Homologous sequence in lumican and fibromodulin LRR 5-7 competes for collagen binding

Sebastian Kalamajski and Åke Oldberg
Department of Experimental Medical Science, University of Lund, SE-221 84 Lund, Sweden

Running title: Lumican – Collagen Type I Interaction
Address correspondence to: Sebastian Kalamajski, Lund University, Department of Experimental Medical Science, BMC B12, 221 84 Lund, Sweden, Tel. +46-(0)462228577; Fax. +46-(0)462220855; E-Mail: Sebastian.Kalamajski@med.lu.se

Lumican and fibromodulin compete for collagen type I binding in vitro and fibromodulin-deficient mice have four-fold more lumican in tendons. These observations indicate that homologous sequences in lumican and fibromodulin bind to collagen type I. Here, we demonstrate that lumican binding to collagen type I is mediated mainly by Asp-213 in LRR 7. The mutation D213N in lumican impairs interaction with collagen, and the lumican fragment spanning LRRs 5-7 is an efficient inhibitor of collagen binding. Also, the lumican LRR 7 sequence-based synthetic peptide CYLDNNKC inhibits the binding to collagen. Homologous collagen-binding site in fibromodulin, located in LRRs 5-7, inhibits the binding of lumican to collagen, and the mutation E251Q in this fibromodulin fragment does not inhibit the lumican-collagen binding. Lumican, but not the the D213N mutation, lowers the melting point and affects the packing of collagen fibrils.

Lumican is expressed in many, mainly loose, connective tissues (1). It belongs to the family of small leucine-rich repeat proteoglycans (SLRPs) that has a varied, often tissue-specific influence on collagen matrices, as demonstrated in several SLRP-deficient mice (2-5). In particular, lumican-deficient mice have fragile skin, as well as opaque corneas with abnormally formed collagen fibrils in the posterior stroma, which results in three-fold increased backscattering of light (6-9). The amount of lumican is also increased in tendons of fibromodulin-deficient mice (10). Fibromodulin is structurally homologous to lumican and their primary sequences are 47% identical. Fibromodulin-deficient mice have deviations in collagen fibril structures in tendons, which provoke secondary phenotypes like osteoarthritis of articular knee cartilage (10,11). In addition, both lumican and fibromodulin can reciprocally inhibit their collagen interaction (12). The increased deposition of lumican in fibromodulin-deficient mice suggests that lumican binds to non-occupied fibromodulin binding sites in collagen I (12).

Some novel functions have recently been reported for lumican. In lumican-deficient mice, toll-like receptor-4 signaling by LPS is diminished, which leads to reduced response to septic shock (13). Since lumican is an extracellular protein and the extracellular domain of toll-like receptor-4 is made of LRR domains, these two LRR proteins could interact and cooperate in a spatial chain of events. Lumican can also prevent cleavage of collagen by MMP-13 in vitro, or at least delay the reaction. In contrast to fibromodulin, lumican is not degraded by this enzyme (14,15). Lumican may also be involved in collagen calcification as it interacts with hydroxyapatite and is present in the areas of bone and tooth ossification (16,17).

Despite their close homology and similar collagen-binding site, fibromodulin and lumican do not appear to be functionally redundant. Judging from the knockout mouse phenotype, fibromodulin is primarily involved in formation of thick collagen fibres, while lumican controls the early formation of thin fibrils (18). Mice deficient in both lumican and fibromodulin have a more severe tendon phenotype than fibromodulin-deficient mice.
In addition, both SLRPs delay the turbidity of spontaneous in vitro collagen fibril formation (20,21). However, it is not known if or how the functional differences relate to the collagen-binding specificity of the two SLRPs.

In this report, we examined the lumican-collagen binding site and how it relates to fibromodulin. We used recombinant and mutated lumican or fibromodulin fragments in direct and in competition collagen-binding assays. We also determined how the lumican mutations influence collagen packing in in vitro assembled collagen fibrils.

Experimental procedures

Homology modelling of lumican – The tertiary structure of lumican was modelled with Swiss-PdbViewer software (22) using the crystal structure of decorin (PDB # 1xku) as template. Expression of GST-tagged lumican and fibromodulin fragments in bacteria – Human lumican cDNA (GenBank Accession Number BC007038) was used as template for PCR-amplification of cDNAs of lumican fragments. The fragments spanned primary sequences: LumN-4 (aa 19-152), Lum5-7 (aa 147-220), Lum8-12 (aa 215-338). The PCR primers are listed in Supplemental Figure 1. Next, the cDNAs were digested with restriction enzymes BamHI and Smal, and ligated into pGEX-4T-3 expression vector (Amersham Biosciences, Sweden). The final constructs were sequenced to confirm the identity, transfected into RosettaGami E. coli (Novagen, USA), and proteins were expressed according to the manufacturer’s instruction. Proteins were purified under native conditions by glutathione-affinity chromatography, as previously described (23) and dialyzed against PBS with 0.05% (v/v) Tween-20. The protein concentration was determined with Coomassie Protein Assay Reagent (Pierce, USA). Similar procedure was used for making fibromodulin, as described earlier (24). The proteins migrated faster on a non-reduced SDS-PAGE than on a reduced gel, indicating presence of disulfide bonds.

Mammalian expression of lumican – Lumican cDNA (BC007038) was amplified by PCR (primers listed in Supplemental Figure 1), digested with Xhol and BamHI, and ligated into pCEP4 BM40-hisEK expression vector (25), attaching lumican cDNA to a N-terminal his-tag. The constructs were confirmed by sequencing and transfected into human embryonic kidney 293 cells by electroporation. Cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% (v/v) fetal calf serum (Invitrogen, Sweden). After 24 h, hygromycin (Invivogen, France) was added to a concentration of 150 µg/mL. After one week, single clones were picked, incubated in medium containing [35S]-sulfate (tagging tyrosine sulphate residues), and analyzed for proteoglycan expression. The proteoglycan-expressing clones were cultured in EX-Cell 325 PF CHO protein-free medium (JRH Biosciences, USA) for 12 days with medium changes every three days. The medium was dialyzed against 300 mM NaCl and 20 mM NaH2PO4 buffer, pH 8.0 and proteoglycans were purified in native form using Ni-NTA agarose (Qiagen, Germany). Lumicans were run on SDS-PAGE to check purity, and also treated with PNGaseF to check for presence of N-linked GAGs (Fig. 3A).

Site-directed mutagenesis and expression of mutated lumican and fibromodulin in bacteria – Wild type lumican cDNA was used as template in PCR (for primers see Supplemental Figure 1), to amplify lumican cDNA with EcoRI and XhoI restriction enzyme flanking sites. After digestion with EcoRI and XhoI , cDNA was ligated into pET-27b(+) expression vector (Novagen, USA), and the his-tagged protein was expressed in RosettaGami E. coli (Novagen, USA). Lumican was purified with Ni-NTA agarose (Qiagen, Germany) under denaturing conditions according to the manufacturer’s instruction. Prior to use, protein was dialyzed against PBS. Similar procedure was used for making fibromodulin, as described earlier (24). The proteins migrated faster on a non-reduced SDS-PAGE than on a reduced gel, indicating presence of disulfide bonds.

Site-directed mutagenesis and expression of mutated lumican and fibromodulin in bacteria – Wild type lumican cDNA (X16485) was used for site-directed mutagenesis using QuikChange II kit with pBluescript® II KS (+/-) cloning vector (Stratagene, Sweden) according to the manufacturer’s instruction. Primers used for lumican D213N and D222N, and fibromodulin E251Q mutations are listed in Supplemental Figure 1. The mutated cDNA was ligated into pET-27b(+) expression vector for bacterial
expression or in pCEP4-BM40 hisEK vector for mammalian expression. Proteins were expressed and purified as described above. For mutation E251Q in Fmod5-7 fragment, we used similar procedure, as described previously (24).

Peptide synthesis- Linear peptide TLYLDNNKIS and the cyclic peptides CYLDNNKC and CYLKNNKC were purchased from Schafer-N (Denmark) and dissolved in TBS with 0.05% (v/v) Tween-20.

Solid-phase collagen-binding assay - Microtiter 96-well Maxisorp plates (Nunc, Denmark) were coated for 16 h using 100 µg/mL acid-soluble collagen. The wells were washed twice with PBS and blocked for 2 h with PBS with 1 mg/mL bovine serum albumin (BSA) and 0.05% (v/v) Tween-20. Next, the wells were washed with incubation buffer (20 mM Tris pH 8.0, with 0.1% (w/v) BSA and 0.05% (v/v) Tween-20), and the GST-tagged lumican fragments or full-length proteins (diluted in incubation buffer) were added and incubated for 4 h. Collagen binding was detected with rabbit anti-GST antibody (ab9085, Abcam, UK) for lumican fragments, or rabbit anti-his antibody (ab9108, Abcam, UK) for full-length lumicans, diluted 1:1000 in the incubation buffer. After 1 h, the wells were washed, and incubated for 1 h with anti-rabbit alkaline phosphatase-conjugated antibody (ab6722, Abcam, UK) diluted 1:1000 in incubation buffer. Lastly, the wells were washed, incubated with phosphatase substrate p-Nitrophenyl Phosphate (Sigma, Sweden), and protein binding was detected by measuring absorbance at 405 nm. KD values were calculated using nonlinear regression sigmoidal dose-response formula

\[ Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{(X - \text{LogEC50})}} \]

for EC50 and assuming one-site binding. Ki values were calculated using one-site competition formula

\[ Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{1 + 10^{(X - \text{LogIC50})}} \]

for IC50. For these calculations, GraphPad Prism (GraphPad Software, Inc.) was used.

Differential scanning calorimetry – Acid-soluble collagen (Vitrogen) was dialyzed against PBS at 4 °C, and then incubated with mammalian-expressed lumican (wild type and D213N-mutated) at a molar ratio 1:10 (lumican:collagen) at 37 °C for 16 h. The samples were then run in a VP-DSC calorimeter (Microcal) in a 30-60 °C temperature gradient, at a scanning rate of 1 °C/min. Sample thermograms were subtracted from blank (buffer only) thermograms and normalized for collagen concentration. The contribution of lumican to the melting thermogram of collagen was negligible due to its low concentration (5 µg/mL). Data were analyzed with Origin software supplied with VP-DSC.

RESULTS

To study the interaction between lumican and collagen, we expressed GST-tagged lumican fragments that covered overlapping parts of the protein (Fig. 1A-B). LumN-4, covering the N-terminal part of lumican and spanning to LRR 4, did not interact with collagen type I in a solid-phase assay, and neither did Lum8-12 that contains LRR 8-12. Only Lum5-7 derived from the central part of the protein, containing LRR 5-7, showed collagen binding (KD ≈ 45 nM) (Fig. 1C). Lum5-7 also inhibited the interaction of full-length mammalian expressed lumican (Ki ≈ 250 nM), which none of the other fragments did (Fig. 2). The fragments did not interact with BSA coat, without collagen (data not shown).

We used site-directed mutagenesis of lumican cDNA, and expressed full-length mutated lumicans that appear to carry short-chained N-linked glycosaminoglycans, indicating that cells produce glycoprotein form of lumican (Fig. 3A). To select putative candidates for mutation, we used sequence alignment of lumican LRRs 5-7 from eleven species deposited in UniProtKB database. We also compared these sequences with the corresponding fibromodulin LRRs 5-7, since Fmod5-7 inhibits lumican-collagen interaction (Supplemental figure 2). We searched for charged residues near the β-sheet – loop region of the LRR domains, since this region was previously implicated in the interaction of SLRPs (26). Asp-213 in human lumican LRR 7 (Fig. 4A) is semi-conserved with Glu-251 in human fibromodulin. Consequently, full-length lumican with D213N mutation (LumD213N) and control (LumD222N) were expressed in mammalian cells, and analyzed for their collagen binding. Wild type lumican and LumD222N bound collagen at KD ≈ 30 nM with a fitted sigmoidal binding curve, while LumD213N interacted weakly with
collagen, with a linear binding curve (Fig. 3B). We also observed that a synthetic linear peptide (TYLDNNKIS) based on lumican LRR 7 sequence did not inhibit the lumican-collagen interaction (data not shown), although the cyclic peptide (CYLDNNKC) was an efficient inhibitor at $K_i \approx 2 \mu M$ (Fig. 3C).

Since the closest homologue of lumican is fibromodulin, and both bind to the same region of collagen, we also tested if any fibromodulin fragment used in our previous study (24) could inhibit the lumican-collagen interaction. Fmod5-7, containing LRRs 5-7, impaired the binding of lumican to collagen ($K_i \approx 250 \text{ nM}$), while other fragments were ineffective. Fmod8-12 – the previously identified main collagen-binding fragment of fibromodulin did not contribute to the inhibitory activity of Fmod5-7 (Fig. 4B). To further explore the competition between lumican and fibromodulin for collagen binding, we introduced E251Q in full-length fibromodulin and in fibromodulin fragment Fmod5-7. This mutation reduced the inhibitory activity of Fmod5-7, but not of full-length fibromodulin (Fig. 4C).

Finally, we studied the effect of the D213N mutation on lumican function during collagen fibrillogenesis. We used differential scanning calorimetry to analyze the denaturation thermograms of collagen pre-incubated with mammalian-expressed lumican (wild type and LumD213N) in PBS, at 37°C for 16 h. We used molar ratio lumican:collagen 1:10, at which the binding of lumican appears to be saturated, since higher concentrations do not further affect the collagen denaturation thermograms. In this functional assay, two melting points are observed: an early 41°C-peak represents the denaturation of monomeric collagen, while the late 52°C-peak corresponds to the denaturation of the fibrils. In this system, wild type lumican reduces the melting point of collagen fibrils by about 1°C, while LumD213N produces a thermogram similar to the control without lumican (Fig. 5).

DISCUSSION

We demonstrate that lumican binding to collagen type I is mediated mainly by Asp-213 in LRR 7. This maps the interaction to the $\beta$-sheet – loop region of the LRR domain, which is similar to other LRR proteins including the close homologues fibromodulin and decorin (24,27). In general, this implies that LRR domains contain interaction sites near the distal parts of the conserved $\beta$-sheet structures and include charged amino acids. In many cases the full-length SLRPs bind stronger than the fragments of the protein to collagen (24). The characteristic curved shape of LRR proteins may engage additional weak collagen interactions mediated by several LRR domains, thus creating a co-operative high-affinity collagen binding.

The mutation D213N in lumican LRR 7 impaired the interaction with collagen, and the fragment spanning LRRs 5-7, as well as the cyclic peptide CYLDNNKC based on the LRR 7 sequence, were both inhibitors of the lumican-collagen interaction. Inhibiting the interaction, suggesteing that the interaction depends on a specific conformation.

Interestingly, fibromodulin does not inhibit lumican-collagen interaction via the high-affinity collagen-binding site located in LRR 11, since Fmod8-12 or the peptide (RLDGNEIKR) are both inactive as inhibitors (24). The low-affinity collagen binding site of Fmod5-7 (LRRs 5-7) contains Glu-251, homologous and topologically related to lumican Asp-213, and inhibits the binding of lumican to collagen (Fig. 4A). The fragment Fmod5-7 with an E251Q mutation does not inhibit lumican binding. In contrast, the full-length fibromodulin with the same E251Q mutation inhibits lumican collagen-binding. This indicates a close proximaty of the Fmod/Lum LRR 5-7 and Fmod LRR 11 collagen-binding sites. The full length fibromodulin, with a functional LRR 11 binding site, may sterically hinder the binding of lumican, despite the mutated LRR 5-7 collagen binding site.

The alternate collagen-binding of fibromodulin and lumican may have a role during the development of collagen matrices. Tendons of fibromodulin-deficient mice have an altered collagen cross-linking pattern (unpublished observation), and the amount of lumican is increased some 4-fold in tendons from these mice (10). The absence of fibromodulin most likely leads to more binding of lumican to unoccupied collagen binding sites in fibromodulin deficient tendons. During tendon development both
lumican and fibromodulin have a role in the initial assembly of intermediates, while fibromodulin facilitates growth steps leading to mature fibrils (18). It has also been demonstrated that the fibromodulin mRNA level is increased 6-8-fold in chicken tendons between day 14 to day 19 of development, which coincide with a dramatic increase in collagen fibril length (28). Increased expression of fibromodulin with a higher affinity for collagen may displace lumican from collagen and appears to have a role in tendon developmental transitions. We propose that the initial cross-linking of collagen can be regulated by both lumican and fibromodulin, but fibromodulin can regulate the fibril assembly further by connecting two assembling collagen units through its two collagen-binding sites. This would later result in regulation of the mature cross-link formation (Fig. 6).

The binding of combinations different SLRPs may shape collagen molecules into specialized matrix networks, such as tendon and cornea. This may occur by binding to different or the same region of collagen. Decorin and fibromodulin bind to separate collagen sites, but here we show that fibromodulin and lumican can bind to and compete for the same collagen binding-site. This SLRP-collagen binding could determine how collagen monomers interact and juxtapose lysine/hydroxylysine residues involved in cross-link formation. SLRP-collagen molecules may thus be aligned or shielded to allow the formation of a specific cross-linking pattern. In this manner, the differential tissue expression of SLRPs could regulate the mechanical properties of collagen matrices.

We also show that lumican binding to collagen regulates the hydration of the fibrils, which can be determined by differential scanning calorimetry (29-31). The bound lumican changes the hydration of the polymer by sterical hindrance, leading to a lower melting temperature of the fibrils assembled without lumican. In contrast, LumD213N (not binding collagen) does not influence the denaturation of the fibrils.
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FOOTNOTES

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1The abbreviations used are: SLRP, small leucine-rich repeat proteoglycan; LRR, leucine-rich repeat; GST, glutathione S-transferase; PBS, phosphate-buffered saline; Ni-NTA, nickel-nitrilotriacetic acid; DMEM, Dulbecco’s modified Eagle’s medium; BSA, bovine serum albumin; KD, constant of dissociation; Ki, constant of inhibition.

FIGURE LEGENDS

Fig. 1. Interactions of lumican fragments with collagen. A. Lumican modelled after decorin crystal structure (1xku.pdb) and energy-minimized. Fragments expressed as GST-fusion proteins are depicted. B. Expression of lumican fragments as GST-fusion proteins. SDS-PAGE on bacterial total lysate (left panel) and purified (right panel) lumican fragments. C. Solid-phase collagen-binding assay. 96-well plates were coated with acid-soluble collagen type I, blocked with BSA, incubated with LumN-4 (■), Lum5-7 (▲), or Lum8-12 (▼), and the binding was detected with rabbit anti-GST followed by alkaline phosphatase-conjugated anti-rabbit antibody (OD 405 nm). Protein concentration is plotted on semi-log scale. Three replicates of each sample were used to calculate means, and standard deviations (error bars).

Fig. 2. Inhibitions of lumican-collagen interaction by lumican fragments. Solid-phase collagen-binding assay. 96-well plates were coated with acid-soluble collagen type I, blocked with BSA, and incubated with 100 ng full-length mammalian-expressed lumican together with increasing concentrations of lumican fragments – LumN-4 (■), Lum5-7 (▲), or Lum8-12 (▼). Binding of lumican was detected by rabbit anti-his followed by alkaline phosphatase-conjugated anti-rabbit antibody (OD 405 nm). Protein concentration is plotted on semi-log scale. Three replicates of each sample were used to calculate means, and standard deviations (error bars).

Fig. 3. Interactions of mutated lumican with collagen and inhibition by synthetic peptides. A. Mammalian-expressed lumicans run on SDS-PAGE, before and after treatment with PNGaseF. B. Solid-phase collagen-binding assay. 96-well plates were coated with acid-soluble collagen type I, blocked with BSA, and incubated with increasing concentrations of full-length wild-type (■) or mutated lumicans: LumD213N (▲), LumD222N (▼). Binding of lumican was detected by rabbit anti-his followed by alkaline phosphatase-conjugated anti-rabbit antibody (OD 405 nm). Protein concentration is plotted on semi-log scale. Three replicates of each sample were used to calculate means, and standard deviations (error bars). C. Inhibition of lumican-collagen interaction by synthetic peptides on solid-phase assay. 96-well plates were coated with acid-soluble collagen type I, blocked with BSA, and incubated with 100 ng mammalian-expressed lumican and increasing concentrations of peptide CYLDNNKC (■), or control peptide CYLKNNKC (▲). The binding of lumican was detected with anti-his antibody followed by alkaline phosphatase-conjugated anti-rabbit antibody (OD 405 nm). Protein concentration is plotted on semi-log scale. Three replicates of each sample were used to calculate means, and standard deviations (error bars).
Fig. 4. Inhibitions of lumican-collagen interaction by fibromodulin fragments. A. Homology model of lumican and fibromodulin with identified collagen-binding sites (shaded). B. Solid-phase collagen-binding assay. 96-well plates were coated with acid-soluble collagen type I, blocked with BSA, and incubated with 100 ng mammalian-expressed lumican together with increasing concentrations of fibromodulin fragments: FmodN-4 (▼), Fmod5-7 (▲), Fmod8-12 (X), or both Fmod5-7 and Fmod8-12 (□). Binding of lumican was detected by rabbit anti-his followed by alkaline phosphatase-conjugated anti-rabbit antibody (OD 405 nm). Protein concentration is plotted on semi-log scale. Three replicates of each sample were used to calculate means, and standard deviations (error bars). C. As in B, but inhibitors of lumican-collagen interactions were Fmod5-7 (▲) and mutated Fmod5-7 E251Q (□).

Fig. 5. Differential scanning calorimetry of collagen fibrils preincubated with lumican. Acid-soluble collagen was dialyzed against PBS at 4 °C, incubated with wild type lumican, LumD213N, or without lumican, at 37 °C for 16 h, and run in VP-DSC. Thermograms were normalized for collagen concentration and subtracted from blank run. Thermograms are: wild type lumican (dashed line), LumD213N (dotted line), or no lumican (control, solid line). Arrows show differences in denaturation temperatures of the respective samples.

Fig. 6. The proposed mechanism of lumican and fibromodulin regulation of collagen fibrillogenesis. A. Lumican (green) binds to collagen monomers through LRR 7 and regulates their initial cross-linking (red). B. Fibromodulin (yellow) binds to collagen monomers preferentially with its high-affinity site in LRR 11, regulates the initial cross-linking, and can compete with collagen binding with the collagen-bound lumican. C. As lumican is dislodged from the collagen by fibromodulin, with the homologous collagen-binding sequence in LRR 7, the two units of initially cross-linked collagens can assemble further in third dimension, and the mature cross-links can be formed between the collagens (red).
SUPPLEMENTAL FIGURE 1

Primers used for PCR amplifications

LUMICAN FRAGMENTS

LumN-4
Forward: AAA GGA TCC CAG TAC TAT GAT TAT GAT TTT CC
REVERSE: AAA CCC GGG CAG CTT GTG GAT CTT GTT ATG A

Lum5-7
Forward: AAA GGA TCC AAC AAG ATC ACA AAG CTG GGC
REVERSE: AAA CCC GGG GAT GTT GCT GAT CTT ATT GTT G

Lum8-12
Forward: AAA GGA TCC AAT AAG ATC AGC AAC ATC CCT
REVERSE: AAA CCC GGG ATT AAG AGT GAC TTC GTT AGC A

BACTERIAL EXPRESSION FULL-LENGTH LUMICAN

Forward: TTT GAA TTC CCA GTA CTA TGA TTA TGA TTT TC
REVERSE: TTT CTC GAG ATT AAG AGT GAC TTC GTT AGC A

MAMMALIAN EXPRESSION FULL-LENGTH LUMICAN

Forward: TTT CTC GAG CCA GTA CTA TGA TTA TGA TTT TC
REVERSE: TTT GGA TCC TTA ATT AAG AGT GAC TTC GTT AGC A

SITE-DIRECTED MUTAGENESIS

Lumican D213N mutation
Forward: CTA ACT CTC TAC TTA AAC AAT AAG ATC AGC AAC ATC CCT G
REVERSE: C AGG GAT GTT GCT GAT CTT ATT GTT GTT TAA GTA GAG AGT TAG

Lumican D222N mutation
Forward: G ATC AGC AAC ATC CCT AAT GAG TAT TTT AGT TTT AAC GC
REVERSE: GC ATT AAA ACG TTT GAA ATA CTC ATT AGG GAT GTT GCT GAT C

Fibromodulin E251Q mutation
Forward: GAG CAG CTG TAC CTG CAG CAC AAC AAC GTC TTC
REVERSE: GAA GAC GTT GTT GTG CTC CAG GTA CAG CTG CTC
SUPPLEMENTAL FIGURE 2

Alignment of lumican LRRs 5-7 in species

Protein sequences retrieved from Protein Knowledgebase (UniProtKB) [http://beta.uniprot.org](http://beta.uniprot.org)
Sequence alignment performed using ClustalW2 software [http://www.ebi.ac.uk/Tools/clustalw2/index.html](http://www.ebi.ac.uk/Tools/clustalw2/index.html)

### Alignment of fibromodulin and lumican LRRs 5-7

| Species                    | Sequence Alignment                                      |
|----------------------------|----------------------------------------------------------|
| Mus musculus               | KGPMQAGLEGHLTHALYLRHKQ---VGSNHGRLSGLSLLKL---LSTYNH3RVPQDGLR-PALCEQLYLMHRYV |
| Rattus norvegicus          | KGPMQAGLEGHLTHALYLRHKQ---VGSNHGRLSGLSLLKL---LSTYNH3RVPQDGLR-PALCEQLYLMHRYV |
| Bos taurus                 | KGPMQAGLEGHLTHALYLRHKQ---VGSNHGRLSGLSLLKL---LSTYNH3RVPQDGLR-PALCEQLYLMHRYV |
| Homo sapiens               | KGPMQAGLEGHLTHALYLRHKQ---VGSNHGRLSGLSLLKL---LSTYNH3RVPQDGLR-PALCEQLYLMHRYV |
| Equus caballus             | KGPMQAGLEGHLTHALYLRHKQ---VGSNHGRLSGLSLLKL---LSTYNH3RVPQDGLR-PALCEQLYLMHRYV |
| Galeus gallus              | KGPMQAGLEGHLTHALYLRHKQ---VGSNHGRLSGLSLLKL---LSTYNH3RVPQDGLR-PALCEQLYLMHRYV |
| Homo sapiens               | --TGSFEGEGLVNL7FL7HQMRLQKE--AVSFAGFAGFVGLLQVTL0---LSFQ141L7PQ6L-YSLLTLYLNYHI |
| Macaca fascicularis        | --TGSFEGEGLVNL7FL7HQMRLQKE--AVSFAGFAGFVGLLQVTL0---LSFQ141L7PQ6L-YSLLTLYLNYHI |
| Mus musculus               | --TGSFEGEGLVNL7FL7HQMRLQKE--AVSFAGFAGFVGLLQVTL0---LSFQ141L7PQ6L-YSLLTLYLNYHI |
| Rattus norvegicus          | --TGSFEGEGLVNL7FL7HQMRLQKE--AVSFAGFAGFVGLLQVTL0---LSFQ141L7PQ6L-YSLLTLYLNYHI |
| Bos taurus                 | --TGSFEGEGLVNL7FL7HQMRLQKE--AVSFAGFAGFVGLLQVTL0---LSFQ141L7PQ6L-YSLLTLYLNYHI |
| Equus caballus             | --TGSFEGEGLVNL7FL7HQMRLQKE--AVSFAGFAGFVGLLQVTL0---LSFQ141L7PQ6L-YSLLTLYLNYHI |
| Galeus gallus              | --TGSFEGEGLVNL7FL7HQMRLQKE--AVSFAGFAGFVGLLQVTL0---LSFQ141L7PQ6L-YSLLTLYLNYHI |
| Cetacea borealis japonica  | TVFYCALEGHLTHALYLRHKQ---VGSNHGRLSGLSLLKL---LSTYNH3RVPQDGLR-PALCEQLYLMHRYV |
| Xenopus tropicalis         | --TGSFEGEGLVNL7FL7HQMRLQKE--AVSFAGFAGFVGLLQVTL0---LSFQ141L7PQ6L-YSLLTLYLNYHI |
| Danio rerio                | --TGSFEGEGLVNL7FL7HQMRLQKE--AVSFAGFAGFVGLLQVTL0---LSFQ141L7PQ6L-YSLLTLYLNYHI |
| Aedes aegypti              | --TGSFEGEGLVNL7FL7HQMRLQKE--AVSFAGFAGFVGLLQVTL0---LSFQ141L7PQ6L-YSLLTLYLNYHI |

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Figure 1

A

B

C

![Diagram A](LumN-4 | Lum5-7 | Lum8-12)

![Diagram B](67 | 43 | 30 | 20)

![Diagram C](Added ligand (nM))

OD 405nm
Homologous sequence in lumican and fibromodulin LRR 5-7 competes for collagen binding
Sebastian Kalamajski and Åke Oldberg

J. Biol. Chem. published online November 13, 2008

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