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(54) ANTIBODY SPECIFIC FOR A CHONDROITIN SULPHATE EPITOPE

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to an antibody and to methods for producing the antibody. Aspects of the invention also relate to diagnostic methods, test kits, and pharmaceutical compositions using the antibody. More particularly, the antibody of the present invention is an antibody to chondroitin sulfate.

BACKGROUND TO THE INVENTION

[0002] Joint diseases resulting from osteoarthritis and other diseases which trigger the destruction of cartilage are causes of economic and social loss, and can be considered as major health problems (1, 2).

[0003] Osteoarthritis results from the degradation of articular cartilage. It is estimated that up to 60% of the population aged over 75 years old suffer from this disease (3). The real cause of osteoarthritis is still unknown but some reports mention that while this disease is developing cartilage, subchondral bone and synovial membrane undergo some changes. The pathogenesis of this disease causes the affected cartilage to lose resistance to outer impact and also the elasticity and smoothness of the joint.

[0004] While rheumatoid arthritis is a progressive destruction of the cartilage similar to osteoarthritis (OA), patients suffering from rheumatoid arthritis (RA) experience the tearing and destruction of macromolecules which are major components of cartilage by proteolytic cleavage; the degradation products are then released to the synovial fluid. The degradation of the surface of articular cartilage and cartilage thickness can be assessed by x-ray diagnosis of the affected joints, but this will only be apparent after a long period of these diseases, which can often be too late for treatment (4, 5).

[0005] Provision of an alternative diagnostic test for arthritis and/or other cartilage degradation would therefore be beneficial to patients. In particular, detection of cartilage degradation products in synovial fluid would assist in the development of diagnostic tests which allow diagnosis at an early stage. Such a test may also assist in monitoring the disease and as a prognosis marker at the different stage of the diseases.

[0006] There is an existing test for diagnosis of degenerative joint diseases (6). Quantitation of proteoglycans and their fragments, especially glycosaminoglycans, can be done by an immunoassay using specific monoclonal antibodies to the proteoglycan fragments and other biochemical markers in the cartilage. Monoclonal antibodies have been produced against proteoglycan fragments such as anti-keratan sulfate peptides (KS-peptides) (7), chondroitin sulfate epitopes (CS-epitope) (8,9), and hyaluronan (HA) (10). Using these antibodies and binding proteins in immunoassays, especially, ELISA-based techniques, can allow a quantitative test of these biomolecules to be performed.

[0007] Furthermore, certain types of cancer are known to over-produce these and related biomolecules. Furthermore, cancers can produce many enzymes that degrade biomolecules in connective tissues surrounding them. So, proteoglycans containing chondroitin sulfate can be a useful marker indicating the existence of cancers and can be a potential marker for a prognosis or treatment of disease activities.

[0008] Chan SS *et al* (30) and Slater Robert R Jr *et al* (31) refer to antibody 3-B-3(-) and to its use is detecting biochemical markers of arthritis in humans.

SUMMARY OF THE INVENTION

[0009] The present inventors have created a hybridoma cell line which produces a monoclonal antibody specific for a particular chondroitin sulfate epitope. The antibody is designated WF6, and the cell line has been deposited under the Budapest Treaty with the American Type Culture Collection (ATCC) on 11 August 2004, under accession number PTA-6157. The cell line will be referred to herein as "the WF6 cell line", while "WF6" alone will refer to the antibody. The antibody is shown herein to specifically recognise a short oligosaccharide epitope comprising chondroitin sulfate, and in particular an epitope including at least one chondroitin 6-sulfate unit. A chondroitin sulfate chain consists of a repeating disaccharide unit, D-glucuronate and N-acetyl-D-galactosamine-sulfate linked in a β 1-3 linkage; the chondroitin sulfate oligosaccharide comprises multiple chondroitin sulfate units. Chondroitin sulfate is found in a number of forms, the main ones of which are chondroitin 4-sulfate (also referred to as chondroitin sulfate A, CSA), chondroitin 6-sulfate (or chondroitin sulfate C, CSC), and chondroitin sulfate D, CSD.

[0010] According to a first aspect of the present invention, there is provided a WF6 antibody produced by a hybridoma cell as deposited in the American Type Culture Collection under accession number PTA-6157, the antibody having specific reactivity to a chondroitin sulfate epitope, the chondroitin sulfate epitope comprising at least eight sugar monomers; a terminal chondroitin 6-sulfate unit; and at least one chondroitin 4-sulfate accessory unit. The at least 8 sugar monomers may comprise a sequence ΔDCCC.

[0011] The invention also comprises functional derivatives of such an antibody, which show the same specificity. Functional derivatives include Fab, Fab' and $F(ab)_2$ fragments; single chain antibodies; and functional fragments obtained by chemical or enzymatic cleavage of the antibody or molecular biological methods such as phage display technique. Chimeric antibodies are also included within the scope of the invention. The invention further comprises labelled antibodies and functional derivatives thereof. Labelled antibodies include enzyme conjugated antibodies, biotinylated antibodies, fluorescently and radioactive labelled antibodies, gold labelled

antibodies, and such like. The skilled person will be aware of suitable techniques for preparing such derivatives; confirming that the antibody has the required specificity may be achieved using the techniques detailed herein which were used to determine the WF6 antibody specificity.

[0012] According to a further aspect of the invention, there is provided a method of producing antibodies, comprising culturing hybridoma cells as deposited with the American Type Culture Collection (ATCC) on 11 August 2004, under accession number PTA-6157 in a suitable growth medium, the hybridoma cells producing growth supernatant comprising antibodies; and harvesting the growth supernatant to thereby extract the antibodies, wherein the antibodies exhibit specific reactivity to a chondroitin sulfate epitope comprising at least eight sugar units having a chondroitin 6-sulfate unit and a chondroitin 4-sulfate accessory unit.

[0013] A further aspect of the present invention relates to a method of detecting cartilage degradation, the method comprising contacting a sample with antibodies produced by a hybridoma cell as deposited in the American Type Culture Collection under accession number PTA-6157, the antibodies having specific reactivity to a chondroitin sulfate epitope, the chondroitin sulfate epitope comprising at least eight sugar monomers; a terminal chondroitin 6-sulfate unit; and a chondroitin-4-sulfate unit; and detecting binding of the antibodies to the sample..

[0014] The detection step may comprise any convenient detection means; for example, ELISA, indirect ELISA, competitive ELISA, sandwich ELISA, radioimmunoassay, fluorescent immunoassay, chemiluminescence immunoassay, immunoblotting, immunohistostaining, immunochromatography, immunodiffusion, flow injection analysis, confocal microscope and the like.

[0015] The present invention also provides a method for detecting immunobinding comprising providing a sample; contacting the sample with one of an antibody and a functional derivative of the antibody, the antibody produced by a hybridoma cell as deposited in the American Type Culture Collection under accession number PTA-6157 and having specific reactivity to a chondroitin sulfate epitope comprising at least eight sugar monomers with a terminal chondroitin 6-sulfate unit and a chondroitin 4-sulfate accessory unit, the functional derivative selected from a group consisting of Fab, Fab', $F(ab)_2$ fragments; single chain antibodies; and functional fragments obtained by chemical or enzymatic cleavage of the antibody or molecular biological methods, the functional derivative exhibiting the same specific reactivity as compared to the antibody; and detecting binding of one of the antibody and the functional derivative with the sample, wherein the detection of binding is performed by one of ELISA, indirect ELISA, competitive ELISA, sandwich ELISA, radioimmunoassay, fluorescent immunoassay, chemiluminescence immunoassay, immunoblotting, immunohistostaining and flow injection analysis.

[0016] The present invention also provides a medicament for facilitating diagnosis of a disease of the connective tissue comprising an antibody produced by a hybridoma cell as deposited in the American Type Culture Collection under accession number PTA-6157, the antibody having binding specificity to a chondroitin sulfate epitope, the chondroitin sulfate epitope comprising at least eight sugar monomers; a terminal chondroitin 6-sulfate unit; and a chondroitin 4-sulfate accessory unit.

[0017] The present invention also provides a medicament for facilitating diagnosis of a disease of the connective tissue comprising a functional derivative of an antibody produced by a hybridoma cell as deposited in the American Type Culture Collection under accession number PTA-6157and having a specifici reactivity to a chondroitin sulfate epitope that comprises at least eight sugar monomers; a terminal chondroitin 6-sulphate unit; and a chondroitin 4-sulfate accessory unit, wherein the functional derivative is selected from a group consisting of Fab, Fab', $F(ab)_2$ fragments; single chain antibodies; and functional fragments obtained by chemical or enzymatic cleavage of the antibody or molecular biological methods.

[0018] The present invention further provides a method of detecting a chondroitin sulfate oligosaccharide epitope comprising at least 8 sugar monomers, with a terminal chondroitin 6-sulfate, the method comprising providing a sample and contacting the sample with one of an antibody and a functional derivative of the antibody, the antibody produced by a hybridoma cell as deposited in the America Type Culture Collection under accession number PTA-6157 and having specific reactivity to a chondroitin sulfate epitope comprising at least eight sugar monomers with a terminal chondroitin 6-sulfate unit and a chondroitin 4-sulfate accessory unit, wherein the functional derivative is selected from a group consisting of Fab, Fab', $F(ab)_2$ fragments; single chain antibodies; and functional fragments obtained by chemical or enzymatic cleavage of the antibody or molecular biological methods..

[0019] A further aspect of the invention provides a diagnostic test kit comprising one of an antibody produced by a hybridoma cell as deposited in the America Type Culture Collection under accession number PTA-6157 and a functional derivative of the antibody, the antibody produced by a hybridoma cell as deposited in the America Type Culture Collection under accession number PTA-6157 and exhibiting binding specificity to a chondroitin sulfate epitope comprising at least 8 sugar monomers having a terminal chondroitin 6-sulfate unit and a chondroitin 4-sulfate accessory unit, wherein the functional derivative is selected from a group consisting of Fab, Fab', $F(ab)_2$ fragments; single chain antibodies; and functional fragments obtained by chemical or enzymatic cleavage of the antibody or molecular biological methods, the functional derivative exhibiting the same specific reactivity as the antibody. The skilled person will be aware of suitable protocols for the detection of binding

of the antibody to a target; for example, various protocols are described herein. The kit may further comprise one or more reagents suitable for the detection of binding of the antibody to a target.

[0020] Also provided by the present invention are pharmaceutical compositions comprising one of an antibody produced by a hybridoma cell as deposited in the America Type Culture Collection under accession number PTA-6157 and a functional derivative of the antibody, the antibody produced by a hybridoma cell as deposited in the America Type Culture Collection under accession number PTA-6157 and exhibiting specific reactivity to a chondroitin sulfate epitope comprising at least 8 sugar monomers having a terminal chondroitin 6-sulfate and a chondroitin 4-sulfate accessory unit, wherein the functional derivative is selected from a group consisting of Fab, Fab', F(ab)2 fragments; single chain antibodies; and functional fragments obtained by chemical or enzymatic cleavage of the antibody or molecular biological methods, the functional derivative exhibiting the same specific reactivity as the antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] These and other aspects of the present invention will now be described by way of example only with reference to the accompanying drawings, in which:

Figure 1 shows inhibition of WF6 antibody activity by various chondroitin sulfates;
Figure 2 shows an absorbance profile of fractions of oligosaccharides containing chondroitin 6-sulfate from chondroitinase ABC digested CS-C eluted by column chromatography;
Figure 3 shows the level of uronic acid in each fraction from Figure 2 showing IC50 against WF6 antibody using competitive ELISA;
Figure 4 shows the column profile of fraction 6 from Figure 2 analysed by ion exchange FPLC;
Figure 5 shows the effect of sulfatase digestion of the substrate on the reactivity of WF6 antibody;
Figure 6 shows immunohistostaining of human skin tissue section using WF6 as primary antibody;
Figure 7 shows uronic acid content of cartilage from an animal model of cartilage degradation;
Figure 8 shows levels of WF6 epitope in samples from the animal model over time;
Figure 9 shows the level of WF6 epitope in serum of human patients with osteoarthritis and rheumatoid arthritis;
Figure 10 shows the effect of chitosan polysulfate on levels of WF6 epitope on a dog model of cartilage degradation;
Figure 11 shows levels of WF6 epitope in cartilage explants induced by retinoic acid; and
Figure 12 shows levels of proteoglycans containing WF6 epitope in serum fron-z cancer patients.

DETAILED DESCRIPTION OF THE DRAWINGS

1. The production of hybridoma cells which produce an antibody against chondroitin 6-sulfate.

[0022] Purified proteoglycans (A1D1 fraction) from embryonic shark cartilage were used as antigen for the induction of an immune response in Balb/c mice. Spleen cells from mice which gave a high titer of antibody to chondroitin 6-sulfate (chondroitin sulfate C; CS-C) were fused with myeloma cell line X63 Ag8.653 in the ratio of 5:1. The fused cells were cultured in medium containing hypoxanthine, aminopterine, and thymine. Cell cultures were then tested for the production of antibodies by ELISA technique using chondroitin 6-sulfate (chondroitin sulfate C) as coating antigen. A positive cell line was isolated as a monoclone using a limiting dilution technique. The resulting cell line which produced antibody specifically against chondroitin 6-sulfate was called WF6, and it was found that the isotype of this antibody is Ig M, with Kappa-light chain. The cell line has been deposited with the American Type Culture Collection (ATCC) on 11 August 2004, under accession number PTA-6157.

2. The production of monoclonal antibody against chondroitin 6-sulfate in ascitic fluid and serum free-medium.

[0023] The mass production of this monoclonal antibody WF6 (MAb WF6) can be performed using hybridoma cells injected into the intraperitoneal cavity of Balb/c mice which have been primed by injection with 0.5 ml pristane one week before. Ascitic fluid samples can be obtained after 2-4 weeks which contain a large quantity of MAb WF6.

[0024] An alternative technique for mass production makes use of serum-free medium. The hybridoma cell WF6 can be cultured in 10 % fetal calf serum Iscove's modified Eagle's medium, with gradual reduction of the percentage of serum to 5%, 1% and 0% along with an increase in commercial serum-free medium. The final medium should be 100% serum-free media which has been formulated with growth factors. The cells were cultured in 5%CO2, and 95% humidity and 37 degree C. The supernatant of this media may be further evaluated for antibody activity by ELISA technique and purified further using ammonium sulfate precipitation and chromatography.

3. Characterization of the monoclonal antibody WF6 by ELISA

[0025]

3.1 Coat plate (polystyrene, Maxisorp Nunc®) with proteoglycans (fraction A1, aggregated form of shark cartilage proteoglycans) and block with 1% BSA
3.2 Prepare the inhibition mixture containing the op-

timal dilution of MAb WF6 and inhibitor, or standards or unknown samples in equal volume (175 + 175 μL each) in plastic tubes (1.5 mL tubes), then incubate at 37 degree C for 1 hour.

3.3 Add the inhibition mixture from 3.2 to the coated and blocked plate at 100 μL/well, and triplicate per sample (three wells), then incubate at 37 degree C for 1 hour.

3.4 Wash plate with washing buffer (PBS-Tween 0.05%) three times and dry before addition of 100 μL per well of peroxidase conjugated anti-Ig M antibody, then incubate at 37 degree C for 1 hour.

3.5 Wash plate with washing buffer three times, and dry before adding OPD substrate (100 μL/well), incubate for 15 minutes in the dark, and then stop the reaction by adding of 4 M sulfuric acid (50 μL/well). Absorbance of the plate was then measured 492/690nm by microplate reader.

3.6 The inhibition graph was plotted showing concentration of inhibitors (or standards) against absorbance (see Figure 1). A range of test compounds were used, being chondroitin sulfate A to E, and the A1D1 proteoglycan fraction.

[0026] It was found that the monoclonal antibody shows reactivity (measured as inhibitory activities) only to chondroitin 6-sulfate (CS-C) and CS-D. It was later determined that the CS-D standard compounds contained some chondroitin 6-sulfate units, such that we believe the WF6 antibody shows specificity for chondroitin 6-sulfate containing compounds having a specific pattern of sulfation.

4. Specificity of monoclonal antibody WF6 against oligosaccharides containing chondroitin 6-sulfate.

[0027]

4.1 Chondroitin 6-sulfate (CS-C) from commercial shark cartilage (Sigma-Aldrich Chemical) was digested with chondroitinase ABC which cleaved the CS-C into small oligosaccharides at various sites. The cleavage products have a double bond at the non-reducing end of the products which has maximal absorption at 232 nm.

4.2 The products from enzyme digestion were separated by column chromatography using a BioGel P-6 column. The absorbance of the column fractions was measured, giving the profile shown in Figure 2.

4.3 The products can be divided into 10 fractions as shown in Figure 2. Each fraction was pooled and concentrated by freeze drying, and the reactivity against MAb WF6 was determined using the method described in section 3. The reactivities from each fraction were compared by calculation of the uronic acid content which gave 50% inhibition (IC50; inhibition concentration at 50%), the results of which are shown in Figure 3. Percentage inhibition can be calculated by % inhibition = 100 [((A(sample) - A (blank))/ (A(control)- A(blank)) x 100)] where A = absorbance.

[0028] It was found that the oligosaccharide fractions which still displayed inhibitory activities were fractions 1 - 6, and by comparison to known standards from previous studies, we determined that fraction number 6 should contain oligosaccharides having 8-12 sugar units (octasaccharides - dodecasaccharides).

4.4 Fraction number 6 of the oligosaccharides was further purified using by ion-exchange chromatography using FPLC and Mono-Q column chromatography using lithium percholate as eluent. The obtained column profile is shown in Figure 4.

[0029] From the experimental results above, it was found that the oligosaccharides from gel filtration consisted of various species of chondroitin 6-sulfate oligosaccharides. This demonstrates that the monoclonal antibody WF6 reacts with oligosaccharides containing chondroitin 6-sulfate that have a special pattern of sulfatation as shown in the fingerprinting of Mono-Q ion-exchange FPLC.

5. A sulfate group at position 6 is significant for reaction against monoclonal antibody WF6

[0030]

5.1 The oligosaccharide fractions d, e and f from the column profile shown in Figure 4 were subjected to digestion with 4- and 6-sulfatase.

5.2 The products from these two enzymes were further evaluated for reactivity against MAb WF6 using competitive ELISA technique as described in section 3.

5.3 The comparison of the reactivities in each product, including a pre-digested sample, is shown in Figure 5. It was found that the sulfate group on the oligosaccharides is required for reactivity against MAb WF6.

Conclusion

[0031] When taken together, the results described in sections 3 to 5 above demonstrate that MAb WF6 has reactivity against oligosaccharides and polysaccharides of at least 8 sugar units containing chondroitin 6-sulfate. Furthermore, this reactivity of MAb WF6 requires the functional group of 6-sulfate at the terminal residue because the 6-sulfatase recognises this position. And 4-sulfate needs to be an accessory group, including the specific sulfating pattern along with the oligosaccharide chain.

6. The use of monoclonal antibody WF6 in immunohistostaining of tissues

[0032]

6.1 Skin tissue samples from the pathological laboratories which had been processed by routine procedures in tissue staining were selected for this study.
6.2 The optimal dilution of MAb WF6 in PBS (for example, 1:500 from ascitic fluid, and 1: 100 tissue culture media) was used as primary antibody in the staining procedure after the tissue section was fixed to the slides.
6.3 Excess antibody was washed from the slide with washing buffer and restained with peroxidase conjugated anti-IgM antibody.
6.4 Excess conjugates and non-reacted antibody were washed from the slides, and insoluble substrate added.
6.5 The enzyme reaction was stopped with acid and excess colour washed out; the sample was then observed under light microscope as shown in Figure 6.

7. The application of monoclonal antibody WF6 for the evaluation of cartilage degradation in an animal model

[0033]

7.1 The animal model was run as previously reported (Kongtawelert et al, 1989) for the induction of cartilage degradation by intra-articular injection of hydrocortisone (Solu-Cortef®). The rabbits were divided into two groups : the control had normal saline solution injection, while the experimental group had hydrocortisone injection of 200mg in every week for up to 12 weeks. Each week before injection the animal was bled for serum preparation.
7.2 The serum samples from each week were assayed for the level of WF6 epitope using the method described in section 3. In addition to this study, the MAb 3B3 (Caterson et al, 1990) has also been used as a biosynthesis marker in cartilage as previously reported.
7.3 At the end of the study, all animals were sacrificed and the articular cartilage were collected for analysis of proteoglycan contents as uronic acid per dried weight of cartilage. This data is shown in Figure 7.
7.5 The level of the WF6 epitope and 3B3 epitope in each week were used to calculate the percentage relative change of each epitope each week. The results are shown in the graph of Figure 8.

[0034] It was found that the monoclonal antibody WF6 can be used or applied to use in the technique of immunoassay (competitive ELISA) for quantitation of proteoglycans containing the chondroitin 6-sulfate epitope, and this assay indicates that it can be used as a marker for degradation of cartilage. Figure 8 shows that the WF6 epitope was found at significantly higher levels in hydrocortisone treated animals than normal saline treated animals, and the Figure also demonstrates the early indication of disease activity.

8. The application of MAb WF6 for quantitation of biomarker in diagnosis of osteoarthritis (OA) and rheumatoid arthritis (RA)

[0035]

8.1 Human serum samples from patients with OA, RA and healthy patients were collected to analyze the WF6 epitope. The samples were diluted 5 folds with 6% BSA-PBS to reduce non-specific binding.
8.2 All serum samples were subjected to analysis using competitive ELISA as described in section 3 using shark proteoglycan fraction A1 as relative standards.
8.3 The age of volunteers in each group was not significantly different.
8.4 The level of WF6 epitope from individual samples were plotted as a scatter graph as shown in Figure 9.

[0036] It was found that the MAb WF6 can be applied to use in a quantitative assay using immunological method for diagnosis of OA, and RA. However, there were still overlapping of the value in some samples of each group, which might be from the variation of the disease stage, and the treatment of the individual person. In addition, this might be interpreted as showing that the level of WF6 epitope can be used to monitor the disease activities and prognosis as well.

9. The application of monoclonal antibody WF6 for monitoring of treatment in an animal model

[0037]

9.1 Cruciate ligament transection is used to induce the degradation of cartilage (OA) in 6 dogs by surgical operation at the hind leg (see Batiste et al, 2004).
9.2 These dogs were divided into 2 group (3 each group), one group were injected intramuscularly with normal saline solution and the other group injected with chitosan polysulfate, which is a potential anti-osteoarthritis agent, at the dose of 3mg/kg body weight. The injection was given every week for eight weeks.
9.3 The third group of dogs was not surgically operated upon, and was injected with chitosan polysulfate intramuscularly at 3 mg/kg body weight.
9.4 All of the animals were observed and cared for by a veterinarian. Blood for serum preparation was collected to use for the determination of WF6 by the

method described in section 3.

9.5 The relative changes of all experimental animals were averaged in each group and the graph was plotted against time of the study as shown in Figure 10.

[0038] It was found that the MAb WF6 can be used in the method of quantitation of its epitope in animal model and can be applied as a marker for monitoring the treatment of the disease, like cartilage degeneration.

10. The application of monoclonal antibody WF6 in the monitoring of cartilage degradation in tissue culture model of cartilage explants

[0039]

10.1 Porcine cartilage was sterile cut into small pieces and cultured in serum-free media under 37 degree C, and 5% carbon dioxide.

10.2 Retinoic acid was added to the culture at the concentration of 10 uM for stimulation of cartilage degradation.

10.3 The media were collected every other day for four weeks for determining the level of WF6 epitope using the method described in section 3.

10.4 The level of WF6 epitope from each week was plotted against time (days) of culture as shown in Figure 11.

[0040] It was found that the MAb WF6 can be used for monitoring the degradation of cartilage *in vitro* by a well established technique using the induction of retinoic acid when compared with the control. This demonstration showed the applicability of MAb WF6 as a research tool for the cartilage degradation *in vitro*.

11. The application of the monoclonal antibody WF6 for the quantitation of a biomarker in ovarian cancer serum samples

[0041] From previous reports (Nash et al, 2002), it was found that there were increased levels of chondroitin sulfate biosynthesis in cancer tissues compared with normal tissues. Furthermore, the cancer cells can produce some kinds of enzymes that can degrade the connective tissues surrounding them. These would make cancer cells intrude through the tissue and metastasize to other areas. We therefore suggest that MAb WF6 could be used for diagnosis of this pathogenesis including the tumour tissues.

11.1 Serum samples were collected from normal patients, patients having a tumour (non-cancer), and patients with ovarian cancers. This study was approved by the Ethics Committee.

11.2 All serum samples were tested for the determination of WF6 epitope level and compared between group of normal, non-cancer (tumour) and cancer. The results are shown in Figure 12

[0042] It was found that the WF6 epitope can be used as a biomarker for the cancerous (ovarian) condition in human.

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Claims

1. A WF6 antibody produced by a hybridoma cell as deposited in the American Type Culture Collection under accession number PTA-6157, the antibody having specific reactivity to a chondroitin sulfate epitope, the chondroitin sulfate epitope comprising:

   at least eight sugar monomers;
   a terminal chondroitin 6-sulfate unit; and
   at least one chondroitin 4-sulfate accessory unit.

2. The antibody of claim 1, the at least 8 sugar monomers comprising a sequence ADCCC.

3. A functional derivative of the antibody of claim 1, the functional derivative being selected from a group consisting of Fab, Fab', $F(ab)_2$ fragments; single chain antibodies; and functional fragments obtained by chemical or enzymatic cleavage of the antibody or molecular biological methods, the functional derivative exhibiting specific reactivity to a chondroitin sulfate epitope comprising at least eight sugar monomers having a terminal chondroitin 6-sulfate unit and a chondroitin 4-sulfate accessory unit.

4. A method of producing antibodies comprising:

   culturing hybridoma cells as deposited with the American Type Culture Collection under accession number PTA-6157 in a suitable growth medium, the hybridoma cells producing growth supernatant comprising antibodies; and
   harvesting the growth supernatant to thereby

extract the antibodies, wherein the antibodies exhibit specific reactivity to a chondroitin sulfate epitope comprising at least eight sugar units having a terminal chondroitin 6-sulfate unit and a chondroitin 4-sulfate accessory unit.

5. A method of detecting cartilage degradation, the method comprising:

contacting a sample with antibodies produced by a hybridoma cell as deposited in the American Type Culture Collection under accession number PTA-6157, the antibodies having specific reactivity to a chondroitin sulfate epitope, the chondroitin sulfate epitope comprising:

at least eight sugar monomers;
a terminal chondroitin 6-sulfate unit; and
a chondroitin-4-sulfate unit; and
detecting binding of the antibodies to the sample.

6. The method of claim 5, wherein the detection of binding of the antibody to the sample is performed by one of ELISA, indirect ELISA, competitive ELISA, sandwich ELISA, radioimmunoassay, fluorescent immunoassay, chemiluminescence immunoassay, immunoblotting, immunohislostaining and flow injection analysis.

7. A method for detecting immunobinding comprising:

contacting a sample with one of an antibody as claimed in claim 1 and a functional derivative of the antibody as claimed in claim 3, the functional derivative exhibiting the same specific reactivity as compared to the antibody; and
detecting binding of one of the antibody and the functional derivative with the sample,
wherein the detection of binding is performed by one of ELISA, indirect ELISA, competitive ELISA, sandwich ELISA, radioimmunoassay, fluorescent immunoassay, chemiluminescence immunoassay, immunoblotting, immunohistostaining and flow injection analysis.

8. A medicament for facilitating diagnosis of a disease of the connective tissue comprising:

an antibody produced by a hybridoma cell as deposited in the American Type Culture Collection under accession number PTA-6157, the antibody having binding specificity to a chondroitin sulfate epitope, the chondroitin sulfate epitope comprising:

at least eight sugar monomers;
a terminal chondroitin 6-sulfate unit; and
a chondroitin 4-sulfate accessory unit.

9. A medicament for facilitating diagnosis of a disease of the connective tissue comprising:

a functional derivative of an antibody produced by a hybridoma cell as deposited in the American Type Culture Collection under accession number PTA-6157and having a specifici reactivity to a chondroitin sulfate epitope that comprises:

at least eight sugar monomers;
a terminal chondroitin 6-sulphate unit; and
a chondroitin 4-sulfate accessory unit,
wherein the functional derivative is selected from a group consisting of Fab, Fab', $F(ab)_2$ fragments; single chain antibodies; and functional fragments obtained by chemical or enzymatic cleavage of the antibody or molecular biological methods.

10. A method of detecting a chondroitin sulfate oligosaccharide epitope comprising at least 8 sugar monomers with a terminal chondroitin 6-sulfate unit and at least one chondroitin 4-sulfate accessory unit, the method comprising:

contacting a sample with one of an antibody as claimed in claim 1 and a functional derivative of the antibody as claimed in claim 3.

11. A diagnostic test kit comprising:

one of an antibody as claimed in claim 1 and a functional derivative of the antibody as claimed in claim 3, the functional derivative exhibiting the same specific reactivity as the antibody.

12. A pharmaceutical composition comprising:

one of an antibody as claimed in claim 1 and a functional derivative of the antibody as claimed in claim 3, the functional derivative exhibiting the same specific reactivity as the antibody.

Patentansprüche

1. WF6-Antikörper, der durch eine Hybridomazelle produziert wird, wie sie in der American Type Culture Collection unter der Eintragsnummer PTA-6157 hinterlegt ist, wobei der Antikörper eine spezifische Reaktivität gegenüber einem Chondroitinsulfat-Epitop hat, wobei das Chondroitinsulfat-Epitop umfasst:

mindestens acht Zucker-Monomere;
eine endständige Chondroitin-6-Sulfat-Einheit;

und
mindestens eine Chondroitin-4-Sulfat-Zusatzeinheit.

2. Antikörper gemäß Anspruch 1, wobei die mindestens 8 Zucker-Monomere eine Sequenz ΔDCCC umfassen.

3. Funktionsfähiges Derivat des Antikörpers gemäß Anspruch 1, wobei das funktionsfähige Derivat ausgewählt ist aus einer Gruppe, welche umfasst: Fab, Fab', $F(ab)_2$-Fragmente; Einzelketten-Antikörper sowie funktionsfähige Fragmente, die durch chemische oder enzymatische Spaltung des Antikörpers oder durch molekularbiologische Verfahren gewonnen werden, wobei das funktionsfähige Derivat eine spezifische Reaktivität gegenüber einem Chondroitinsulfat-Epitop zeigt, das mindestens acht Zucker-Monomeren umfasst und eine endständige Chondroitin-6-Sulfat-Einheit und eine Chondroitin-4-Sulfat-Zusatzeinheit aufweist.

4. Verfahren zur Herstellung von Antikörpern, umfassend:

das Kultivieren von Hybridomazellen, wie sie in der American Type Culture Collection unter der Eintragsnummer PTA-6157 hinterlegt sind, in einem geeigneten Nährmedium, wobei die Hybridomazellen ein Wachstums-Supernatant produzieren, welches Antikörper umfasst; und
das Ernten des Wachstums-Supernatanten um daraus die Antikörper zu extrahieren, wobei die Antikörper eine spezifische Reaktivität gegenüber einem Chondroitinsulfat-Epitop zeigen, das mindestens acht Zucker-Monomeren umfasst und eine endständige Chondroitin-6-Sulfat-Einheit und eine Chondroitin-4-Sulfat-Zusatzeinheit aufweist.

5. Verfahren zur Erkennung von Knorpelabbau, das Verfahren umfassend:

das Kontaktieren einer Probe mit Antikörpern, die durch eine Hybridomazelle produziert wird, wie sie in der American Type Culture Collection unter der Eintragsnummer PTA-6157 hinterlegt ist, wobei die Antikörper eine spezifische Reaktivität gegenüber einem Chondroitinsulfat-Epitop haben, wobei das Chondroitinsulfat-Epitop umfasst:

mindestens acht Zucker-Monomere;
eine endständige Chondroitin-6-Sulfat-Einheit; und
eine Chondroitin-4-Sulfat-Einheit; und

das Nachweisen der Bindung der Antikörper an die Probe.

6. Verfahren gemäß Anspruch 5, wobei das Erkennen der Bindung des Antikörpers an die Probe durch eines der Folgenden durchgeführt wird: ELISA, indirekter ELISA, kompetitiver ELISA, Sandwich-ELISA, Radio-Immunassay, Fluoreszenz-Immunassay, Chemolumineszenz-Immunassay, Immun-Blotting, immunhistochemische Färbung und Fließ-Injektions-Analyse.

7. Verfahren zum Nachweisen von Immunbindungen, umfassend:

das Kontaktieren einer Probe entweder mit einem Antikörper gemäß Anspruch 1 oder einem funktionsfähigen Derivat des Antikörpers gemäß Anspruch 3, wobei das funktionsfähige Derivat dieselbe spezifische Reaktivität zeigt wie der Antikörper; und
das Nachweisen der Bindung von entweder dem Antikörper oder dem funktionsfähigen Derivat an die Probe,
wobei das Nachweisen der Bindungen durch eines der Folgenden durchgeführt wird: ELISA, indirekter ELISA, kompetitiver ELISA, Sandwich-ELISA, Radio-Immunassay, Fluoreszenz-Immunassay, Chemolumineszenz-Immunassay, Immun-Blotting, immunhistochemische Färbung und Fließ-Injektions-Analyse.

8. Medikament zur Erleichterung der Diagnose einer Erkrankung des Bindegewebes, umfassend:

einen Antikörper, der durch eine Hybridomazelle produziert wird, wie sie in der American Type Culture Collection unter der Eintragsnummer PTA-6157 hinterlegt ist, wobei der Antikörper eine Bindespezifität gegenüber einem Chondroitinsulfat-Epitop hat, wobei das Chondroitinsulfat-Epitop umfasst:

mindestens acht Zucker-Monomere;
eine endständige Chondroitin-6-Sulfat-Einheit; und
eine Chondroitin-4-Sulfat-Zusatzeinheit.

9. Medikament zur Erleichterung der Diagnose einer Erkrankung des Bindegewebes, umfassend:

ein funktionsfähiges Derivat eines Antikörpers, der durch eine Hybridomazelle produziert wird, wie sie in der American Type Culture Collection unter der Eintragsnummer PTA-6157 hinterlegt ist, und der eine spezifische Reaktivität gegenüber einem Chondroitinsulfat-Epitop hat, welches umfasst:

mindestens acht Zucker-Monomere;
eine endständige Chondroitin-6-Sulfat-Einheit; und
eine Chondroitin-4-Sulfat-Zusatzeinheit,
wobei das funktionsfähige Derivat ausgewählt ist aus einer Gruppe, welche umfasst: Fab, Fab', $F(ab)_2$-Fragmente; Einzelketten-Antikörper sowie funktionsfähige Fragmente, die durch chemische oder enzymatische Spaltung des Antikörpers oder durch molekularbiologische Verfahren gewonnen werden.

10. Verfahren zum Nachweisen eines Chondroitin-Sulfat-Oligosaccharid-Epitops, das mindestens 8 Zucker-Monomere umfasst sowie eine endständige Chondroitin-6-Sulfat-Einheit und mindestens eine Chondroitin-4-Sulfat-Zusatzeinheit aufweist, das Verfahren umfassend:

das Kontaktieren einer Probe mit entweder einem Antikörper gemäß Anspruch 1 oder einem funktionsfähigen Derivat des Antikörpers gemäß Anspruch 3.

11. Diagnostisches Testkit, umfassend:

entweder einen Antikörper gemäß Anspruch 1 oder ein funktionsfähiges Derivat des Antikörpers gemäß Anspruch 3, wobei das funktionsfähige Derivat dieselbe spezifische Reaktivität zeigt wie der Antikörper.

12. Pharmazeutische Zusammensetzung, umfassend:

entweder einen Antikörper gemäß Anspruch 1 oder ein funktionsfähiges Derivat des Antikörpers gemäß Anspruch 3, wobei das funktionsfähige Derivat dieselbe spezifische Reaktivität zeigt wie der Antikörper.

Revendications

1. Un anticorps WF6 produit par une cellule d'hybridome telle que déposée auprès de l'American Type Culture Collection sous le numéro d'entrée PTA-6157, l'anticorps ayant une réactivité spécifique à un épitope chondroïtine sulfate, l'épitope chondroïtine sulfate comprenant :

au moins huit monomères de sucre ;
une unité chondroïtine 6-sulfate terminale ; et
au moins une unité accessoire chondroïtine 4-sulfate.

2. L'anticorps de la revendication 1, les au moins 8 monomères de sucre comprenant une séquence ΔDCCC.

3. Un dérivé fonctionnel de l'anticorps de la revendication 1, le dérivé fonctionnel étant sélectionné dans un groupe consistant en fragments Fab, Fab', $F(ab)_2$; anticorps à chaîne unique ; et fragments fonctionnels obtenus par clivage enzymatique ou chimique de l'anticorps ou des procédés biologiques moléculaires, le dérivé fonctionnel présentant une réactivité spécifique à un épitope chondroïtine sulfate comprenant au moins huit monomères de sucre ayant une unité chondroïtine 6-sulfate terminale et une unité accessoire chondroïtine 4-sulfate.

4. Un procédé pour produire des anticorps comprenant :

faire une culture de cellules d'hybridome telles que déposées auprès de l'American Type Culture Collection sous le numéro d'entrée PTA-6157 dans un milieu de croissance approprié, les cellules d'hybridome produisant un surnageant de croissance comprenant des anticorps ; et
récolter le surnageant de croissance pour ainsi extraire les anticorps, les anticorps présentant une réactivité spécifique à un épitope chondroïtine sulfate comprenant au moins huit unités de sucre ayant une unité chondroïtine 6-sulfate terminale et une unité accessoire chondroïtine 4-sulfate.

5. Un procédé pour détecter une dégradation de cartilage, le procédé comprenant :

faire entrer en contact un échantillon avec des anticorps produits par une cellule d'hybridome telle que déposée auprès de l'American Type Culture Collection sous le numéro d'entrée PTA-6157, les anticorps ayant une réactivité spécifique à un épitope chondroïtine sulfate, l'épitope chondroïtine sulfate comprenant :

au moins huit monomères de sucre ;
une unité chondroïtine 6-sulfate terminale ; et
une unité chondroïtine-4-sulfate terminale ; et
détecter une liaison des anticorps à l'échantillon.

6. Le procédé de la revendication 5, la détection de liaison de l'anticorps à l'échantillon étant réalisée par une méthode parmi : ELISA, ELISA indirecte, ELISA de compétition, sandwich ELISA, dosage radioimmunologique, dosage par immunofluorescence, dosage par chimioluminescence, immunoempreinte, coloration immunohistochimique et analyse par in-

jection de flux.

7. Un procédé pour détecter une immunoliaison comprenant :

faire entrer en contact un échantillon avec un élément parmi un anticorps tel que revendiqué dans la revendication 1 et un dérivé fonctionnel de l'anticorps tel que revendiqué dans la revendication 3, le dérivé fonctionnel présentant la même réactivité spécifique par comparaison avec l'anticorps ; et
détecter une liaison d'un élément parmi l'anticorps et le dérivé fonctionnel avec l'échantillon, la détection de liaison étant réalisée par une méthode parmi : ELISA, ELISA indirecte, ELISA de compétition, sandwich ELISA, dosage radioimmunologique, dosage par immunofluorescence, dosage par chimioluminescence, immunoempreinte, coloration immunohistochimique et analyse par injection de flux.

8. Un médicament pour faciliter le diagnostic d'une maladie du tissu conjonctif comprenant :

un anticorps produit par une cellule d'hybridome telle que déposée auprès de l'American Type Culture Collection sous le numéro d'entrée PTA-6157, l'anticorps ayant une spécificité de liaison à un épitope chondroïtine sulfate, l'épitope chondroïtine sulfate comprenant :

au moins huit monomères de sucre ;
une unité chondroïtine 6-sulfate terminale ; et
une unité accessoire chondroïtine 4-sulfate.

9. Un médicament pour faciliter le diagnostic d'une maladie du tissu conjonctif comprenant :

un dérivé fonctionnel d'un anticorps produit par une cellule d'hybridome telle que déposée auprès de l'American Type Culture Collection sous le numéro d'entrée PTA-6157 et ayant une réactivité spécifique à un épitope chondroïtine sulfate qui comprend :

au moins huit monomères de sucre ;
une unité chondroïtine 6-sulfate terminale ; et
une unité accessoire chondroïtine 4-sulfate.
le dérivé fonctionnel étant sélectionné dans un groupe consistant en fragments Fab, Fab', $F(ab)_2$; anticorps à chaîne unique ; et fragments fonctionnels obtenus par clivage enzymatique ou chimique de l'anticorps ou des procédés biologiques moléculaires.

10. Un procédé pour détecter un épitope chondroïtine sulfate oligosaccharide comprenant au moins 8 monomères de sucre avec une unité chondroïtine 6-sulfate terminale et au moins une unité accessoire chondroïtine 4-sulfate, le procédé comprenant :

faire entrer en contact un échantillon avec un élément parmi un anticorps tel que revendiqué dans la revendication 1 et un dérivé fonctionnel de l'anticorps tel que revendiqué dans la revendication 3.

11. Un nécessaire de test de diagnostic comprenant :

un élément parmi un anticorps tel que revendiqué dans la revendication 1 et un dérivé fonctionnel de l'anticorps tel que revendiqué dans la revendication 3, le dérivé fonctionnel présentant la même réactivité spécifique que l'anticorps.

12. Une composition pharmaceutique comprenant :

un élément parmi un anticorps tel que revendiqué dans la revendication 1 et un dérivé fonctionnel de l'anticorps tel que revendiqué dans la revendication 3, le dérivé fonctionnel présentant la même réactivité spécifique que l'anticorps.

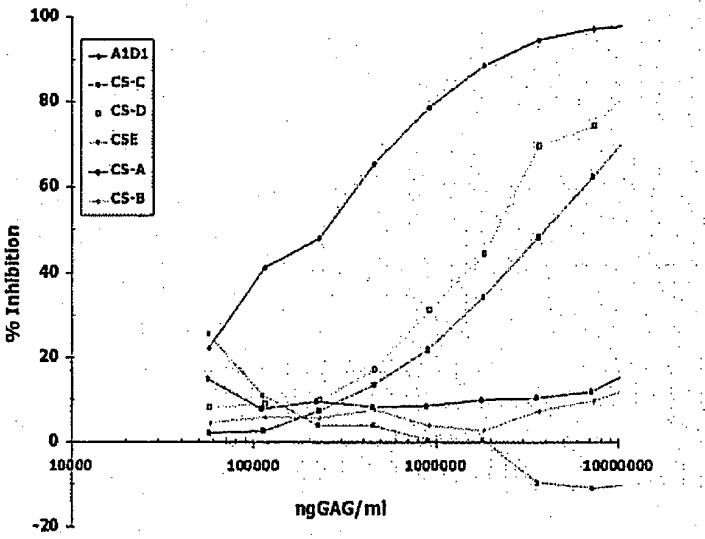


Figure 1 Graph showing the characteristics of monoclonal antibody WF6 reacting with various chondroitin sulfates

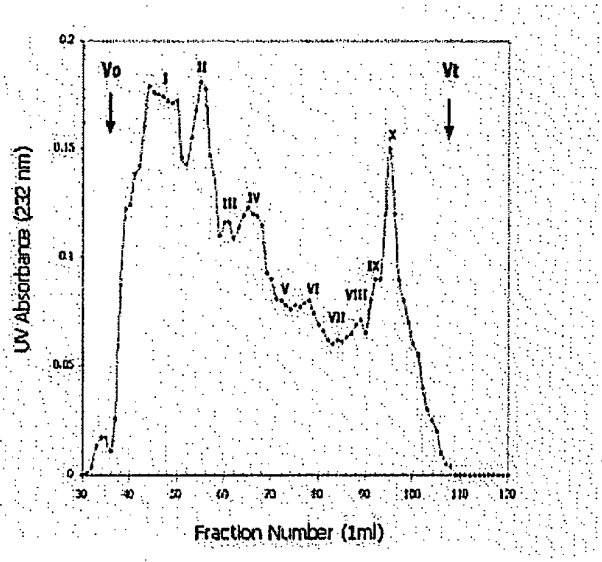


Figure 2 The column profile of oligosaccharides containing chondroitin 6-sulfate from chondroitinase ABC digested CS-C on BioGel P-6 gel filtration

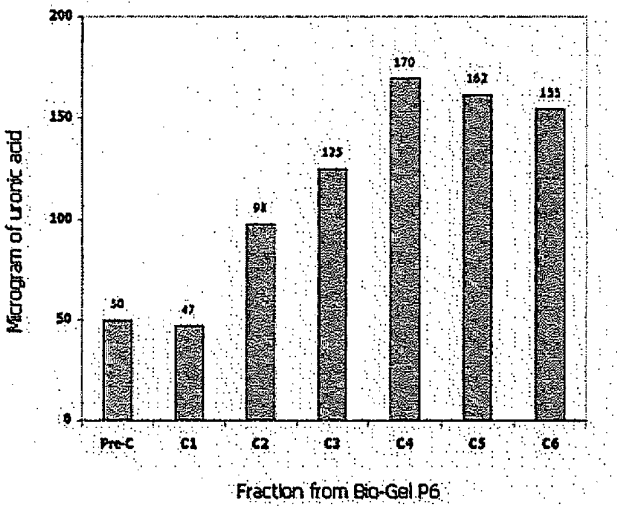


Figure 3. The level of uronic acid in each fraction of oligosaccharides from BioGel P-6 column chromatography which gives reactivities against MAb WF6 at 50 % inhibition (IC50) using competitive ELISA. The number shown in the graph is the amount of uronic acid in microgram.

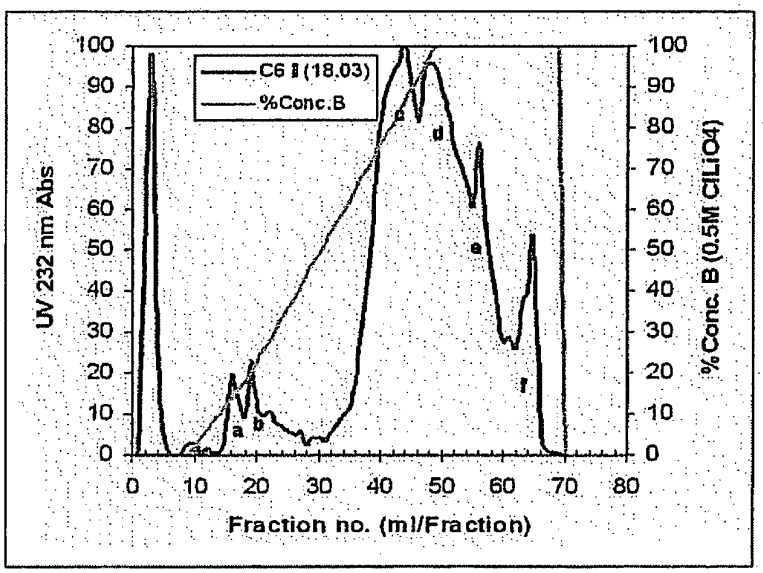


Figure 4 Graph demonstrating the column profile of fraction 6 (from BioGel P-6) on Mono-Q ion-exchange FPLC

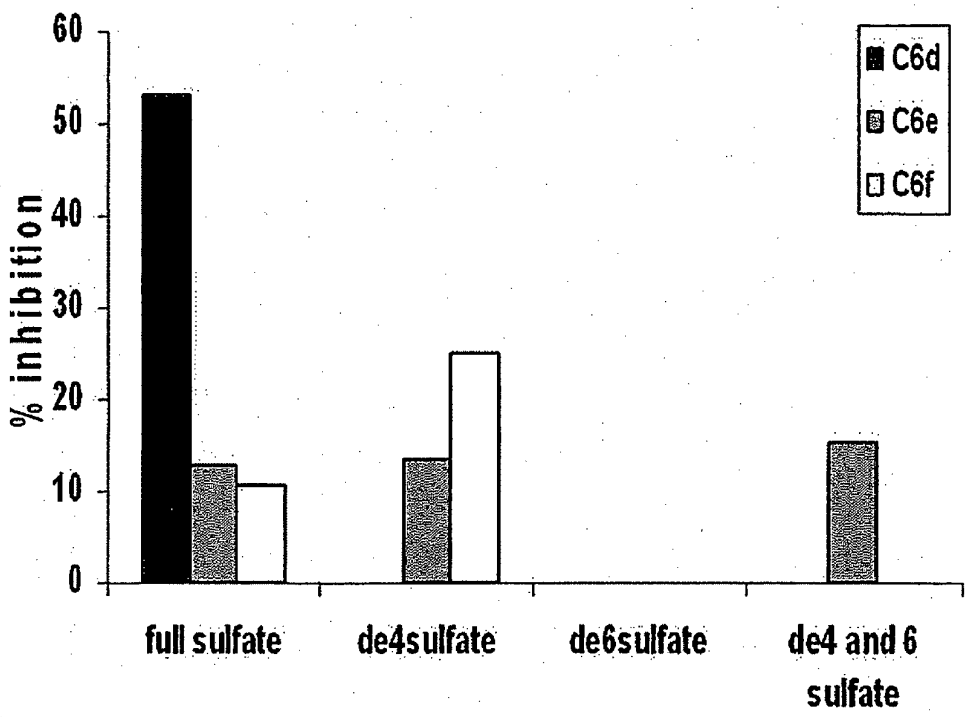


Figure 5 Graph demonstrating the effect of sulfatase enzyme on the reactivities of oligosaccharides digested with 4- and 6-sulfatase against MAb WF6

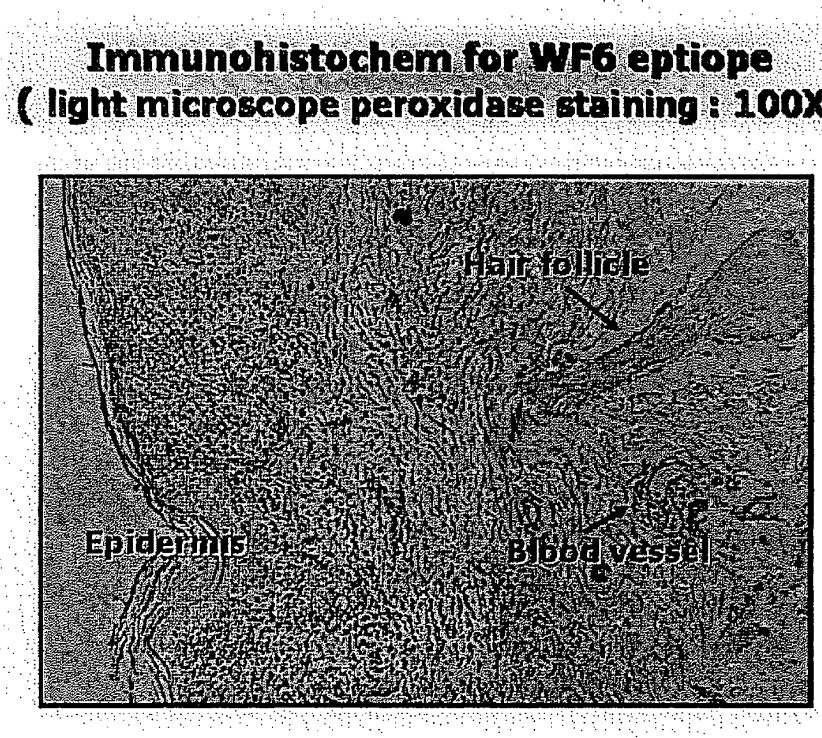


Figure 6. The immunohistostaining of human skin tissue section with MAb WF6 as primary antibody.

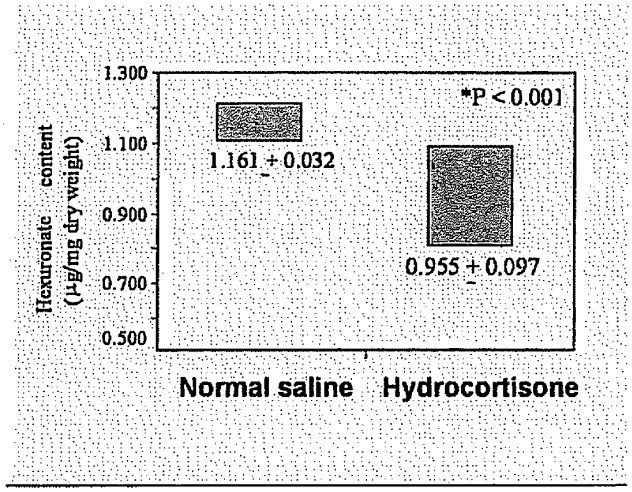


Figure 7 The proteoglycan content (as uronic acid) in cartilage from the animal treated with intra-articular injection of normal saline solution and hydrocortisone

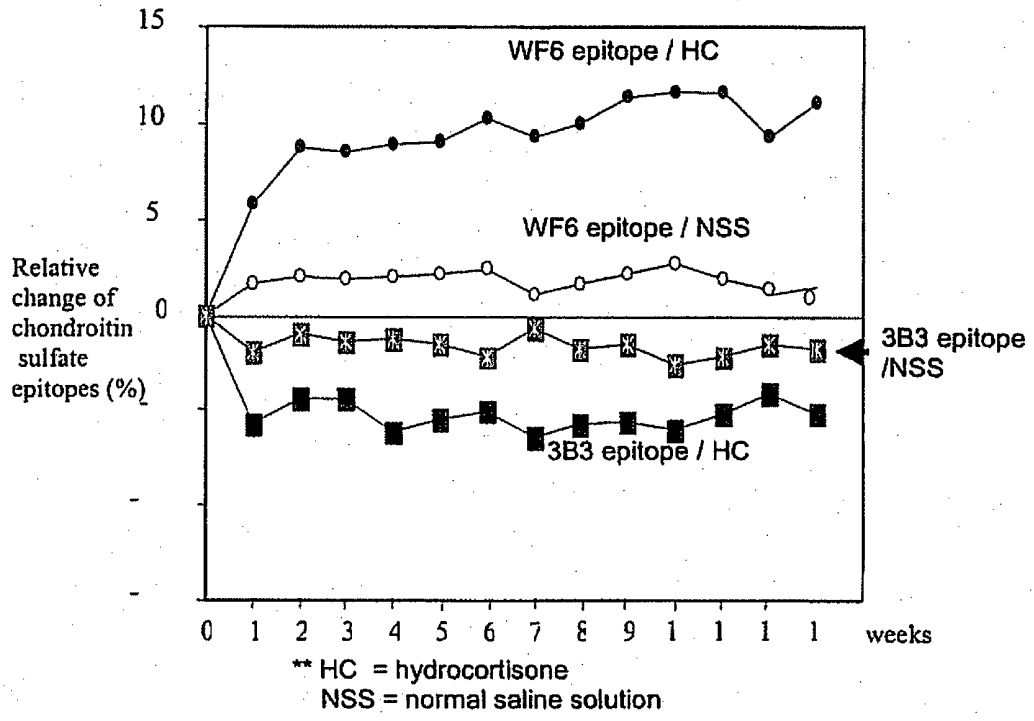
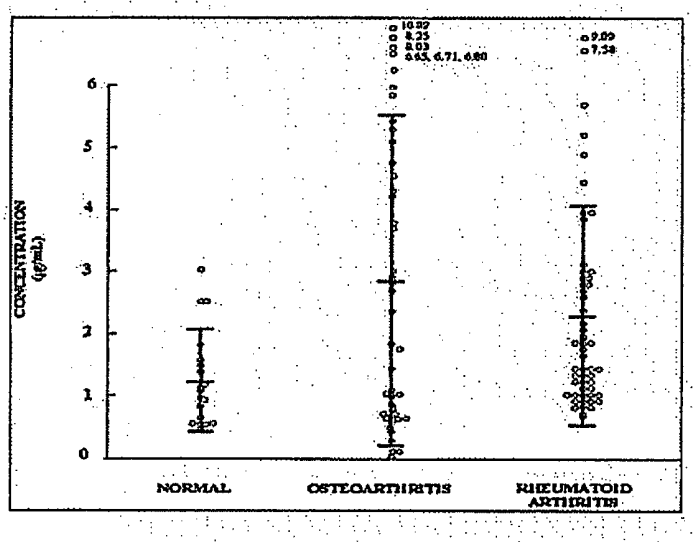


Figure 8 The level of WF6 epitope as catabolic marker and 3B3 epitope (anabolic marker) in the animals treated with normal saline solution and hydrocortisone group



	WF6 epitope (μg/ml)
Normal	1.18 ± 0.81 (N = 20)
OA	2.73 ± 2.63 * (N = 49)
RA	2.29 ± 1.70 * (N = 56)

* Significantly difference ($p < 0.05$)

Figure 9 A scatter graph showing the level of WF6 epitope in serum of normal (N = 20), OA (N = 49) and RA (N= 56), the significant level is $p < 0.05$ using student t-test.

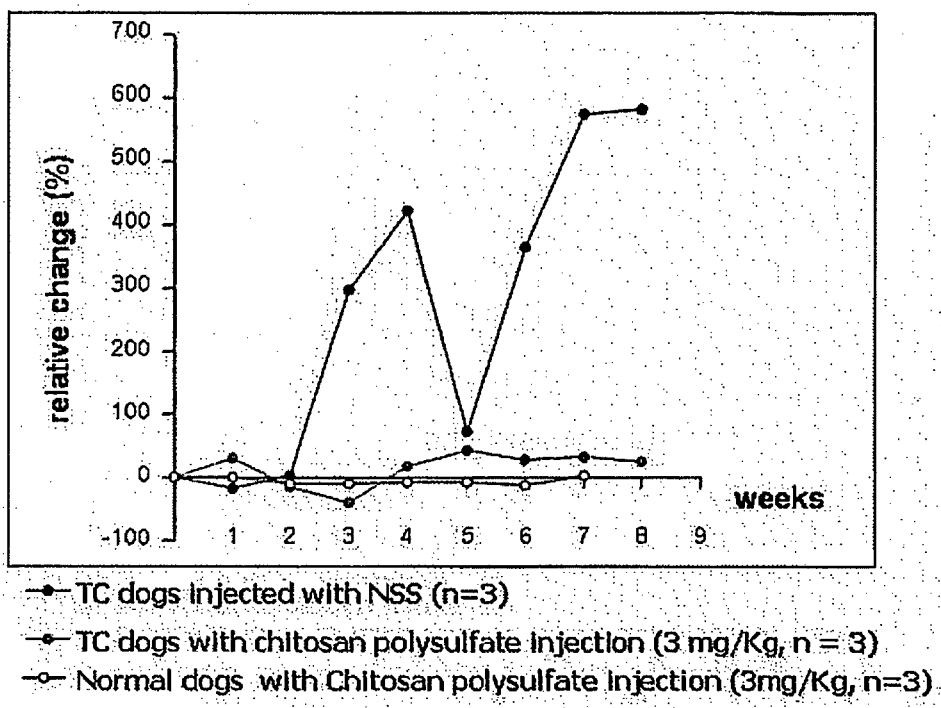


Figure 10. Comparison of the effect of chitosan polysulfate on the level of WF6 epitope and normal saline solution in the cruciate ligament transection in dog model. The relative change is determined by percentage of the level against the pre-treated period (week 0).

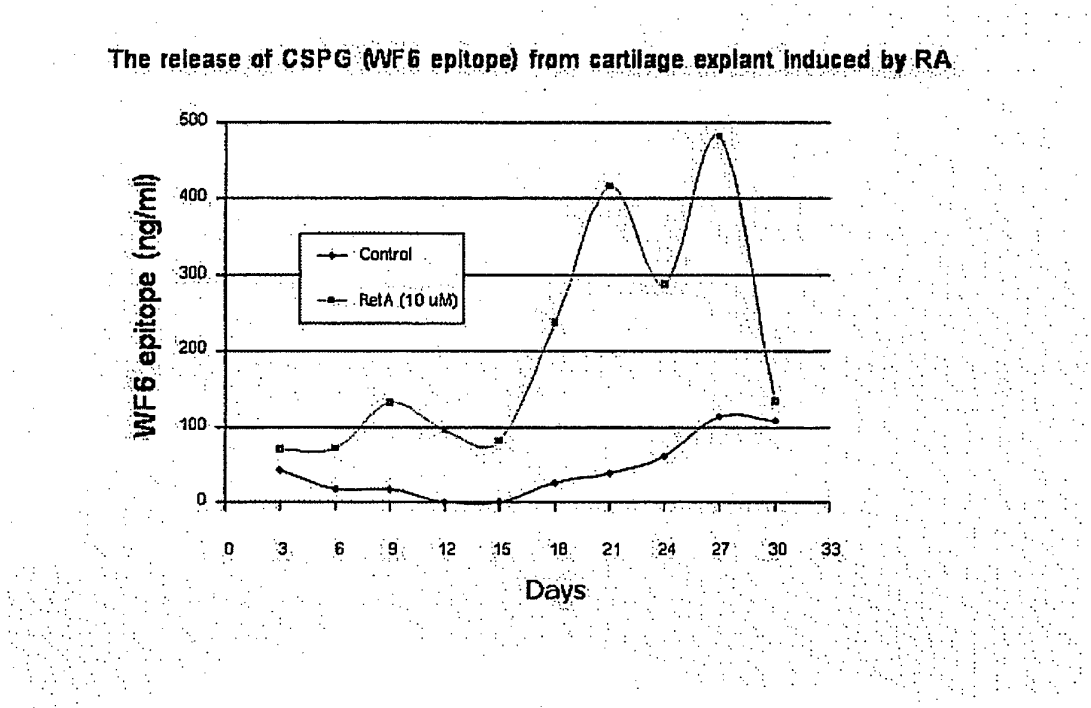


Figure 11 The level of WF6 epitope in cartilage explants culture which have been quantitated by competitive ELISA using monoclonal antibody WF6 for the monitoring the degradation by retinoic acid induction

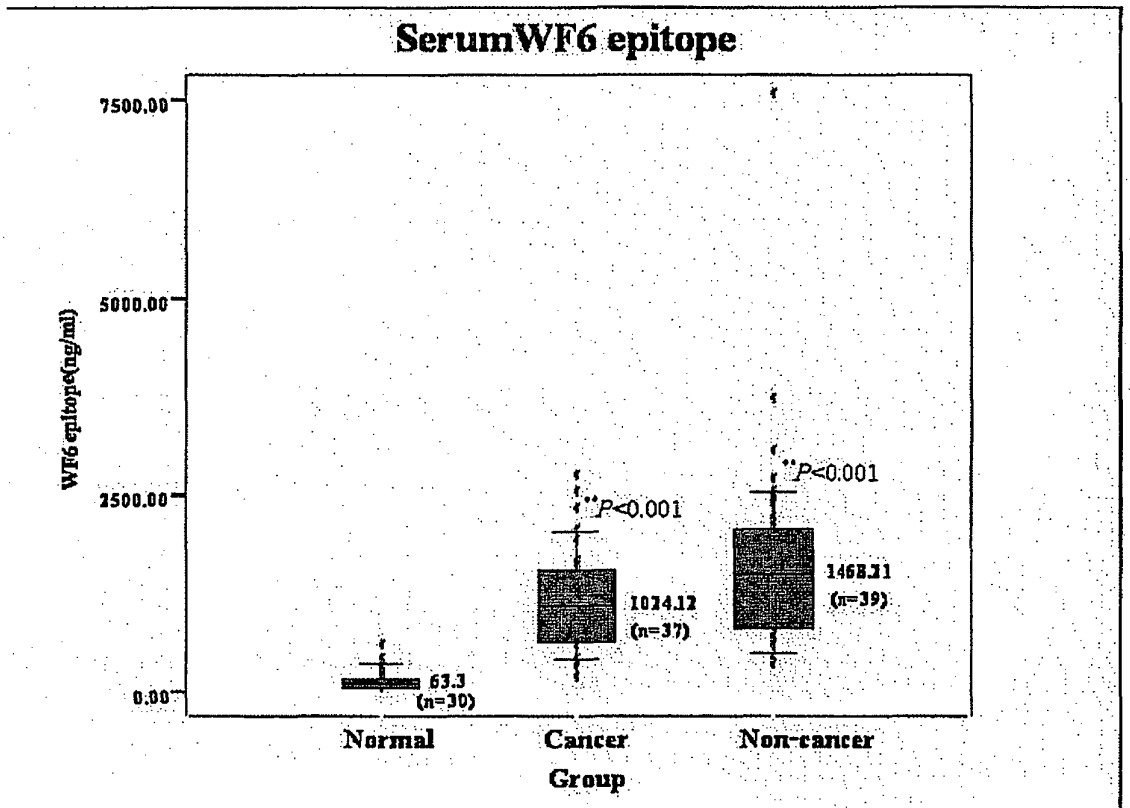


Figure 12. The level of proteoglycan containing chondroitin 6-sulfate WF6 epitope in serum from normal, ovarian cancer group and ovarian non-cancer (tumour). The significantly difference between group shown as $p < 0.05$ by student t-test.

REFERENCES CITED IN THE DESCRIPTION

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专利名称(译)	特异于硫酸软骨素表位的抗体		
公开(公告)号	EP1778727B1	公开(公告)日	2014-08-06
申请号	EP2005748554	申请日	2005-06-01
[标]申请(专利权)人(译)	在NAT RES理事会泰国 泰国启发式算法基金 清迈大学		
申请(专利权)人(译)	国家研究理事会泰国 泰国研究基金 清迈大学		
当前申请(专利权)人(译)	国家研究理事会泰国 泰国研究基金 清迈大学		
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发明人	KONGTAWELERT, PRACHYA HARDINGHAM, TIM ONG-CHAI, SIRIWAN SUGAHARA, KAZUYUKI POTHACHAROEN, PERAPHAN TIENGBURANATHUM, NATTHACHAI		
IPC分类号	C07K16/18 C07K16/30 C12N5/20 G01N33/53 A61K39/395 A61P19/02 A61P19/04 G01N33/574 G01N33/68		
CPC分类号	A61P19/02 A61P19/04 A61P29/00 C07K16/18 G01N33/57407 G01N33/6887 G01N2400/40 G01N2800/10 G01N2800/102		
优先权	2004018415 2004-08-18 GB		
其他公开文献	EP1778727A1		
外部链接	Espacenet		

摘要(译)

描述了对硫酸软骨素表位特异的抗体，以及产生这种抗体的杂交瘤细胞系。该抗体可用于诊断和治疗结缔组织疾病，例如关节炎和肉瘤。还描述了测试试剂盒和药物组合物。

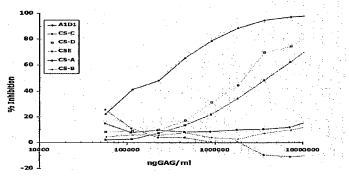

Figure 1 Graph showing the characteristics of monoclonal antibody WF6 reacting with various chondroitin sulfates

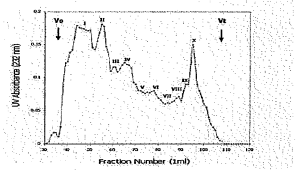

Figure 2 The column profile of oligosaccharides containing chondroitin 6-sulfate from chondroitinase ABC digested CS-C on BioGel P-6 gel filtration