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(54) Title: SYNDECAN INTERACTING PROTEINS AND THE USE THEREOF

(57) **Abrégé/Abstract:**

The invention relates to the identification of a protein that binds to the cytoplasmic domain of the syndecan. This protein, now called syntenin, contains a tandem repeat of PDZ-domains that reacts with the FYA C-terminal amino acid sequence of the syndecans (Trends Biochem.Sci., 20, p. 350, 1995: Origin of PDZ {DHR, GLGF}, by M.B. Kennedy). Endogenous syntenin appears to be localized to the cytoskeleton. GFP-syntenin fusion-proteins decorate the plasma membrane and intracellular vesicles, where they co-localize and -segregate with syndecan cytoplasmic domains. Syntenin, therefore, is an unexpected candidate for connecting the cytoskeleton to heparan sulfate-assisted signal transduction pathways. The syndecan cytoplasmatic link (sycl) protein or syndecan interacting protein is referred to by the name "syntenin", meaning "putting tension on the syndecans".

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(54) Title: SYNDECAN INTERACTING PROTEINS AND THE USE THEREOF (57) Abstract The invention relates to the identification of a protein that binds to the cytoplasmic domain of the syndecan. This protein, now called syntenin, contains a tandem repeat of PDZ-domains that reacts with the FYA C-terminal amino acid sequence of the syndecans (Trends Biochem.Sci., 20, p. 350, 1995: Origin of PDZ {DHR, GLGF}, by M.B. Kennedy). Endogenous syntenin appears to be localized to the cytoskeleton. GFP-syntenin fusion-proteins decorate the plasma membrane and intracellular vesicles, where they co-localize and -segregate with syndecan cytoplasmic domains. Syntenin, therefore, is an unexpected candidate for connecting the cytoskeleton to heparan sulfate-assisted signal transduction pathways. The syndecan cytoplasmatic link (sycl) protein or syndecan interacting protein is referred to by the name "syntenin", meaning "putting tension on the syndecans".		

Syndecan interacting proteins and the use thereof

The present invention relates to syndecan interacting protein(s) and the use thereof.

The invention relates to the identification of a protein that binds to the cytoplasmic domain of the syndecan. This protein, now called syntenin, contains a tandem repeat of PDZ-domains that reacts with the FYA C-terminal amino acid sequence of the syndecans (Trends Biochem.Sci., 20, p. 350, 1995: Origin of PDZ {DHR, GLGF}, by M.B.Kennedy). Endogenous syntenin appears to be localized to the cytoskeleton.

GFP-syntenin fusion-proteins decorate the plasma membrane and intracellular vesicles, where they co-localize and -segregate with syndecan cytoplasmic domains. Syntenin, therefore, is an unexpected candidate for connecting the cytoskeleton to heparan sulfate-assisted signal transduction pathways.

The syndecan cytoplasmatic link (sycl) protein or syndecan interacting protein is referred to by the name "syntenin", meaning "putting tension on the syndecans".

Heparan sulfate proteoglycans (HSPG) are proteins which are mostly associated with the cell membrane. The most characterizing feature of these proteins is that they are substituted with different heparan sulfate sugar derivatives which strongly determine the function of said protein.

Basically there are two classes of membrane heparan sulfate proteoglycans. Firstly the best characterized membrane proteoglycans are the syndecans, which have a

membrane spanning core protein. The second class is the glypican family which are located at the cell surface and anchored in the cell membrane through GPI (glycosyl phosphatidyl inositol).

The existence of two distinct highly conserved multigene families of cell surface proteoglycans, the syndecan and glypican families respectively, suggests two distinctive cellular and/or subcellular pathways wherein these proteins will function, and ramifications within these pathways that are specified by the variations on a basic structural theme that are realized by the various members of these families.

Above mentioned HSPGs are involved in several physiological processes and play definitely a role in the transmission of signals from outside a cell into the cell itself.

Probably their activities are characterized by the specific binding of heparan sulfate molecules to proteinase inhibitors, cell adhesion molecules or growth factors.

If the structure of HSPG is disturbed, abnormal cell growth and abnormal morphogenesis occur. Therefore it is of utmost importance to know and understand the structure-function relation of the proteoglycans and the way they transmit outside signals through the cell membrane into the cell, the so-called signal transduction cascade.

As mentioned above syndecans are transmembrane proteoglycans that place structurally heterogeneous heparan sulfate chains at the cell surface and a highly conserved polypeptide in the cytoplasm. Their versatile heparan sulfate moieties support various processes of molecular recognition, signaling and trafficking.

The cell surface heparan sulfate proteoglycans are at the cross-section of several different pathways. Their heparan sulfate moieties bind various differentiation-, growth-, and scatter factors, facilitate the occupation and activation

of the corresponding signal-transducing receptors, and are involved in the internalisation and clearance of the signaling complexes from the cell surface. They also assist receptors that are involved in cell-cell and cell-matrix adhesion, and assist scavenging receptors that are involved in the endocytosis and transcytosis of lipoproteins and lipases. They also bind and activate serine proteinase inhibitors and accelerate the reactions of these inhibitors with their targets. Proteolysis, lipolysis, mesoderm-induction, gastrulation, angiogenesis, neuritogenesis all appear to be regulated by or to depend on heparan sulfate, because this glycosaminoglycan is needed for the allosteric activation, approximation and compartmentalisation of the reactants that are engaged in these processes.

In most cells syndecans represent the major source of cell surface heparan sulfate. The four known syndecans are small type I membrane proteins, with similar and simple domain organisations: a single ectodomain, membrane-span, and cytoplasmic domain. Except for the presence of three or four consensus sites for heparan sulfate attachment, near the amino-termini of the proteins, and a dibasic, presumably protease-sensitive site at the junctions with the membrane spanning segments, the ectodomains of the different syndecans have little in common. The structures of these ectodomains have also not been evolutionary conserved, except again for these shared structural elements. The membrane-spanning and the small cytoplasmic domains of the syndecans, in contrast, show extensive structural similarity (60% sequence identity) and have been highly conserved during evolution. All four vertebrate syndecans and the single *Drosophila* syndecan share the amino acid sequence RM(K/R)KKDEGSY- depicted in one-letter code- in the membrane-proximal segments of their cytoplasmic domains, and the amino acid sequence

EFYA -depicted in one letter code- at their C-termini. This suggests that the extracellular heparan sulfate moieties, the cytoplasmic protein moieties, and the contiguity of these moieties are essential for syndecan function. The syndecans may provide for a transmembrane link between extracellular heparin-steered processes and intracellular structural or regulatory proteins, and mediate outside-in or inside-out effects on signaling or effector systems.

In order to understand above mechanism/cascade a search for syndecan cytoplasmic links (sycls) was initiated using the cytoplasmic domains of four different syndecans as baits in a yeast two-hybrid screening assay. The insert of one apparent truly positive clone (yielding HIS⁺ and LacZ⁺ phenotypes in combination with all four syndecan/Gal4 DNA-binding domain fusion constructs, but not with Gal4 DNA-binding domain alone or with p53 fused to the Gal4 DNA-binding domain) was sequenced, and used as a starting point to obtain a cDNA coding for the corresponding full length sycl protein.

The present invention concerns syndecan interacting protein(s) obtainable by a two-hybrid screening assay whereby as bait a cytoplasmic domain comprising the amino acid sequence FYA as C-terminal sequence as occurring in syndecan and as prey a cDNA library are used. For the purpose of the invention the cDNA library can be any suitable cDNA library, but preferably be a mammalian, more preferably a human and most preferably a human liver cDNA library.

The full length sycl thus obtained consists of 298 aminoacids, and can be divided in three or four parts. The first amino-terminal region (aa 1-109) shows no striking homology to any known structural motif. It is relatively rich in proline and contains

five tyrosines, while the remainder of the protein is free of tyrosine. Based on sequence alignments, the second (aa 101-193) and third (aa 194-274) regions of sycl appear to correspond to a tandem repeat of two PDZ domains. The sequence coding for the putative second PDZ domain is extended by 24 amino acids (aa 275-298), which may still be part of the second PDZ domain or compose a fourth separate C-terminal domain. PDZ domains have recently been recognized as one of the conserved modular structures that support protein-protein interactions and networking.

PDZ domains mediate protein-protein interactions by binding to the carboxy-terminal ends of target proteins, and often occur in association with other functional modules, such as SH3 domains, protein tyrosine phosphatase domains, domains related to guanylate kinase (GUK), to band 4.1 protein, leucine zipper motifs, and additional PDZ domains. PDZ domains have now been discovered in a variety of proteins, and shown to bind to membrane channels, receptors (e.g. wingless and Notch), tumor suppressor proteins (APC), GAPs and GEFs. These interactions appear to be involved in the formation of multimeric protein complexes that influence receptor positioning and clustering, and the connections of receptors and receptor-associated molecules to cytoskeletal proteins and downstream signal effectors. Further tests were aimed at investigating the involvement of the sycl PDZ domains in the syndecan-sycl interaction, and potential further links of sycl to the intracellular cytoskeleton.

The Genbank accession number for this sycl/syntenin cDNA and protein sequence according to the invention is AF000652.

The yeast two-hybrid system (Chien et al, 1991; P.N.A.S.,88, p.9578-9582) was used to identify the domains of sycl and the residues in the syndecan cytoplasmic domains that are responsible for the syndecan-sycl interaction. Different parts of the original clone (3p11) and of the sycl cDNA were subcloned in the pGAD10 vector (Clontech Laboratories), to code for fusions between the activating domain of Gal4 (Gal4 AD) and various full length, truncated, deleted, point-mutated, or epitope-tagged versions of sycl.

Full length sycl (tagged in the C-terminus with myc-epitope or not) and the original isolate (missing the first 91 aa of sycl) were able to interact with the full length syndecan (2 or 3) cytoplasmic domains, as revealed by growth on His- plates and β -gal activity. Even the fragment missing the first 112 amino acids of sycl is able to interact with the full length of syndecan. The N-terminal domain alone and either of the two PDZ domains alone were all inactive, suggesting that the paired PDZ domains are required for the binding interaction. Deletion of the complete N-terminal region (aa position 1-113), however, also abolished the interaction, indicating that the two PDZ domains and part of the N-terminal sequence are required (to allow the correct folding of the PDZ domains or for the interaction itself). It is not clear why the PDZ domains of sycl are not or less active separately. But a requirement for paired PDZ domains (for instance two PDZ1 or two PDZ2 domains) has also been observed for the interactions of the GRIP adaptor protein with AMPA receptors¹² and the interaction of hDlg with with the cytoplasmic domain of the Shaker-type K⁺channel.

The sycl binding sites in the syndecan cytoplasmic domain were deduced from the testing of a series of deletion- and alanine-substitution mutants of the syndecan-2 cytoplasmic domain. These mutants were cloned in pAS2, as Gal4 DNA-binding domain fusion proteins, and expressed as partners for the Gal4 activation domain-full length sycl fusion protein. All the C-terminal deletions and the substitutions of alanine for the F-residue at -2 and the Y-residue at -1, but not the alanine substitution for the E-residue at -3,abolished the binding interaction. Deletion of all but the last four residues from the cytoplasmic domain, only partially abolished the interaction (His-,but gal+). This localizes the binding site to the C-terminal FYA sequence of the syndecans, and appears consistent with the concept that PDZ proteins mediate protein-protein interactions by binding to the C-termini of proteins and discriminate amongst these sequences.

To examine the interaction between the syndecan cytoplasmic domain and sycl in systems other than yeast, the various sycl and syndecan constructs were also expressed as GST-fusion proteins (Glutathione S-Transferase), and used in ligand blot-assays and surface plasmon resonance experiments. In ligand blots, GST-sycl full length (FL) and GST-sycl M92 (sycl sequence starting at M92) fusion proteins failed to bind to GST itself, to themselves, or to fusion proteins composed of GST and to the C-terminal deletion, the F(C30)A or Y(C31)A mutants (at positions 30 and 31 respectively), of the syndecan-2 cytoplasmic domain. The same GST-sycl constructs, in contrast, bound to fusion proteins composed of GST and any of the other syndecan-2 mutants or four different wild type syndecan cytoplasmic domains. In biosensor experiments, a streptavidin-immobilized biotinylated synthetic peptide, representing the 32 cytoplasmic amino acids of syndecan-2, bound the GST-sycl FL

and GST- sycl M92 proteins, but not fusion proteins composed of GST and parts of sycl containing only one of the two PDZ domains.

Unlike the yeast two-hybrid assay, however, the biosensor experiment showed some binding of sycl that missed all of the N-terminal domain but retained both PDZ domains. A calculation of the k_{on} and k_{off} values for the binding of sycl, from curves obtained at different concentrations of the GST-sycl FL or M92 fusion proteins and using the BIAcore2000 software, proved less relevant since the data were not consistent with a simple binding interaction, but the data suggest rapid dissociation ($k_{off} = 10^{-3}$, if one would accept a $A+B \rightleftharpoons AB$ interaction model). The sycl moiety itself, isolated from the fusion protein with the help of factor Xa, also bound to the synthetic peptide, indicating that the GST-moiety itself was not needed. No sycl-peptide binding interaction could be observed, however, when the GST-sycl proteins were immobilized on a biosensor chip coated with an anti-GST antibody and the peptide was perfused over the immobilized sycl. These data suggest that the sycl-syndecan interaction may be very transient and depend on cooperative syndecan interactions. This implies that sycl-syndecan interactions may require the clustering of the syndecans.

The subcellular expression of sycl and its potential colocalization with syndecan was investigated with the help of monoclonal antibodies raised against sycl fusion proteins.

Association was investigated by immunohistochemistry, and through the overexpression of syndecans and Green Fluorescent Protein-fusion (GFP) proteins in CHO-cells. In non-transfectant CHO cells most of the sycl-immunoreactivity was associated with cytoplasmic fibrils, identifying sycl as a cytoskeleton-associated

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protein. These sycl-fibrils or sycl-decorated fibrils ran criss-cross through the cytoplasm, with a perinuclear rather than a subcortical distribution. Little or no membrane staining was observed in these cells, indicating sycl was scarce at this site or undetectable with the current tools. To investigate possible co-localisations and interactions of syndecans and sycl, CHO cells that were stably transfected with an expression plasmid coding for human syndecan-1 (chosen because of the availability of a monoclonal antibody for the syndecan-1 cytoplasmic domain) or syndecan-2 were transiently supertransfected with plasmids coding for eGFP (enhanced green fluorescent protein) or an eGFP-sycl (enhanced green fluorescent protein-syndecan cytoplasmic ligand) fusion-protein. CHO cells overexpressing eGFP-sycl showed a striking change in morphology in comparison to non-transfected cells and cells expressing eGFP (see figure 1 and 2 respectively). These cells were larger and flatter, and were decorated with numerous membrane projections or filopodia. Often, these cells were also multinucleated. The eGFP-sycl protein autofluorescence was localized to the cytoplasm, to small intracellular vesicular structures, to the plasmamembrane, and partially colocalized with F-actin filaments, as revealed by staining with phalloidin-Texas Red-X. Green fluorescent eGFP-sycl transfectants were intensely stained by anti-sycl monoclonal antibody, indicating marked overexpression of sycl protein in these cells. Staining of the syndecan-1 double transfectants with antibody directed towards the cytoplasmic domain of syndecan-1 and Texas Red-X-labeled secondary antibody, showed partial colocalization of this syndecan domain and eGFP-sycl. No colocalization of green and red fluorescence was observed in control cells transfected with eGFP. Capping

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of the syndecan-1 cytoplasmic domain could be induced by mild and brief trypsinization of the cells at 4°C, followed by a 10-30 min reincubation of the cells at 37°C. Massive co-capping of the red (syndecan-1 cytoplasmic domain) and green

fluorescence (GFP) was observed in eGFP-sycl expressing cells, but not in eGFP expressing cells, indicating colocalization of sycl and this syndecan domain during the capping process. Similar, but less perfect, colocalizations were observed with a syndecan-1 ectodomain antibody in the syndecan-1 transfectants, and with a syndecan-2 ectodomain antibody in the syndecan-2 transfectants. These data show that sycl associates with the cytoskeleton and with the cytoplasmic domains of the syndecans, and function as an adaptor molecule that links syndecan-supported recognition processes to intracellular signal-transduction, -control, and -effector systems.

The current invention thus concerns an isolated nucleic acid sequence comprising the nucleotide sequence as provided in SEQ ID NO. 2 coding for a syndecan interacting protein or a functional fragment thereof. "Functional fragment" in this context means that said fragment to which subject it relates has substantially the same activity as the subject itself, although the form, length or structure may vary.

Furthermore a recombinant expression vector comprising said isolated nucleic acid sequence (in sense or anti-sense orientation) operably linked to a suitable control sequence belongs to the present invention and cells transfected or transduced with said recombinant expression vector also belong to the scope of the invention.

The current invention is not limited to the exact isolated nucleic acid sequence comprising the nucleotide sequence as mentioned in SEQ ID NO. 2 but also a nucleic acid sequence hybridizing to said nucleotide sequence as provided in SEQ ID NO. 2 or a functional part thereof and encoding a syndecan interacting protein or a functional fragment thereof belongs to the present invention.

To clarify, with "hybridization" is meant conventional hybridization conditions known to the skilled person, preferably appropriate stringent hybridization conditions. Hybridization techniques for determining the complementarity of nucleic acid sequences are known in the art.

The stringency of hybridization is determined by a number of factors during hybridization, including temperature, ionic strength length of time and composition of the hybridization buffer. These factors are outlined in, for example, Maniatis et al. (1982) Molecular Cloning; A laboratory manual (Cold Spring Harbor Press, Cold Spring Harbor, N.Y.)

Another aspect of the invention is a polypeptide comprising the amino acid sequence according to SEQ.ID.NO.1 or a functional fragment thereof.

Specifically the polypeptide fragments with the amino acid sequence located between position 92 and 298 and more specifically between 113 and 298 belong to the current invention.

To the scope of the present invention also belong variants or homologues of amino acids enclosed in the polypeptide wherein said amino acids are substituted by other amino acids obvious for a person skilled in the art without losing their activity.

The polypeptide or functional fragments thereof according to the present invention are not necessarily translated from the nucleic acid sequence according to the invention but may be generated in any manner, including for example, chemical synthesis, or expression of a recombinant expression system.

A pharmaceutical composition comprising above mentioned nucleic acid(s) or a pharmaceutical composition comprising said polypeptide(s) also belong to the current invention. The nucleic acid and/or polypeptide according to the invention can be optionally used for appropriate gene therapy purposes.

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According to one aspect of the present invention, there is provided an isolated syndecan interacting protein characterized in that said syndecan interacting protein (a) contains a tandem repeat of PDZ-domains reacting with the
5 FYA C-terminal amino acid sequence of syndecan, (b) is represented by a 2.19 kb cDNA encoding a polypeptide of 298 amino acids of SEQ ID NO: 1, (c) contains five tyrosine residues in the amino terminal region represented by amino acids 1-109 of SEQ ID NO: 1, and (d) said syndecan
10 interacting protein containing no tyrosine residues in amino acids 110-298 of SEQ ID NO: 1.

According to another aspect of the present invention, there is provided an isolated polypeptide comprising the amino acid sequence according to SEQ ID NO:1
15 or a functional fragment thereof comprising at least one PDZ domain or the N-terminal region (amino acid positions 1-113 of SEQ ID NO: 1), wherein said fragment or N-terminal region binds to syndecan.

According to still another aspect of the present
20 invention, there is provided a composition comprising a polypeptide comprising the amino acid sequence according to SEQ ID NO: 1 or a functional fragment thereof comprising at least one PDZ domain or the N-terminal region (amino acid positions 1-113 of SEQ ID NO: 1), wherein said fragment or
25 N-terminal region binds to syndecan and a carrier.

According to yet another aspect of the present invention, there is provided A method for screening compounds that interfere with the interaction between

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syndecan and syndecan interacting protein, said method comprising: (a) adding a compound to a solution comprising syndecan and syndecan interacting protein; and (b) monitoring the interaction between syndecan and syndecan
5 interacting protein.

In addition a method for diagnosing, prognosis and/or follow-up of a disease by using the nucleic acid(s) according to the invention or by using the polypeptide(s) also form part of the current invention.

A method of screening for components which affect the interaction between syndecan and syndecan interacting protein can be developed having the current knowledge of these syndecan interacting factors according to the invention.

A diagnostic kit comprising the nucleic acid(s) and/or polypeptide(s) according to the invention for performing above mentioned method for diagnosing, prognosis and/or follow-up of a disease clearly belong to the invention as well.

Some diseases in this respect are for instance Alzheimer disease, inflammatory diseases but also screening for cell malignancies and the activity of cytostatica thereupon.

A transgenic animal harbouring the nucleic acid(s) according to the invention in its genome also belong to the scope of this invention.

With transgenic animal is meant a non-human animal which have incorporated a foreign gene (called transgene) into their genome; because this gene is present in germ line tissues, it is passed from parent to offspring establishing lines of transgenic animals from a first founder animal.

It will be appreciated that when a nucleic acid construct is introduced into an animal to make it transgenic the nucleic acid may not necessarily remain in the form as introduced.

By "offspring" is meant any product of the mating of the transgenic animal whether or not with another transgenic animal, provided that the offspring carries the transgene.

In order to clarify what is meant in this description by some terms a further explanation is hereunder given.

The polypeptides of the present invention are not necessarily translated from a designated nucleic acid sequence; the polypeptides may be generated in any manner, including for example, chemical synthesis, or expression of a recombinant expression system, or isolation from a suitable viral system. The polypeptides may include one or more analogs of amino acids, phosphorylated amino acids or unnatural amino acids. Methods of inserting analogs of amino acids into a sequence are known in the art. The polypeptides may also include one or more labels, which are known to those skilled in the art.

The terms "gene(s)", "polynucleotide", "nucleic acid sequence", "nucleotide sequence", "DNA sequence" or "nucleic acid molecule(s)" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, and RNA. It also includes known types of modifications, for example, methylation, "caps" substitution of one or more of the naturally occurring nucleotides with an analog.

An "expression vector" is a construct that can be used to transform a selected host cell and provides for expression of a coding sequence in the selected host.

A "coding sequence" is a nucleotide sequence which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, recombinant nucleotide sequences or genomic DNA, while introns may be present as well under certain circumstances.

"Control sequence" refers to regulatory DNA sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of

such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoter, ribosomal binding site, and terminators. In eukaryotes generally control sequences include promoters, terminators and, in some instances, enhancers, transactivators or transcription factors. The term "control sequence" is intended to include, at a minimum, all components the presence of which are necessary for expression, and may also include additional advantageous components.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. In case the control sequence is a promoter, it is obvious for a skilled person that double-stranded nucleic acid is used.

The terms "protein" and "polypeptide" used in this application are interchangeable. "Polypeptide" refers to a polymer of amino acids (amino acid sequence) and does not refer to a specific length of the molecule unless otherwise specified in the description. Thus peptides and oligopeptides are included within the definition of polypeptide. This term does also refer to or include post-translational modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

"Fragment of a sequence" or "part of a sequence" means a truncated sequence of the original sequence referred to. The truncated sequence (nucleic acid or protein sequence) can vary widely in length; the minimum size being a sequence of sufficient size to provide a sequence with at least a comparable function and/or activity of the original sequence referred to, while the maximum size is not critical. In some applications, the maximum size usually is not substantially greater than that required to provide the desired activity and/or function(s) of the original sequence.

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The term "antibody" includes, without limitation, chimeric antibodies, altered antibodies, univalent antibodies, bi-specific antibodies, Fab proteins or single-domain antibodies. In many cases, the binding phenomena of antibodies to antigens is equivalent to other ligand/anti-ligand binding. The antibody can be a monoclonal or a polyclonal antibody.

"Sense strand" refers to the strand of a double-stranded DNA molecule that is homologous to a mRNA transcript thereof. The "anti-sense strand" contains an inverted sequence which is complementary to that of the "sense strand".

"Expression" means the production of a protein or nucleotide sequence in the cell itself or in a cell-free system. It includes transcription into an RNA product, post-transcriptional modification and/or translation to a protein product or polypeptide from a DNA encoding that product, as well as possible post-translational modifications.

"Foreign" with regard to a DNA sequence means that such a DNA is not in the same genomic environment in a cell, transformed with such a DNA in accordance with this invention, as is such DNA when it is naturally found in a cell from which such a DNA originates.

In the description of the current invention reference is made to the following sequences of the Sequence Listing and Figures.

SEQ ID NO. 1: amino acid sequence (position 1-298) for syndecan interacting protein (one letter code)

SEQ ID NO. 2: cDNA sequence (position 1-2193) encoding for the amino acid sequence of SEQ ID NO: 1, of 298 amino acids; ATG start codon at position 149

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SEQ ID NO. 3: cDNA sequence together with amino acid sequence encoding
syndecan interacting protein

Figure 1 (control) and figure 2 represent an eGFP (enhanced green fluorescent protein) and an eGFP-sycl (enhanced green fluorescent protein-syndecan cytoplasmic ligand) fusion-protein staining of CHO cells overexpressing sycl protein showing a striking change in morphology.

METHODS

Yeast two-hybrid screening

Baits, comprising at least a cytoplasmic domain FYA (corresponds to Phe-Tyr-Ala) as C-terminal sequence of syndecans fused in frame to the DNA-binding domain of Gal4, were constructed by PCR, using the BamHI/Sall restriction sites of the pAS2 vector (Clontech Laboratories), and used to screen a human liver cDNA library (Clontech Laboratories) cloned in pGAD10. The pAS2 plasmids and library were cotransfected in CG1945 yeast cells, and positive clones were selected on triple minus plates (Leu-, Trp-, His-) and assayed for β -galactosidase activity. Positive cDNA clones were cotransfected with all four bait vectors and with the original pAS2 vector cells to confirm the interaction. Mutations in the syndecan and sycl constructs were made by PCR, using the QuickChange^{*} site-directed mutagenesis protocol and reagents (Stratagene). All the constructs and mutants that were tested in the matings were confirmed by sequencing.

* Trade-mark

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Cloning of sycl

The insert from the selected clone (3p11) was recloned in pBluescript^{*} and completely sequenced. Full length cDNA for sycl was obtained from the sequencing and merging of the I.M.A.G.E. clones #33203 and #285265 (obtained through Research Genetics), identified as containing parts of the sycl sequence (from a BLAST search of the NCBI database of EST Sequences).

Production of GST-fusion proteins

Fusion proteins between glutathione S-transferase (GST) and sycl or parts of sycl, and wild-type or mutant syndecan cytoplasmic domains were produced by cloning the cDNAs into the pGEX-5X-2 vector (Pharmacia Biotech) and expression into E. coli BL21 cells. Cells, induced for 3 h with 10 mM IPTG, were suspended in 100 mM NaCl, 1 mM EDTA, 50 mM Tris-HCl, pH 8.0, supplemented with the proteinase inhibitors aprotinine, benzamidine, leupeptine, pepstatin, 6-aminohexanoic acid and PMSF. After treatment with lysozyme for 1 h, on ice, the fusion protein was recovered from the water soluble phase, and purified by affinity chromatography over glutathione-Sepharose 4B^{*} (Pharmacia Biotech).

Surface Plasmon Resonance measurements

300 RU of biotinylated synthetic peptide (32aa), corresponding to the cytoplasmic domain of syndecan-2, were immobilized on the Fc2 surface of a Streptavidin (SA)-sensor chip. Analyte was perfused (10 µl/min) over the Fc1(control) and Fc2 (capture) surfaces in running buffer (100 mM NaCl, 0.005% surfactant P20, 10 mM HEPES, pH 7.4). Binding was monitored in a Biacore 2000^{*} instrument (Pharmacia

* Trade-mark

Biosensor), and measured as the difference between Fc2 and Fc1 binding curves. The surface was regenerated through three 1 min pulses of 1M NaCl, 0.05 M NaOH.

Production of sycl antibodies

Sycl was tagged with a myc-epitope at its C-terminus and cloned into the pQE-11 prokaryotic expression vector (Qiagen), introducing a 6xHis-tag at the N-terminus of the protein. After expression in E.coli, the 6xHis-sycl-myc protein was extracted in 4M guanidinium chloride and purified by chromatography over a Ni-NTA column (Qiagen), using the myc-tag to monitor the purification of the protein. The protein was used for the immunization of mice and the production of sycl-specific monoclonal antibody, using standard hybridoma technology.

Cell transfections with eGFPsy클

The full length sy클 and 3p11 cDNAs were subcloned in pEGFP-C1 using the XhoI restriction sites of the vector.

Additional data relating to the syndecan-interacting protein "syntenin"

1. The second PDZ-domain of syntenin is crucial for the syndecan-syntenin binding interaction:

Prior deletion experiments had indicated that the N-terminal domain of syntenin alone and either of the two PDZ domains of syntenin alone were inactive in binding (the cytoplasmic domain of the) syndecans. Syndecan-binding was limited to the combination of the two PDZ domains (and possibly part of the N-terminal region).

Yet, point mutagenesis of K119 (PDZ-1), K203 (PDZ-2), and of the alpha-B helices of PDZ-1 and PDZ-2 in (full length) syntenin shows that syndecan-syntenin binding involves PDZ-2 (only the mutagenesis of PDZ-2 reduced the binding of GST-syntenin to immobilized syndecan-2 peptide in BIAcore experiments). If syndecan binds directly and only to PDZ-2, it remains to be explained why the isolated PDZ-2 domain of syntenin is less active than the paired domains. Together with the failure of (immobilized) syntenin to bind free syndecan-peptide (added in solution) and the evidence that residues 92-298 of syntenin interact with full length syntenin and with the isolated N-terminal domain of syntenin, this may indicate that the syndecan-syntenin interaction requires oligomerisation of both syndecan and syntenin.

2. While syndecan binds syntenin via its t-FYA sequence, the specificity of the syndecan-syntenin interaction may involve additional syndecan structural elements:

Syntenin binds to a peptide representing the full length of the syndecan-2 cytoplasmic domain (yeast two-hybrid, GST-fusion protein overlays, and BIAcore experiments) and to a peptide comprising EFYA as its C-terminus. In combination with the mutagenesis experiments (deletions and point mutations of the syndecan-2 peptide sequence) this shows that syndecan-syntenin binding involves the C-terminal FXA sequence of the syndecans. Yet, while some PDZ-proteins other than syntenin interact with Gal4-EFYA fusion proteins in the yeast-two-hybrid assay, these do not interact with Gal4-syndecan baits. This demonstrates that the context upstream of the t-FYA sequence needs to be "permissive" and may help determining the specificity of the syndecan-syntenin (peptide-PDZ) interaction. This implies that, in addition to syndecans, several, but not all proteins terminating with t-FXA may be binding partners (and compete with syndecans) for syntenin.

3. The syndecan-syntenin interaction occurs in situ, but may not be "constitutive":

Clear membrane-recruitment of transiently expressed eGFP-syntenin (fusion protein) and colocalization of this eGFP-syntenin with syndecans was observed in cells that overexpress syndecans. To investigate the possible interactions of the natural molecules, mouse monoclonal antibodies were raised against recombinant human syntenin and used for immuno-purification of endogenous syntenin and determining its associations. In Western blots, these antibodies react with GST-syntenin fusion proteins, but not with the isolated GST-moiety of these fusion proteins. In immuno-fluorescence microscopy, these antibodies stain cells that overexpress eGFP-syntenin (colocalization of red anti-mouse and green eGFP-fluorescence) more intensely than non green-fluorescent cells or cells that express eGFP. This establishes the reactivity of these antibodies with the syntenin peptide. Immunoprecipitation of syntenin from (MDCK) cell extracts with one of these antibodies (mAb 4F6), and development of the immunoprecipitates with anti-syndecan-1 antibody indicates that syndecan-1 co-precipitates with syntenin in subconfluent (spreading, unpolarized) MDCK cells, but not (or clearly less) in confluent (polarized) MDCK cells. This suggests that syntenin-syndecan association occurs in situ, but that it may be conditional upon the state of the cells.

4. Syntenin is a component of the cytoskeleton:

In immuno-fluorescence microscopy of permeabilized (human fibroblasts, CHO, Swiss-3T3, MDCK) cells, two mAbs (4F6 and 4D12, directed against different syntenin epitopes) stain primarily the microtubular network (colocalization with anti-tubulin). Association of (at least a fraction or particular pool of) syntenin with

microtubuli is supported by the copurification (and specific enrichment) of the 4F6 epitope with microtubuli during successive cycles of polymerization and depolymerization of microtubuli from pig brain extracts (a classical source and procedure for the isolation of these cytoskeletal structures). A second set of anti-syntenin mAbs (1C8 and 9C10), in contrast, decorates the F-actin-containing stress fibers (colocalization with falloidin-FITC) of (CHO) cells, but not the microtubuli of these cells. An anti-syndecan-1 antibody, finally, stains primarily the lateral plasma membranes of confluent (MDCK) cells, but reacts primarily with small intracellular vesicles that align with the microtubular network in spreading (MDCK) cells. Whereas none of the transiently expressed eGFP-tagged syntenin constructs (N- or C-terminal tag) colocalized with microtubuli (microfilament association could not be assessed because of some association with microfilaments of eGFP itself), transiently expressed C-terminal myc-tagged M92 syntenin (syntenin lacking the first 91 aminoacids, the N-terminal domain) also aligned with stress fibers, but not with microtubuli (as assessed by anti-myc mAb). Myc-tagged full length syntenin, in contrast, was more cytoplasmic. These data show that syntenin associates with both the actin and the tubulin filament systems of the cells, and that the syntenin-microfilament and syntenin-microtubule associations may involve different parts and conformations of syntenin.

5. Conclusion:

Syntenin links syndecans and syndecan-containing membrane complexes to the cytoskeleton. Syndecans bind to syntenin via their C-terminal ends. These syndecan ends engage the second PDZ domain of syntenin. Theoretically, this leaves the first PDZ domain of syntenin available for the C-terminal peptide from a second syntenin

binder, probably a non-syndecan molecule present in the membranes or cytoplasm of the cells, and leaves the N-terminal domain of syntenin available for yet additional binding partners. The documented self-interaction of the paired PDZ domains of syntenin, and their interaction with the N-terminal domain of syntenin suggest that the molecular associations of syntenin are determined by complex intra- and inter-molecular interactions. Syntenin, possibly different pools or conformations of syntenin, directly or indirectly associates with both microfilaments and microtubuli. This demonstrates that syntenin functions as an adaptor molecule that links syndecans (and possibly other molecules) to the cytoskeleton, e.g. during membrane internalisation (actin filament-associated syntenin) and intracellular membrane traffic (microtubule-associated syntenin).

6. Appendix: Other syntenin localisations.

Preliminary results, obtained by immuno-fluorescence microscopy using the anti-syntenin mAbs, suggest that (a fraction of) syntenin may also occur in the nucleus (of human lung fibroblast) cells. This suggests that syntenin may also participate in nuclear processes (e.g. regulation of transcription).

SEQUENCE LISTING**SEQ ID NO. 1**

(one letter amino acid code)

MSLYPSLEDLKVDKVIQAQTAFSANPANPAILSEASAPIPHDGNLYPRLYPELSQYMGLS	60
LNEEEIRASVAVVSGAPLQGQLVARPSSINYMVAPVTGNDVGIRRAEIK <u>QGIREVILCK</u> *D	120
<u>QDGKIGLRLKSIDNGIFVQLVQANSPASLVGLRFGDQVLQINGENCAGWSSDKAHKVLKQ</u>	180
<u>AFGEKITMTIRDRPFERTITMHK</u> *DSTGHVGFIFKNGKITSIVKDSSAARNGLLTEHNICE	240
<u>INGQNVIGLKDSQIADILSTSGTVVTITIMPAFIFEHIIKRMAPSIMKSLMDHTIPEV</u>	298

Structure of sycl (syntenin). The two PDZ domains are indicated by the single and double underlinings. The lysine/arginine residues that are involved in the stabilization of the C-terminal carboxylates are indicated by *

SEQ ID NO. 2

GGCACGAGGC GGGGGCGGTG CATGACGCGC CTCGGGGGCG GTCCTCGGGC GCGCACCGCT	60
CTCTTACACT CGGGCCTCAG AAGTCCGTGC CAGTGACCGG AGCGGCGGCG GCGAGCGGTT	120
CCTTGTGGGC TAGAAGAATC CTGCAAAAAT GTCTCTCTAT CCATCTCTCG AAGACTTGAA	180
GGTAGACAAA GTAATTCAGG CTCAAACCTGC TTTTCTGCA AACCTGCCA ATCCAGCAAT	240
TTTGTCAGAA GCTTCTGCTC CTATCCCTCA CGATGGAAAT CTCTATCCCA GACTGTATCC	300
AGAGCTCTCT CAATACATGG GGCTGAGTTT AAATGAAGAA GAAATACGTG CAAGTGTGGC	360
CGTGGTTTCT GGTGCACCAC TTCAGGGGCA GTTGGTAGCA AGACCTTCCA GTATAAACTA	420
TATGGTGGCT CCTGTAACCTG GTAATGATGT TGGAATTCGT AGAGCAGAAA TTAAGCAAGG	480
GATTCGTGAA GTCATTTTGT GTAAGGATCA AGATGGAAAA ATTGGACTCA GGCTTAAATC	540
AATAGATAAT GGTATATTTG TTCAGCTAGT CCAGGCTAAT TCTCCAGCCT CATTGGTTGG	600
TCTGAGATTT GGGGACCAAG TACTTCAGAT CAATGGTGAA AACTGTGCAG GATGGAGCTC	660
TGATAAAGCG CACAAGGTGC TCAAACAGGC TTTTGGAGAG AAGATTACCA TGACCATTCTG	720
TGACAGGCCC TTTGAACGGA CGATTACCAT GCATAAGGAT AGCACTGGAC ATGTTGGTTT	780
TATCTTTAAA AATGGAAAAA TAACATCCAT AGTGAAAGAT AGCTCTGCAG CCAGAAATGG	840
TCTTCTCACG GAACATAACA TCTGTGAAAT CAATGGACAG AATGTCATTG GATTGAAGGA	900
CTCTCAAATT GCAGACATAC TGTCAACATC TGGGACTGTA GTTACTATTA CAATCATGCC	960
TGCTTTTATC TTTGAACATA TTATTAAGCG GATGGCACCA AGCATTATGA AAAGCCTAAT	1020

24

GGACCACACC ATTCCTGAGG TTAAAATTC ACGGCACCAT GGAAATGTAG CTGAACGTCT 1080
 CCAGTTTCCT TCTTTGGCAA CTTCTGTATT ATGCACGTGA AGCCTTCCCG GAGCCAGCGA 1140
 GCATATGCTG CATGAGGACC TTTCTATCTT ACATTATGGC TGGGGATCTT ACTCTTTCAT 1200
 CTGATACCTT GTTCAGATTT CAAAATAGTT GTAGCCTTAT CCTGGTTTTA CAGATGTGAA 1260
 CTTTCAAGAG ATTTACTGAC TTTCCTAGAA TAGTTTCTCT ACTGGAAACC TGATGCTTTT 1320
 ATAAGCCATT GTGATTAGGA TGACTGTTAC AGGCTTAGCT TTGTGTGAAA ACCAGTCACC 1380
 TTTCTCCTAG GTAATGAGTA GTGCTGTCAT ATTACTTTAG TTCTATAGCA TACTTGCATC 1440
 TTTAACATGC TATCATAGTA CATTAGAAT GATTGCCTTT GATTTTTTTT TTAAAATTCT 1500
 GTGTGTGTGT GTGTAAAATG CCAATTAAGA AACTGGTTT CATTCCATGT AAGCATTAAA 1560
 CAGTGTATGT AGGTTTCAAG AGATTGTGAT GATTCTTAAA TTTAACTAC CTTCACTTAA 1620
 TATGCTTGAA CTGTCGCCTT AACTATGTTA AGCATCTAGA CTAAAAGCCA AAATATAATT 1680
 ATTGCTGCCT TTCTAAAAAC CAAAATGTA GTTCTCTATT AACCTGAAAT GTACACTAGC 1740
 CCAGAACAGT TTAATGGTAC TTAAGTGGT ATAGCATAGC TGCTTAGTTG TTTTGTGAGT 1800
 TTTTAGTCA ACACATAATG GAACTTCTT TCTTCTAAAA GTTGCCAGTG CCACTTTTAA 1860
 GAAGTGAATC ACTATATGTG ATGTAAAAGT TATTACACTA AACAGGATAA ACTTTTGAAT 1920
 CCCCTTTTGT TCATTTGTGG ATTAAGTGGT ATAATACTTA ATTTTGGCAT TTGACTCTTA 1980
 AGATTATGTA ACCTAGCTAC TTTGGGATGG TCTTAGAATA TTTTCTGAT AACTTGTTCC 2040
 TTTTCCTGAC TCCTCCTTGC AAACAAAATG ATAGTTGACA CTTTATCCTG ATTTTTTTCT 2100
 TTTTTTTGGT TTATGTCTAT TCTAATTAAA TATGTATAAA TAAAGTTACA TTTTAGTCTG 2160
 TCAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAA 2193

SEQ ID NO. 3

GGCACGAGGC GGGGGCGGTG CATGACGCGC CTCGGGGGCG GTCCTCGGGC GCGCACCGCT 60
 CTCTTACACT CGGGCCTCAG AAGTCCGTGC CAGTGACCGG AGCGGCGGGC GCGAGCGGTT 120
 CCTTGTGGGC TAGAAGAATC CTGCAAAAAT GTCTCTCTAT CCATCTCTCG AAGACTTGAA 180
 M S L Y P S L E D L K 11
 GGTAGACAAA GTAATTCAGG CTCAAAGTGC TTTTCTGCA AACCTGCCA ATCCAGCAAT 240
 V D K V I Q A Q T A F S A N P A N P A I 31
 TTTGTCAGAAGCTTCTGCTCCTATCCCTCACGATGGAAATCTCTATCCCAGACTGTATCC 300
 L S E A S A P I P H D G N L Y P R L Y P 51
 AGAGCTCTCTCAATACATGGGGCTGAGTTTAAATGAAGAAGAAATACGTGCAAGTGTGGC 360
 E L S Q Y M G L S L N E E E I R A S V A 71
 CGTGGTTTCTGGTGCACCACTTCAGGGGCAGTTGGTAGCAAGACCTTCCAGTATAAACTA 420
 V V S G A P L Q G Q L V A R P S S I N Y 91
 TATGGTGGCTCCTGTAAGTGGTAATGATGTTGGAATTCGTAGAGCAGAAATTAAGCAAGG 480
 M V A P V T G N D V G I R R A E I K Q G 111
 GATTCGTGAAGTCATTTTGTGTAAGGATCAAGATGGAAAAATTGGACTCAGGCTTAAATC 540
 I R E V I L C K D Q D G K I G L R L K S 131
 AATAGATAATGGTATATTTGTTTCAGCTAGTCCAGGCTAATTCTCCAGCCTCATTGGTTGG 600

25

I D N G I F V Q L V Q A N S P A S L V G 151
TCTGAGATTTGGGGACCAAGTACTTCAGATCAATGGTGAAAAGTGTGCAGGATGGAGCTC 660
L R F G D Q V L Q I N G E N C A G W S S 171
TGATAAAGCGCACAAGGTGCTCAAACAGGCTTTTGGAGAGAAGATTACCATGACCATTTCG 720
D K A H K V L K Q A F G E K I T M T I R 191
TGACAGGCCCTTTGAACGGACGATTACCATGCATAAGGATAGCACTGGACATGTTGGTTT 780
D R P F E R T I T M H K D S T G H V G F 211
TATCTTTAAAAATGGAAAAATAACATCCATAGTGAAAGATAGCTCTGCAGCCAGAAATGG 840
I F K N G K I T S I V K D S S A A R N G 231
TCTTCTCACGGAACATAACATCTGTGAAATCAATGGACAGAATGTCATTGGATTGAAGGA 900
L L T E H N I C E I N G Q N V I G L K D 251
CTCTCAAATTGCAGACATACTGTCAACATCTGGGACTGTAGTTACTATTACAATCATGCC 960
S Q I A D I L S T S G T V V T I T I M P 271
TGCTTTTATCTTTGAACATATTATTAAGCGGATGGCACCAAGCATTATGAAAAGCCTAAT 1020
A F I F E H I I K R M A P S I M K S L M 291
GGACCACACCATTCTGAGGTTTAAAATTCACGGCACCATGGAAATGTAGCTGAACGTCT 1080
D H T I P E V * 298
CCAGTTTCCTTCTTTGGCAACTTCTGTATTATGCACGTGAAGCCTTCCCGGAGCCAGCGA 1140
GCATATGCTGCATGAGGACCTTTCTATCTTACATTATGGCTGGGGATCTTACTCTTTCAT 1200
CTGATACCTTGTTGAGATTTCAAATAGTTGTAGCCTTATCCTGGTTTTACAGATGTGAA 1260
CTTTCAAGAGATTTACTGACTTTCTAGAAATAGTTTCTCTACTGGAAACCTGATGCTTTT 1320
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TTTCTCCTAGGTAATGAGTAGTGCTGTCATATTACTTTAGTTCTATAGCATACTTGCATC 1440
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GTGTGTGTGTGTGTGAAAATGCCAATTAAGAACACTGGTTTCATTCCATGTAAGCATTAAA 1560
CAGTGTATGTAGGTTTCAAGAGATTGTGATGATTCTTAAATTTTAACTACCTTCACTTAA 1620
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TTTTTAGTCAACACATAATGGAAACTTCTTTCTTCTAAAAGTTGCCAGTGCCACTTTTAA 1860
GAAGTGAATCACTATATGTGATGTAAAAGTTATTACACTAAACAGGATAAACTTTTGACT 1920
CCCCTTTTGTTCAATTTGTGGATTAAGTGGTATAATACTTAATTTTGGCATTGACTCTTA 1980
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TTTTCCTGACTCCTCCTTGCAAACAAAATGATAGTTGACACTTTATCCTGATTTTTTCT 2100
TTTTTTTGGTTTATGTCTATTCTAATTAAATATGTATAAATAAAGTTACATTTTAGTCTG 2160
TCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 2193

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: VLAAMS INTERUNIVERSITAIR INSTITUUT VOOR
BIOTECHNOLOGIE
- (ii) TITLE OF INVENTION: SYNDECAN INTERACTING PROTEINS AND THE USE
THEREOF
- (iii) NUMBER OF SEQUENCES: 3
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: FETHERSTONHAUGH & CO.
 - (B) STREET: P.O. BOX 2999, STATION D
 - (C) CITY: OTTAWA
 - (D) STATE: ONT
 - (E) COUNTRY: CANADA
 - (F) ZIP: K1P 5Y6
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: ASCII (text)
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: CA 2,288,376
 - (B) FILING DATE: 22-APR-1998
 - (C) CLASSIFICATION:
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 - (A) APPLICATION NUMBER: EP 97201233.0
 - (B) FILING DATE: 25-APR-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: FETHERSTONHAUGH & CO.
 - (B) REGISTRATION NUMBER:

(C) REFERENCE/DOCKET NUMBER: 29775-1

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (613)-235-4373

(B) TELEFAX: (613)-232-8440

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 298 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Ser	Glu	Ala	Ser	Ala	Pro	Ile	Pro	His	Asp	Gly	Asn	Leu	Tyr	Pro	Arg	35	40	45	
Leu	Tyr	Pro	Glu	Leu	Ser	Gln	Tyr	Met	Gly	Leu	Ser	Leu	Asn	Glu	Glu	50	55	60	
Glu	Ile	Arg	Ala	Ser	Val	Ala	Val	Val	Ser	Gly	Ala	Pro	Leu	Gln	Gly	65	70	75	80
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Thr	Gly	Asn	Asp	Val	Gly	Ile	Arg	Arg	Ala	Glu	Ile	Lys	Gln	Gly	Ile	100	105	110	
Arg	Glu	Val	Ile	Leu	Cys	Lys	Asp	Gln	Asp	Gly	Lys	Ile	Gly	Leu	Arg	115	120	125	
Leu	Lys	Ser	Ile	Asp	Asn	Gly	Ile	Phe	Val	Gln	Leu	Val	Gln	Ala	Asn	130	135	140	
Ser	Pro	Ala	Ser	Leu	Val	Gly	Leu	Arg	Phe	Gly	Asp	Gln	Val	Leu	Gln	145	150	155	160

Ile	Asn	Gly	Glu	Asn	Cys	Ala	Gly	Trp	Ser	Ser	Asp	Lys	Ala	His	Lys			
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Arg	Pro	Phe	Glu	Arg	Thr	Ile	Thr	Met	His	Lys	Asp	Ser	Thr	Gly	His			
		195					200					205						
Val	Gly	Phe	Ile	Phe	Lys	Asn	Gly	Lys	Ile	Thr	Ser	Ile	Val	Lys	Asp			
	210					215					220							
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225					230					235					240			
Ile	Asn	Gly	Gln	Asn	Val	Ile	Gly	Leu	Lys	Asp	Ser	Gln	Ile	Ala	Asp			
				245					250					255				
Ile	Leu	Ser	Thr	Ser	Gly	Thr	Val	Val	Thr	Ile	Thr	Ile	Met	Pro	Ala			
			260					265					270					
Phe	Ile	Phe	Glu	His	Ile	Ile	Lys	Arg	Met	Ala	Pro	Ser	Ile	Met	Lys			
		275					280					285						
Ser	Leu	Met	Asp	His	Thr	Ile	Pro	Glu	Val									
	290					295												

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2193 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGCACGAGGC	GGGGGCGGTG	CATGACGCGC	CTCGGGGGCG	GTCCTCGGGC	GCGCACCGCT	60
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CCTTGTGGGC	TAGAAGAATC	CTGCAAAAAT	GTCTCTCTAT	CCATCTCTCG	AAGACTTGAA	180
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TTTGTCAGAA	GCTTCTGCTC	CTATCCCTCA	CGATGGAAAT	CTCTATCCCA	GACTGTATCC	300

AGAGCTCTCT	CAATACATGG	GGCTGAGTTT	AAATGAAGAA	GAAATACGTG	CAAGTGTGGC	360
CGTGGTTTCT	GGTGCACCAC	TTCAGGGGCA	GTTGGTAGCA	AGACCTTCCA	GTATAAACTA	420
TATGGTGGCT	CCTGTAAC TG	GTAATGATGT	TGGAATTCGT	AGAGCAGAAA	TTAAGCAAGG	480
GATTCGTGAA	GTCATTTTGT	GTAAGGATCA	AGATGGAAAA	ATTGGACTCA	GGCTTAAATC	540
AATAGATAAT	GGTATATTTG	TTCAGCTAGT	CCAGGCTAAT	TCTCCAGCCT	CATTGGTTGG	600
TCTGAGATTT	GGGGACCAAG	TACTTCAGAT	CAATGGTGAA	AACTGTGCAG	GATGGAGCTC	660
TGATAAAGCG	CACAAGGTGC	TCAAACAGGC	TTTTGGAGAG	AAGATTACCA	TGACCATTCTG	720
TGACAGGCCC	TTTGAACGGA	CGATTACCAT	GCATAAGGAT	AGCACTGGAC	ATGTTGGTTT	780
TATCTTTAAA	AATGGAAAAA	TAACATCCAT	AGTGAAAGAT	AGCTCTGCAG	CCAGAAATGG	840
TCTTCTCACG	GAACATAACA	TCTGTGAAAT	CAATGGACAG	AATGTCATTG	GATTGAAGGA	900
CTCTCAAATT	GCAGACATAC	TGTCAACATC	TGGGACTGTA	GTTACTATTA	CAATCATGCC	960
TGCTTTTATC	TTTGAACATA	TTATTAAGCG	GATGGCACCA	AGCATTATGA	AAAGCCTAAT	1020
GGACCACACC	ATTCCTGAGG	TTTAAAATTC	ACGGCACCAT	GGAAATGTAG	CTGAACGTCT	1080
CCAGTTTCCT	TCTTTGGCAA	CTTCTGTATT	ATGCACGTGA	AGCCTTCCCG	GAGCCAGCGA	1140
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CTTTCAAGAG	ATTTACTGAC	TTTCCTAGAA	TAGTTTCTCT	ACTGGAAACC	TGATGCTTTT	1320
ATAAGCCATT	GTGATTAGGA	TGACTGTTAC	AGGCTTAGCT	TTGTGTGAAA	ACCAGTCACC	1380
TTTCTCCTAG	GTAATGAGTA	GTGCTGTCAT	ATTACTTTAG	TTCTATAGCA	TACTTGCATC	1440
TTTAACATGC	TATCATAGTA	CATTTAGAAT	GATTGCCTTT	GATTTTTTTT	TTTAAATTCT	1500
GTGTGTGTGT	GTGTAAAATG	CCAATTAAGA	ACACTGGTTT	CATTCCATGT	AAGCATTAAA	1560
CAGTGTATGT	AGGTTTCAAG	AGATTGTGAT	GATTCTTAAA	TTTTAACTAC	CTTCACTTAA	1620
TATGCTTGAA	CTGTCGCCTT	AACTATGTTA	AGCATCTAGA	CTAAAAGCCA	AAATATAATT	1680
ATTGCTGCCT	TTCTAAAAAC	CCAAAATGTA	GTTCTCTATT	AACCTGAAAT	GTACACTAGC	1740
CCAGAACAGT	TTAATGGTAC	TTACTGAGCT	ATAGCATAGC	TGCTTAGTTG	TTTTTGAGAT	1800
TTTTTAGTCA	ACACATAATG	GAAACTTCTT	TCTTCTAAAA	GTTGCCAGTG	CCACTTTTAA	1860
GAAGTGAATC	ACTATATGTG	ATGTAAAAGT	TATTACACTA	AACAGGATAA	ACTTTTGACT	1920
CCCCTTTTGT	TCATTTGTGG	ATTAAGTGGT	ATAATACTTA	ATTTTGGCAT	TTGACTCTTA	1980
AGATTATGTA	ACCTAGCTAC	TTTGGGATGG	TCTTAGAATA	TTTTTCTGAT	AACTTGTTCC	2040

TTTTCCTGAC	TCCTCCTTGC	AAACAAAATG	ATAGTTGACA	CTTTATCCTG	ATTTTTTTTCT	2100
TTTTTTTGGT	TTATGTCTAT	TCTAATTAA	TATGTATAAA	TAAAGTTACA	TTTTAGTCTG	2160
TCAAAAAAAAA	AAAAAAAAAAA	AAAAAAAAAAA	AAA			2193

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2193 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 149..1042

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGCACGAGGC	GGGGGCGGTG	CATGACGCGC	CTCGGGGGCG	GTCCTCGGGC	GCGCACCGCT	60										
CTCTTACACT	CGGGCCTCAG	AAGTCCGTGC	CAGTGACCGG	AGCGGCGGCG	GCGAGCGGTT	120										
CCTTGTGGGC	TAGAAGAATC	CTGCAAAA	ATG	TCT	CTC	TAT	CCA	TCT	CTC	GAA	172					
			Met	Ser	Leu	Tyr	Pro	Ser	Leu	Glu						
			1				5									
GAC	TTG	AAG	GTA	GAC	AAA	GTA	ATT	CAG	GCT	CAA	ACT	GCT	TTT	TCT	GCA	220
Asp	Leu	Lys	Val	Asp	Lys	Val	Ile	Gln	Ala	Gln	Thr	Ala	Phe	Ser	Ala	
	10					15					20					
AAC	CCT	GCC	AAT	CCA	GCA	ATT	TTG	TCA	GAA	GCT	TCT	GCT	CCT	ATC	CCT	268
Asn	Pro	Ala	Asn	Pro	Ala	Ile	Leu	Ser	Glu	Ala	Ser	Ala	Pro	Ile	Pro	
25					30				35						40	
CAC	GAT	GGA	AAT	CTC	TAT	CCC	AGA	CTG	TAT	CCA	GAG	CTC	TCT	CAA	TAC	316
His	Asp	Gly	Asn	Leu	Tyr	Pro	Arg	Leu	Tyr	Pro	Glu	Leu	Ser	Gln	Tyr	
				45				50						55		
ATG	GGG	CTG	AGT	TTA	AAT	GAA	GAA	GAA	ATA	CGT	GCA	AGT	GTG	GCC	GTG	364
Met	Gly	Leu	Ser	Leu	Asn	Glu	Glu	Glu	Ile	Arg	Ala	Ser	Val	Ala	Val	
			60				65						70			

GTT	TCT	GGT	GCA	CCA	CTT	CAG	GGG	CAG	TTG	GTA	GCA	AGA	CCT	TCC	AGT	412
Val	Ser	Gly	Ala	Pro	Leu	Gln	Gly	Gln	Leu	Val	Ala	Arg	Pro	Ser	Ser	
		75					80					85				
ATA	AAC	TAT	ATG	GTG	GCT	CCT	GTA	ACT	GGT	AAT	GAT	GTT	GGA	ATT	CGT	460
Ile	Asn	Tyr	Met	Val	Ala	Pro	Val	Thr	Gly	Asn	Asp	Val	Gly	Ile	Arg	
	90					95					100					
AGA	GCA	GAA	ATT	AAG	CAA	GGG	ATT	CGT	GAA	GTC	ATT	TTG	TGT	AAG	GAT	508
Arg	Ala	Glu	Ile	Lys	Gln	Gly	Ile	Arg	Glu	Val	Ile	Leu	Cys	Lys	Asp	
105					110					115					120	
CAA	GAT	GGA	AAA	ATT	GGA	CTC	AGG	CTT	AAA	TCA	ATA	GAT	AAT	GGT	ATA	556
Gln	Asp	Gly	Lys	Ile	Gly	Leu	Arg	Leu	Lys	Ser	Ile	Asp	Asn	Gly	Ile	
				125					130					135		
TTT	GTT	CAG	CTA	GTC	CAG	GCT	AAT	TCT	CCA	GCC	TCA	TTG	GTT	GGT	CTG	604
Phe	Val	Gln	Leu	Val	Gln	Ala	Asn	Ser	Pro	Ala	Ser	Leu	Val	Gly	Leu	
			140					145					150			
AGA	TTT	GGG	GAC	CAA	GTA	CTT	CAG	ATC	AAT	GGT	GAA	AAC	TGT	GCA	GGA	652
Arg	Phe	Gly	Asp	Gln	Val	Leu	Gln	Ile	Asn	Gly	Glu	Asn	Cys	Ala	Gly	
		155					160					165				
TGG	AGC	TCT	GAT	AAA	GCG	CAC	AAG	GTG	CTC	AAA	CAG	GCT	TTT	GGA	GAG	700
Trp	Ser	Ser	Asp	Lys	Ala	His	Lys	Val	Leu	Lys	Gln	Ala	Phe	Gly	Glu	
	170					175					180					
AAG	ATT	ACC	ATG	ACC	ATT	CGT	GAC	AGG	CCC	TTT	GAA	CGG	ACG	ATT	ACC	748
Lys	Ile	Thr	Met	Thr	Ile	Arg	Asp	Arg	Pro	Phe	Glu	Arg	Thr	Ile	Thr	
185					190					195					200	
ATG	CAT	AAG	GAT	AGC	ACT	GGA	CAT	GTT	GGT	TTT	ATC	TTT	AAA	AAT	GGA	796
Met	His	Lys	Asp	Ser	Thr	Gly	His	Val	Gly	Phe	Ile	Phe	Lys	Asn	Gly	
				205					210					215		
AAA	ATA	ACA	TCC	ATA	GTG	AAA	GAT	AGC	TCT	GCA	GCC	AGA	AAT	GGT	CTT	844
Lys	Ile	Thr	Ser	Ile	Val	Lys	Asp	Ser	Ser	Ala	Ala	Arg	Asn	Gly	Leu	
			220					225					230			
CTC	ACG	GAA	CAT	AAC	ATC	TGT	GAA	ATC	AAT	GGA	CAG	AAT	GTC	ATT	GGA	892
Leu	Thr	Glu	His	Asn	Ile	Cys	Glu	Ile	Asn	Gly	Gln	Asn	Val	Ile	Gly	
		235					240					245				
TTG	AAG	GAC	TCT	CAA	ATT	GCA	GAC	ATA	CTG	TCA	ACA	TCT	GGG	ACT	GTA	940
Leu	Lys	Asp	Ser	Gln	Ile	Ala	Asp	Ile	Leu	Ser	Thr	Ser	Gly	Thr	Val	
	250					255					260					
GTT	ACT	ATT	ACA	ATC	ATG	CCT	GCT	TTT	ATC	TTT	GAA	CAT	ATT	ATT	AAG	988
Val	Thr	Ile	Thr	Ile	Met	Pro	Ala	Phe	Ile	Phe	Glu	His	Ile	Ile	Lys	
265					270					275					280	
CGG	ATG	GCA	CCA	AGC	ATT	ATG	AAA	AGC	CTA	ATG	GAC	CAC	ACC	ATT	CCT	1036
Arg	Met	Ala	Pro	Ser	Ile	Met	Lys	Ser	Leu	Met	Asp	His	Thr	Ile	Pro	
				285					290					295		

GAG GTT TAAAATTCAC GGCACCATGG AAATGTAGCT GAACGTCTCC AGTTTCCTTC 1092
Glu Val

TTTGGCAACT TCTGTATTAT GCACGTGAAG CCTTCCCGGA GCCAGCGAGC ATATGCTGCA 1152

TGAGGACCTT TCTATCTTAC ATTATGGCTG GGGATCTTAC TCTTTCATCT GATACCTTGT 1212

TCAGATTTCA AAATAGTTGT AGCCTTATCC TGGTTTTACA GATGTGAACT TTCAAGAGAT 1272

TTACTGACTT TCCTAGAATA GTTTCTCTAC TGGAAACCTG ATGCTTTTAT AAGCCATTGT 1332

GATTAGGATG ACTGTTACAG GCTTAGCTTT GTGTGAAAAC CAGTCACCTT TCTCCTAGGT 1392

AATGAGTAGT GCTGTCATAT TACTTTAGTT CTATAGCATA CTTGCATCTT TAACATGCTA 1452

TCATAGTACA TTTAGAATGA TTGCCTTTGA TTTTTTTTTT TAAATTCTGT GTGTGTGTGT 1512

GTAAAATGCC AATTAAGAAC ACTGGTTTCA TTCCATGTAA GCATTAAACA GTGTATGTAG 1572

GTTTCAAGAG ATTGTGATGA TTCTTAAATT TTAACCTACCT TCACTTAATA TGCTTGAAC 1632

GTCGCCTTAA CTATGTTAAG CATCTAGACT AAAAGCCAAA ATATAATTAT TGCTGCCTTT 1692

CTAAAAACCC AAAATGTAGT TCTCTATTAA CCTGAAATGT AACTAGCCC AGAACAGTTT 1752

AATGGTACTT ACTGAGCTAT AGCATAGCTG CTTAGTTGTT TTTGAGATTT TTTAGTCAAC 1812

ACATAATGGA AACTTCTTTC TTCTAAAAGT TGCCAGTGCC ACTTTTAAGA AGTGAATCAC 1872

TATATGTGAT GTAAAAGTTA TTACACTAAA CAGGATAAAC TTTTGACTCC CCTTTTGTTT 1932

ATTTGTGGAT TAAGTGGTAT AATACTTAAT TTTGGCATTT GACTCTTAAG ATTATGTAAC 1992

CTAGCTACTT TGGGATGGTC TTAGAATATT TTTCTGATAA CTTGTTTCCTT TTCCTGACTC 2052

CTCCTTGCAA ACAAATGAT AGTTGACACT TTATCCTGAT TTTTTTCTTT TTTTGGTTT 2112

ATGTCTATTC TAATTAAATA TGTATAAATA AAGTTACATT TTAGTCTGTC AAAAAAAAAA 2172

AAAAAAAAAA AAAAAAAAAA A 2193

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CLAIMS:

1. An isolated syndecan interacting protein characterized in that said syndecan interacting protein

(a) contains a tandem repeat of PDZ-domains
5 reacting with the FYA C-terminal amino acid sequence of syndecan,

(b) is represented by a 2.19 kb cDNA encoding a polypeptide of 298 amino acids of SEQ ID NO: 1,

(c) contains five tyrosine residues in the amino
10 terminal region represented by amino acids 1-109 of SEQ ID NO: 1, and

(d) contains no tyrosine residues in amino acids 110-298 of SEQ ID NO: 1.

2. An isolated polypeptide comprising the amino acid
15 sequence according to SEQ ID NO:1 or a functional fragment thereof comprising at least one PDZ domain or the N-terminal region (amino acid positions 1-113 of SEQ ID NO:1), wherein said fragment or N-terminal region binds to syndecan.

3. The isolated polypeptide of claim 2, wherein said
20 functional fragment is located between position 92 and 298 of SEQ ID NO: 1.

4. The isolated polypeptide functional fragment of claim 2, wherein said functional fragment is located between positions 113 and 298 of SEQ ID NO: 1.

25 5. A composition comprising (a) a polypeptide comprising the amino acid sequence according to SEQ ID NO: 1 or a functional fragment thereof comprising at least one PDZ domain or the N-terminal region (amino acid positions 1-113

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of SEQ ID NO: 1), wherein said fragment or N-terminal region binds to syndecan, and (b) a carrier.

6. A method for screening compounds that interfere with the interaction between syndecan and syndecan interacting protein, said method comprising:

(a) adding a compound to a solution comprising syndecan and syndecan interacting protein; and

(b) monitoring the interaction between syndecan and syndecan interacting protein.

10 7. The method according to claim 6, wherein said monitoring comprises a two-hybrid screening assay.

8. The method according to claim 6 wherein said monitoring comprises ligand blot-assays.

15 9. The method according to claim 6, wherein said monitoring comprises surface plasmon resonance experiment.

Application number / numéro de demande: 22 88 376

Figures: 1

Pages: _____

Unscannable items
received with this application
(Request original documents in File Prep. Section on the 10th floor)

Documents reçu avec cette demande ne pouvant être balayés
(Commander les documents originaux dans la section de préparation des dossiers au
10^{ème} étage)