygous loss of FSTL1, results in various skeletal defects including the development of the limbs, axial skeleton, sesamoid bones and cartilage of the tracheal rings [177 Sylva, M (2011)]. Like in zebrafish dorso-ventral axis establishment, a parallel between chordin and FSTL1 function can be drawn in mouse skeletal development, since both KO mice suffer from decreased tracheal cartilage formation, and hypoplastic development of the first cervical vertebra, or atlas [211 Bachiller, D (2003)], [177 Sylva, M (2011)], [135 Geng, Y (2011)]. Digit defects in FSTL1 KO mice comprise loss of digit numbers due to the bony fusion of digits as well as abnormal fusion of digit joints. Both processes are under control of BMP signaling and show a similar phenotype in absence of normal
BMP inhibition [212 Khokha, MK (2003)], [213 Seemann, P (2009)]. Recently mutations in another SPARC family member, SMOC-1, were shown to be causal for Waardenburg-Anophthalmia-Syndrome, also known as Ophthalmo-Acromelic-Syndrome [214 Rainger, J (2011)], [215 Abouzeid, H (2011)], [216 Okada, I (2011)]. Apart from the ocular manifestations, patients with Waardenburg-Anophthalmia, also suffer from various digit defects including the bony fusion of digits and bony fusions of digit joints. In knockdown experiments in Xenopus it was demonstrated that SMOC-1 functioned as a BMP inhibitor [217 Thomas, JT (2009)]. However, unlike the mechanism proposed for FSTL1 (see below) and all other known secreted BMP inhibitors, SMOC-1 acts on the intracellular part of the BMP signaling cascade, by stimulating ERK1/2 mediated phosphorylation of SMAD1/5/8 [217 Thomas, JT (2009)]. The missense mutations reported in Waardenburg-Anophthalmia are all located in the thyroglobulin module of the protein, which is not shared between FSTL1 and SMOC-1, which further underscores the different modes of action between FSTL1 and SMOC-1 in their effect on BMP signaling [214 Rainger, J (2011)].

Heterozygous FSTL1 KO mice suffer from asymmetrical rib sternum attachments, which strikingly is not observed in mice with increased BMP signaling, but is present in mice with decreased BMP signaling, namely the BMP7 KO mice. Additionally, FSTL1 KO mice display campomelia, which encompasses bending of the long bones, a phenotype observed in cases of heterozygous sex determining region Y-box 9 (SOX9) loss as seen in Campomelic Dysplasia in humans and heterozygous deletion of Sox9 in mice [218 Wagner, T (1994)], [219 Bi, W (2001)], [220 Foster, JW (1994)]. No BMP inhibitor or ligand mutant is known to have this condition [177 Sylva, M (2011)]. Other human diseases involving the bending of the long bones, do not appear to have mutations in BMP regulating genes [221 Cormier-Daire, V (2004)].

**Lung development**

In FSTL1 KO mice the development of the lungs is abnormal. In this condition increased proliferation of the epithelial cells, results in thickened alveolar membranes [177 Sylva, M (2011)], [135 Geng, Y (2011)]. Decreased differentiation of Alveolar Epithelial Cells (AEC) -I cells and immature AEC-II cells as well as impaired surfactant maturation was observed in FSTL1 KO lungs. The combination of impaired lung maturation and absence of most of the tracheal cartilage most likely results in the perinatal death of the FSTL1 KO mice. Lung development can be subdivided in different steps. During the first “pseudoglandular” stage, in which, initiated by fibroblast growth factor 10 (FGF10), the ventral part of the foregut buds off from the future oesophagus. Then, by a stereotyped form of branching, the embryonic lung is formed. During the “canalicular” and “saccular” stages of lung development, the embryonic lung matures into an efficient gas-exchange unit, by developing numerous alveoli with thin membranes and production of surfactant (reviewed in [222 Morrisey, EE (2010)]). It is most likely that the abnormalities observed in the FSTL1
KO lungs develop in the latter two stages. From the budding of the future trachea onwards BMP signaling plays important roles in lung development determining the branching pattern during early development [223 Cardoso, WV (2006)]. In later stages of lung development inhibition of normal BMP signaling by over-expression of the BMP inhibitor noggin or a dominant negative BMP receptor (dnALK6) results in abnormal alveoli formation [224 Weaver, M (1999)]. Mice deficient for the BMP inhibitor Gremlin also die at birth in respiratory distress and display deficient alveolar formation [225 Michos, O (2004)]. An additional mechanism of BMP regulation in lung development has been suggested. The FGF10 downstream target Cathepsin H was suggested to regulate BMP4 post-translationally by determining the eventual protein concentrations, as chemical inhibition of Cathepsin H, resulted in increased BMP4 protein concentrations in cultured lungs [226 Lu, J (2007)]. Interestingly, one of the proteins regulating surfactant maturation is Cathepsin H [227 Buhling, F (2011)], illustrating a possible link between abnormal BMP signaling and immature surfactant production observed in FSTL1 KO mice.

Lungs of FSTL1KO mice showed increased levels of phosphorylated SMAD1/5/8 indicative of increased BMP signaling activity. Culture of FSTL1 KO lungs in the presence of the BMP inhibitor noggin resulted in a decrease of the lung defects, further underscoring that uninhibited BMP signaling underlies the observed malformations. In in-vitro assays, FSTL1 over-expression was shown to inhibit activation of a BMP reporter plasmid, by BMP4 [135 Geng, Y (2011)].

Co-immunoprecipitation experiments demonstrated that FSTL1 can bind to both the BMP receptor BMPRII and BMP4. Based on these results it was proposed that FSTL1 interferes with receptor ligand interactions, and by doing so inhibits BMP-signaling.

**FSTL1 in ureter development.**

In FSTL1 KO mice hydroureters are observed from ED16 onwards [203 Xu, J (2012)]. Defects in ureter development are part of the disease group: “congenital anomalies of the kidney and urinary tract (CAKUT)”, which are as prevalent as 0.2-2% of newborns. Congenital obstructive nephropathy is reported as one of the most common causes for renal dysfunction in children. Urine collected in the renal pelvis is transported to the bladder by the ureter. For the ureter to function normally, smooth muscle cells propel the urine in a peristaltic fashion towards the bladder. Defects in ureter development that result in abnormal peristalsis or ureter-bladder connections, cause hydroureter and obstructive nephropathy [228 Airik, R (2007)].

The ureter buds from the caudal part of the Wolffian duct and is, at this stage, not directly connected to the cloaca, which will later form the bladder. The part of the Wolffian duct caudal to the ureter budding site, connects the embryonic ureter to the cloaca, is termed the common nephric duct (CND) and will not be part of the eventual uretero-vesical
junction (UVJ), as demonstrated by creLox lineage tracing studies [229 Mendelsohn, C (2009)]. The CND will disappear via apoptosis, a crucial event for formation of normal ureter bladder connections [230 Batourina, E (2005)]. Eventually the ureter runs diagonally through the bladder wall which results in a sphincteric function of the distal part of the ureter, preventing backflow of urine.

Abnormal budding locations of the ureter, or delayed connection of the Wolffian duct to the cloaca (or disorders in apoptosis surrounding the bladder) will result in abnormal connections of the ureter to the bladder, giving rise to ureteral reflux, due to insufficient sphincteric functions of the UVJ.

The mesenchyme surrounding the developing ureter is important for ureter development in at least two distinct ways, namely by providing the smooth muscle cells that will surround the ureter and propel the urine towards the bladder peristaltically and by providing signals to the ureter epithelium, which will cause its differentiation into an urothelium. The mesenchyme surrounding the ureter, unlike the renal mesenchyme, originates from tail bud-derived mesenchyme, at least in chick [228 Airik, R (2007)], [231 Brenner-Anantharam, A (2007)].

BMP signaling is imperative to urinary tract development, regulating different processes, involving both kidney formation as well as development of the ureter and its surrounding smooth muscle cells [228 Airik, R (2007)]. Due to this multitude of BMP effects on urinary tract development, distinct yet linked defects are observed in BMP mouse mutants. In BMP4 heterozygous mice the ureter buds from the Wolffian duct more cranially, resulting in a longer common metanephric duct, and eventually an abnormal location of the UVJ, which likely contributes to the hydroureter phenotype [232 Miyazaki, Y (2000)]. Also the development of the peri-ureteric smooth muscle cells is impaired in BMP4 heterozygous mice, adding to the causes for hydroureter [233 Miyazaki, Y (2003)].

In FSTL1 KO mice, peristalsis of the ureter appeared to be normal, but the UVJ was displaced posteriorly, likely resulting in impaired sfincteric function of the UVJ. However, when the events that might cause the abnormal position of the UVJ, were investigated, the budding site of the ureter from the Wolfian duct was normal, as were the levels of smooth muscle cells surrounding the ureter. Also, no abnormalities in apoptosis were observed in the mesenchyme surrounding the CND. Therefore, although both BMP4 KO mice and FSTL1 KO mice display hydroureter and abnormal UVJ connections, the underlying mechanism appears to be different between the two mouse mutants. Decreased rates of proliferation were measured in FSTL1 KO ureters and it was suggested that this might have been causal for the development of the abnormal UVJ positions.

However, another factor in determining the UVJ is the primitive bladder itself. The ureter enters the bladder in a hilus-like structure, together with the connecting bladder vessels. In absence of the ureter the hilus-like structures still develops, with the connecting vessel running through this hilus, suggesting that the entry site of the ureter forms independent
of a connecting ureter [234 Viana, R (2007)].
In humans, defects in the transcription factor homeobox A13 (HOXA13) give rise to the hand-foot-genital syndrome, which involves multiple defects of the urogenital sinus, like hypospadias and bicornuate uterus. HOXA13 is expressed in the developing cloaca and not in the developing ureters. Interestingly, in hand-foot-genital syndrome, hydroureters occur, due to abnormal sphincteric function of the UVJ. This is demonstrated by the fact that the hydroureters can be resolved by surgical re-implantation of the ureters [235 Verp, MS (1989)]. The ureter defects in hand foot genital syndrome therefore demonstrate the importance of normal cloaca development for proper UVJ formation. In mice, HOXA13 deletion alone is not sufficient to induce hydroureter, but an additional deletion of the highly related HOXD13 gene results in a phenotype comparable to the human situation [236 Innis, JW (2004)], [237 Scott, V (2005)], indicating that mouse models are inadequate to study this disease.
In addition to the urogenital defects, reduced finger or toe numbers, as seen in the FSTL1 KO mice [177 Sylva, M (2011)], are also part of the hand foot genital syndrome. Moreover, in a screen for genes downstream of HOXA13, FSTL1 was shown to be one of the genes regulated by HOXA13 in vitro [200 Williams, TM (2005)]. In vivo, FSTL1 was then shown to be down regulated in HOXA13 KO limb buds [200 Williams, TM (2005)]. The above-mentioned studies suggest that FSTL1 is an important downstream factor of HOXA13 in the etiology of the hand-foot-genital syndrome.
Possible mechanism of BMP inhibition by FSTL1

**BMP signaling and inhibitors**

In most developmental defects observed in FSTL1 KO mice, BMP signaling appears to be the main pathway affected. Normal BMP signaling takes place when BMP molecules bind to a type 1 receptor on the cell membrane, than a type 2 receptor is recruited to form a complex, which results in the intracellular phosphorylation of R-SMAD1,5,8 molecules. These molecules, with coSMAD4, influence transcription regulation.

Inhibitors of BMP signaling can act both extra- and intra-cellularly, due to the secreted nature of FSTL1 it is assumed that it functions outside of the cell. Extracellular inhibitors of BMP signaling, like chordin, noggin or gremlin, function by scavenging the BMP ligands, preventing them to bind to the BMP receptors. A somewhat different model is proposed for the function of FSTL1 [135 Geng, Y (2011)], [203 Xu, J (2012)]. Unfortunately, confusion is raging in the field. It has been shown that FSTL1 can bind to BMPs like BMP4 and BMP2 [135 Geng, Y (2011)], [184 Tanaka, M (2010)], [208 Zhou, J (2006)] and also to their BMP receptors. It has therefore been put forward that FSTL1 functions as a BMP inhibitor by inhibiting the formation of the BMP ligand-receptor complex, and not as a normal scavenger [135 Geng, Y (2011)], [203 Xu, J (2012)]. It has been suggested that the BMP type 2 receptor is specifically bound to FSTL1 and not the type 1 ALK6 receptor [135 Geng, Y (2011)]. Whereas others showed that not the type 2, but the type 1 receptors, bind to FSTL1 [203 Xu, J (2012)]. In the latter study co-immuno precipitation experiments, over-expressing FSTL1 and various type 1 BMP receptors, gave rise to different results, depending on the cell lines used [203 Xu, J (2012)]. In all cell lines ALK6 was co-immunoprecipitated, but only in some cases ALK3 was also precipitated. Just as puzzling are the results of FSTL1 BMP ligand interaction experiments, where it is shown that FSTL1 can interact with not only the BMP ligands, but also with other TGFβ super family members, Activin A or TGFβ1, the latter having the highest affinity for FSTL1 of all tested ligands [135 Geng, Y (2011)], [184 Tanaka, M (2010)], [208 Zhou, J (2006)]. Yet, these interactions have not been shown to have effects on their downstream signaling [135 Geng, Y (2011)], [238 Oshima, Y (2009)]. The observation that the phenotypes of FSTL1 KO mice do not always resemble the phenotypes of other BMP inhibitor KO mice, but sometimes, on the contrary, are similar to phenotypes of decreased BMP signaling, like in the case of asymmetrical rib sternum attachments [239 Luo, G (1995)], adds to the confusion.

Perhaps the most puzzling, is the fact that no developmental defects have been described in zebrafish [118 Dal-Pra, S (2006)], frogs [166 Okabayashi, K (1999)], or mice [157 Liu, S (2010)] upon over-expression of FSTL1, which, if FSTL1 were a “simple” BMP inhibitor molecule, seems unlikely. What it is, that makes FSTL1 such an ambiguous BMP inhibitor and on what factors the specificity of FSTL1 to the ligands and their receptors rely, is still unknown.
Concluding remarks

FSTL1 is a secreted protein affecting many different biological processes including development. The proposed mechanisms of the developmental defects are related to disturbed BMP signaling. The mode of FSTL1 affecting BMP signaling in development, however, remains highly confusing. Moreover, what underlies the fact that in development FSTL1 seems to signal through BMP signaling, in cardiac disease through the AKT and AMPK pathway, in immunological diseases through binding to CD14 and TLR4 and in the nervous system via activation of the Na/K ATPase, is completely unknown. Much more work is needed to unravel the functions of FSTL1 and integrate the different pathways this protein affects.

Acknowledgements

We would like to gratefully acknowledge Professor R.C.M. Hennekam and Professor A. Kispert for critical reading of the manuscript and their advice.

References

<table>
<thead>
<tr>
<th>References</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adams, Larman, Oxburgh; 2007, Gene Expr.Patterns. 491-500(7); Developmental expression of mouse Follistatin-like 1 (Fst1): Dynamic regulation during organogenesis of the kidney and lung.</td>
</tr>
<tr>
<td>2</td>
<td>Adams, Karolak, Larman, Liaw, Nolin, Oxburgh; 2010, Am.J.Physiol Renal Physiol F1320-F1327(299); Follistatin-like 1 regulates renal IL-1beta expression in cisplatin nephrotoxicity.</td>
</tr>
<tr>
<td>4</td>
<td>Belecky-Adams, Scheurer, Adler; 1999, Dev Biol. 107-123(210); Activin family members in the developing chick retina: expression patterns, protein distribution, and in vitro effects.</td>
</tr>
<tr>
<td>5</td>
<td>Bradshaw; 2012, Int.J Biochem.Cell Biol. 480-488(44); Diverse biological functions of the SPARC family of proteins.</td>
</tr>
<tr>
<td>6</td>
<td>Brekken, Sage; 2001, Matrix Biol. 816-827(19); SPARC, a matricellular protein: at the crossroads of cell-matrix communication.</td>
</tr>
<tr>
<td>7</td>
<td>Chaly, Marinov, Oxburgh, Bushnell, Hirsch; 2012, Arthritis Rheum. 1082-1088(64); FSTL1 promotes arthritis in mice by enhancing inflammatory cytokine/chemokine expression.</td>
</tr>
<tr>
<td>8</td>
<td>Chan, Masui, Krakovska, Belozerov, Voisin, Ghanny, Chen, Moyez, Zhi, Evans, McDermott, Siu; 2011, Mol Cell Proteomics. M110(10); Identification of differentially regulated secretome components during skeletal myogenesis.</td>
</tr>
<tr>
<td>9</td>
<td>Chan, Ngan, Ip, Liu, Xue, Cheung; 2009, Carcinogenesis 114-121(30); Tumor suppressor effect of follistatin-like 1 in ovarian and endometrial carcinogenesis: a differential expression and functional analysis.</td>
</tr>
<tr>
<td>10</td>
<td>Clutter, Wilson, Marinov, Hirsch; 2009, J Immunol. 234-239(182); Follistatin-like protein 1 promotes arthritis by up-regulating IFN-gamma.</td>
</tr>
<tr>
<td>11</td>
<td>Dal-Pra, Furthauer, Van-Celst, Thisse, Thisse; 2006, Dev.Biol. 514-526(298); Noggin1 and Follistatin-like2 function redundantly to Chordin to antagonize BMP activity.</td>
</tr>
<tr>
<td>13</td>
<td>Dean, Butler, Hamma-Kourbali, Delbe, Briggstock, Court, Overall; 2007, Mol Cell Biol. 8454-8465(27); Identification of candidate angiogenic inhibitors processed by matrix metalloproteinase 2 (MMP-2) in cell-based proteomic screens: disruption of vascular endothelial growth factor (VEGF)/heparin affin regulatory peptide (pleiotrophin) and VEGF/Connective tissue growth factor angiogenic inhibitory complexes by MMP-2 proteolysis.</td>
</tr>
<tr>
<td>14</td>
<td>Dixon, Bruce; 2009, Development 1675-1685(136); Short- and long-range functions of Goosecoid in zebrafish axis formation are independent of Chordin, Noggin 1 and Follistatin-like 1b.</td>
</tr>
</tbody>
</table>
Follistatin-like 1 in vertebrate development


16 Drabovich, Diamandis; 2010, J Proteome.Res. 1236-1245(9); Combinatorial peptide libraries facilitate development of multiple reaction monitoring assays for low-abundance proteins.

17 Drummond, Glynn, Fry, Dhanani, Volpi, Rasmussen; 2009, J Nutr. 2279-2284(139); Essential amino acids increase microRNA-499, -208b, and -23a and downregulate myostatin and myocyte enhancer factor 2C mRNA expression in human skeletal muscle.

18 Ehara, Sakurai, Tsuchiya, Nakano, Yamaguchi, Tokunaga; 2004, Clin.Exp.Rheumatol. 707-712(22); Follistatin-related protein gene (FRP) is expressed in the synovial tissues of rheumatoid arthritis, but its polymorphisms are not associated with genetic susceptibility.

19 El-Armouche, Ouchi, Tanaka, Doros, Wittkopper, Schulze, Eschenhagen, Walsh, Sam; 2011, Circ.Heart Fail. 621-627(4); Follistatin-like 1 in chronic systolic heart failure: a marker of left ventricular remodeling.

20 Esterberg, Delalande, Fritz; 2008, Development 3891-3901(135); Tailbud-derived Bmp4 drives proliferation and inhibits maturation of zebrafish chordamesoderm.

21 Geng, Dong, Yu, Zhang, Yan, Sun, Qiao, Geng, Nakajima, Furuichi, Ikegawa, Gao, Chen, Jiang, Ning; 2011, Proc.Natl. Acad.Sci U.S.A 7058-7063(108); Follistatin-like 1 (Fst1) is a bone morphogenetic protein (BMP) 4 signaling antagonist in controlling mouse lung development.

22 Genovese, Spadaccio, Rivello, Toyoda, Patel; 2009, Cytotherapy. 448-456(11); Electrostimulated bone marrow human mesenchymal stem cells produce follistatin.

23 Geske, Zhang, Patel, Ornitz, Stappenbeck; 2008, Development 2959-2968(135); Fgf9 signaling regulates small intestinal elongation and mesenchymal development.


25 Goo, Liu, Ryu, Shaffer, Malmstrom, Page, Nguyen, Doneanu, Goodlett; 2009, Prostate 49-61(69); Identification of secreted glycoproteins of human prostate and bladder stromal cells by comparative quantitative proteomics.

26 Gorelik, Wilson, Cloonan, Shulman, Hirsch; 2012, J Pediatr. 116-119(161); Plasma follistatin-like protein 1 is elevated in Kawasaki disease and may predict coronary artery aneurysm formation.


28 Hambrock, Kaufmann, Muller, Hanisch, Nose, Paulsson, Maurer, Hartmann; 2004, J Biol.Chem. 11727-11735(279); Structural characterization of TSC-36/Flik: analysis of two charge isoforms.


30 Ihara, Suzuki, Kitt, Jones, Ikeda; 2002, Cell Calcium 21-29(32); Modulation of gene expression in transgenic mouse hearts overexpressing calsequestrin.

31 Javerzat, Franco, Herbert, Platonova, Peille, Pantesco, De, Assou, Bicknell, Bikfalvi, Hagedorn; 2009, PLOS ONE e7856(4); Correlating global gene regulation to angiogenesis in the developing chick extra-embryonic vascular system.


33 Kawabata, Tanaka, Fujii, Umehara, Fujita, Yoshifuji, Mimori, Ozaki; 2004, Arthritis Rheum. 660-668(50); Ameliorative effects of follistatin-related protein/TSC-36/FSTL1 on joint inflammation in a mouse model of arthritis.

34 Kim, Eun, Lee, Nam, Rhe, Koh, Kim; 2011, Exp.Mol Pathol. 201-209(90); Gene expression changes in patient-matched gastric normal mucosa, adenomas, and carcinomas.

35 Kim, An, Nam, Jeon, Suh; 2012, J Rheumatol. 1399-1406(39); Serum S100A8/A9, but not follistatin-like protein 1 and interleukin 18, may be a useful biomarker of disease activity in adult-onset Still’s disease.

36 Komatsu, Watakabe, Hashikawa, Tochitani, Yamamori; 2005, Cereb.Cortex 96-108(15); Retinol-binding protein gene is highly expressed in higher-order association areas of the primate neocortex.

37 Lara-Pezzi, Felkin, Birks, Sarathchandra, Panse, George, Hall, Yacoub, Rosenthal, Barton; 2008, Endocrinol. 5822-5827(149); Expression of follistatin-related genes is altered in heart failure.
Le Luduec, Condamine, Louvet, Thebault, Heslan, Heslan, Chiffoleau, Cuturi; 2008, Am.J Transplant. 2297-2306(8); An immunomodulatory role for follistatin-like 1 in heart allograft transplantation.

Lehnert, Reverter, Byrne, Wang, Nattrass, Hudson, Greenwood; 2007, BMC Dev Biol. 95(7); Gene expression studies of developing bovine longissimus muscle from two different beef cattle breeds.


Li, Wang, Zhong, Lu, Wang, Xiao, Bao, Zhang; 2011, Cell Res. 697-699(21); Reduction of follistatin-like 1 in primary afferent neurons contributes to neuropathic pain hypersensitivity.

Li, Zhang, Li, Wang, Yu, Zhong, Zhang, Lu, Wang, Ma, Yao, Wang, Lin, Han, Zhang, Kuner, Xiao, Bao, Gao, Zhang; 2011, Neuron 974-987(69); Follistatin-like 1 suppresses sensory afferent transmission by activating Na+,K+-ATPase.

Liu, Wang, Wang, Lin, Han, Sun, Guo, Sun, Wu; 2006, Exp. Mol Pathol. 132-140(80); TSC-36/FRP inhibits vascular smooth muscle cell proliferation and migration.

Liu, Shen, Xu, Liu, Zhao, Guo, Du; 2010, Am J Physiol Endocrinol. Metab E351-E363(299); FRP inhibits ox-LDL-induced endothelial cell apoptosis through an Akt-NF-(kappa)B-Cbl-2 pathway and inhibits endothelial cell apoptosis in an apoE-knockout mouse model.

Lombardi, van den Hoff, Ruijter, Luijierink, Buffing, Markman, Moorman, Lekanne Deprez; 2003, J Biochem Biophys Methods 17-33(57); Expression analysis of subtractively enriched libraries (EASEL): a widely applicable approach to the identification of differentially expressed genes.

Mashimo, Maniwa, Sugino, Nose; 1997, Cancer Lett. 213-219(113); Decrease in the expression of a novel TGF beta1-inducible and ras-recision gene, TSC-36, in human cancer cells.

Maurer, Hohenadl, Hohenester, Gohring, Timpl, Engel; 1995, J Mol Biol. 347-357(253); The C-terminal portion of BM-40 (SPARC/osteonectin) is an autonomously folding and crystallisable domain that binds calcium and collagen IV.

Meehan, Holland, Dawkins; 2002, Prostate 54-63(50); Proteomic analysis of normal and malignant prostate tissue to identify novel proteins lost in cancer.

Miyamae, Marinov, Sowders, Wilson, Devlin, Boudreau, Robbins, Hirsch; 2006, J. Immunol. 4758-4762(177); Follistatin-like protein-1 is a novel proinflammatory molecule.

Mokashery; 2012, Osteoarthritis Cartilage. 1451-1464(20); Osteoarthritis year 2012 in review: biomarkers.

Mukhopadhyay, Greene, Pisano; 2006, Birth Defects Res. A Clin. Mol Teratol. 528-543(76); Expression profiling of transforming growth factor beta superfamily genes in developing orofacial tissue.


Ogura, Ouchi, Ohashi, Shibata, Kataoka, Kambara, Kito, Maruyama, Yuasa, Matsuo, Enomoto, Uemura, Miyabe, Ishii, Yamamoto, Shimizu, Walsh, Murohara; 2012, Circulation 1728-1738(126); Therapeutic impact of follistatin-like 1 on myocardial ischemic injury in preclinical models.

Ohashi, Sato, Yoshiki, Kusakabe; 1997, Calcif. Tissue Int. 400-403(61); TSC-36 (follistatin-related polypeptide) gene expression in estrogen receptor positive osteoblastic cell line, CD07F.


Oshima, Ouchi, Sato, Izumiya, Pimentel, Walsh; 2008, Circulation 3099-3108(117); Follistatin-like 1 is an Akt-regulated cardioprotective factor that is secreted by the heart.

Ouchi, Asaumi, Ohashi, Higuchi, Sono-Romanelli, Oshima, Walsh; 2010, J. Biol. Chem. 7127-7134(285); DIP2A functions as a FSTL1 receptor.

Ouchi, Oshima, Ohashi, Higuchi, Ikegami, Izumiya, Walsh; 2008, J. Biol. Chem. 32802-32811(283); Follistatin-like 1, a secreted muscle protein, promotes endothelial cell function and revascularization in ischemic tissue through a nitric-oxide synthase-dependent mechanism.

Follistatin-like 1 in vertebrate development

61 Pedersen, Febbraio; 2012, Nat.Rev.Endocrinol. 457-465(8); Muscles, exercise and obesity: skeletal muscle as a secretory organ.


63 Rosenberg, Georges, Asawachaicharn, Analau, Tapscott; 2006, J Cell Biol. 77-85(175); MyoD inhibits Fstl1 and Utrn expression by inducing transcription of miR-206.


65 Rosenberg, Georges, Asawachaicharn, Analau, Tapscott; 2006, J Cell Biol. 77-85(175); MyoD inhibits Fstl1 and Utrn expression by inducing transcription of miR-206.

66 Serao, Delfino, Southey, Beever, Rodriguez-Zas; 2008, BMC.Med.Genomics 49(4); Cell cycle and aging, morphogenesis, and response to stimuli genes are individualized biomarkers of glioblastoma progression and survival.

67 Shiranuma, Mashimo, Mita, Kuroki, Nose; 1993, Eur.J.Biochem. 13-19(217); Cloning from a mouse osteoblastic cell line of a transforming-growth-factor-beta 1-regulated gene, one of which seems to encode a follistatin-related polypeptide.

68 Shimano, Ouchi, Nakamura, van, Ohashi, Asaumi, Higuchi, Pimentel, Sam, Murohara, van den Hoff, Walsh; 2011, Proc.Natl.Acad.Sci.U.S.A E899-E906(108); Cardiac myocyte follistatin-like 1 functions to attenuate hypertrophy following pressure overload.

69 Slany, Haudek, Zwickl, Gundacker, Grusch, Weiss, Seir, Rodgarkia-Dara, Hellerbrand, Gerner; 2010, J Proteome.Res. 6-21(9); Cell characterization by proteome profiling applied to primary hepatocytes and hepatocyte cell lines Hep-G2 and Hep-3B.


71 Takahata, Shukia, Yamamori, Kaas; 2012, Cereb.Cortex 2313-2321(22); Differential expression patterns of striate cortex-enriched genes among Old World, New World, and prosimian primates.

72 Takahata, Hashikawa, Tochitani, Yamamori; 2010, J Chem.Neuroanat. 112-122(40); Differential expression patterns of OCC1-related, extracellular matrix proteins in the lateral geniculate nucleus of macaque monkeys.

73 Takahata, Komatsu, Watakabe, Hashikawa, Tochitani, Yamamori; 2009, Cereb.Cortex 1937-1951(19); Differential expression patterns of occ1-related genes in adult monkey visual cortex.


75 Takahata, Komatsu, Watakabe, Hashikawa, Tochitani, Yamamori; 2006, Cereb.Cortex 929-940(16); Activity-dependent expression of occ1 in excitatory neurons is a characteristic feature of the primate visual cortex.


80 Tochitani, Hashikawa, Yamamori; 2003, Neurosci.Lett. 105-108(346); Occ1 mRNA expression reveals a characteristic feature in the hippocampal CA2 field of adult macaques.

81 Tochitani, Hashikawa, Yamamori; 2003, Neurosci.Lett. 114-116(337); Expression of occ1 mRNA in the visual cortex during postnatal development in macaques.


Trojan, Schaaf, Steidler, Haak, Thalmann, Knoll, Gretz, Alken, Michel; 2005, Anticancer Res. 183-191(25); Identification of metastasis-associated genes in prostate cancer by genetic profiling of human prostate cancer cell lines.


van den Berg, Somi, Buffing, Moorman, van den Hoff; 2007, Anat.Rec.(Hoboken.) 783-787(290); Patterns of expression of the Follistatin and Follistatin-like1 genes during chicken heart development: a potential role in valvulogenesis and late heart muscle cell formation.


Walsh; 2009, Circ.J 13-18(73); Adipokines, myokines and cardiovascular disease.


Watakabe, Komatsu, Nawa, Yamamori; 2006, Genes Brain Behav. 38-43(S Suppl 1); Gene expression profiling of primate neocortex: molecular neuroanatomy of cortical areas.


Williams, Williams, Kuick, Misek, McDonagh, Hanash, Innis; 2005, Dev Biol. 462-480(279); Candidate downstream regulated genes of HOX group 13 transcription factors with and without monomeric DNA binding capability.

Wilson, Marinov, Blair, Bushnell, Thompson, Chaly, Hirsch; 2010, Arthritis Rheum. 2510-2516(62); Follistatin-like protein 1 is a mesenchyme-derived inflammatory protein and may represent a biomarker for systemic-onset juvenile rheumatoid arthritis.

Wu, Pang, Sahlin, Blanck, Norstedt, Flores-Morales; 2003, Biol.Reprod. 1308-1317(69); Gene expression profiling of the effects of castration and estrogen treatment in the rat uterus.

Wu, Zhou, Smas; 2010, Mech.Dev 183-202(127); Downregulated expression of the secreted glycoprotein follistatin-like 1 (Fstl1) is a robust hallmark of preadipocyte to adipocyte conversion.

Xu, Qi, Gong, Yu, Zhang, Sha, Gao; 2012, PLoS.ONE. e32554(7); Fstl1 antagonizes BMP signaling and regulates ureter development.

Yamamori, Rockland; 2006, Neurosci.Res. 11-27(55); Neocortical areas, layers, connections, and gene expression.

Yang, Liu, Mao, Hu, Yan, Zhao; 2009, Gene Expr.Patterns. 532-540(9); The expression pattern of Follistatin-like 1 in mouse central nervous system development.

Zendaoui, Lachance, Roussel, Couet, Arsenault; 2011, Circ.Heart Fail. 207-213(4); Usefulness of carvedilol in the treatment of chronic aortic valve regurgitation.

Zhao, Han, Zhang; 2011, Int.J Biochem.Cell Biol. 1459-1468(43); Suppression of lung cancer cell invasion and metastasis by connexin43 involves the secretion of follistatin-like 1 mediated via histone acetylation.

Zhou, Liao, Hatta, Tanaka, Xuan, Fujisaki; 2006, Gene 191-198(372); Identification of a follistatin-related protein from the tick Haemaphysalis longicornis and its effect on tick oviposition.