

[Non-patent Document 3] Gerald W. Hart *et al.*, Nature 446: 1017-1022 (2007)

[Non-patent Document 4] Ajit Varki, Nature 446: 1023-1029 (2007)

[Non-patent Document 5] Joseph R. Bishop *et al.*, Nature 446: 1030-1037 (2007)

[Non-patent Document 6] Christopher N. *et al.*, Nature 446: 1038-1045 (2007)

5 [Non-patent Document 7] Peter H. Seeberger and Daniel B. Werz, Nature 446: 1046-1051 (2007)

Summary

[Problems to be Solved by the Disclosure]

10 The present disclosure is based on a new finding obtained through studies on sugar chain-related genes. An objective of the present disclosure is to provide novel uses of sugar chain-related genes. Specifically, the objective is to provide agents that suppress fibrogenesis at the physiological tissue level by inhibiting the function of sugar chain-related genes, and methods of screening for the agents.

[Means for Solving the Problems]

Sugar chains have been suggested to play a very important role *in vivo*. However, little is known about the *in vivo* functions of sugar chains.

As described above, several types of sugars constituting sugar chains are known.

20 Sugars are variously chemically-modified at multiple sites, and such modifications are considered to assume important physiological effects *in vivo*.

As described above, there are various types of sugars that constitute sugar chains, many types of chemical modifications that target sugars, and many chemical modification sites in the sugars. This suggests that possible sugar chain variations are innumerable. Thus, it is very difficult to determine a sugar chain structure that plays a certain role, and it is also extremely difficult to reveal the relationship between a pathological condition caused by a disease and a specific action of a sugar chain.

The present inventors conducted dedicated studies, and as a result succeeded for the first time in discovering that tissue fibrogenesis can be suppressed at the physiological level by inhibiting sulfation at position 4 or 6 of GalNAc, a sugar that constitutes sugar chains. Furthermore, by studies using various animal disease models, the present inventors demonstrated that inhibitors of sulfation at position 4 or 6 of GalNAc produce therapeutic effects against diseases caused by tissue fibrogenesis (tissue fibrogenic disorders).

35 The present disclosure relates to agents that suppress fibrogenesis at the physiological tissue level by inhibiting the functions of sugar chain-related genes, and methods of screening for the agents. Specifically, the present disclosure provides:

(a) contacting a test compound with a cell or cell extract containing a DNA wherein a reporter gene is operably linked to the transcriptional regulatory region of the gene encoding a sulfotransferase that transfers a sulfate to position 4 or 6 of N-acetylgalactosamine;

(b) determining the expression level of the reporter gene; and

5 (c) selecting a compound that reduces the expression level of the reporter gene as compared to when the test compound is not contacted; and

[12] a method of producing a pharmaceutical composition for treating or preventing a fibrogenic disorder, which comprises the steps of:

10 (a) selecting a tissue fibrogenesis suppressing-agent from test compounds by the method of any one of claims 7 to 11; and

(b) combining the agent with a pharmaceutically acceptable carrier.

The present disclosure also provides:

[13] a method of suppressing tissue fibrogenesis, which comprises the step of administering an inhibitor of sulfation at position 4 or 6 of N-acetylgalactosamine to an individual;

15 [14] use of an inhibitor of sulfation at position 4 or 6 of N-acetylgalactosamine in the manufacture of a tissue fibrogenesis suppressing-agent; and

[15] an inhibitor of sulfation at position 4 or 6 of N-acetylgalactosamine for use in suppressing tissue fibrogenesis.

20 In one aspect, there is provided a pharmaceutical composition for treating or preventing inflammatory bowel disease, comprising an siRNA as an ingredient which suppresses the expression of sulfotransferase, GalNAc4S-6ST, wherein the siRNA consists of one RNA strand consisting of nucleotide sequence of SEQ ID No: 25 and another RNA strand consisting of nucleotide sequence of SEQ ID No: 26, and the RNA strands are complementarily bound to each
25 other.

In another aspect, there is provided an siRNA consisting of one RNA strand consisting of nucleotide sequence of SEQ ID No: 25 and another RNA strand consisting of nucleotide sequence of SEQ ID No: 26, wherein the RNA strands are complementarily bound to each other.

30 Brief Description of the Drawings

Fig. 1 shows the result of quantitative reverse transcription PCR method for gene expressions of cardiomyopathy model mice in which cardiomyopathy was induced by intratracheal administration of DOX. The examined items were GalNAc4S-6ST as an siRNA target gene, and α -SMA and type I collagen, both of which are fibrogenesis markers. The graph indicates relative
35 ratios between a target gene and a house keeping gene (ribosome 18S). *P<0.001 (t-test)

Fig. 2 shows the result analyzing the heart weight/body weight ratio of cardiomyopathy model mice in which cardiomyopathy was induced by intraperitoneal administration of Doxorubicin hydrochloride (DOX; Kyowa Hakko). *P<0.05 (t-test)

5 Fig. 3 depicts photographs showing histological observations indicating the suppressive effect on type I collagen deposition in an siRNA-treated group of cardiomyopathy model mice in which cardiomyopathy was induced by intratracheal administration of DOX. The magnification is 100 fold.

10 Fig. 4 depicts photographs showing histological observations indicating the suppressive effect on type III collagen deposition in an siRNA-treated group of cardiomyopathy model mice in which cardiomyopathy was induced by intratracheal administration of DOX. The magnification is 100 fold.

CLAIMS

1. A pharmaceutical composition for treating or preventing inflammatory bowel disease, comprising an siRNA as an ingredient which suppresses the expression of sulfotransferase, GalNAc4S-6ST, wherein the siRNA consists of one RNA strand consisting of nucleotide sequence of SEQ ID No: 25 and another RNA strand consisting of nucleotide sequence of SEQ ID No: 26, and the RNA strands are complementarily bound to each other.
2. The pharmaceutical composition according to claim 1, wherein the inflammatory bowel disease is Crohn's disease or ulcerative colitis.
3. An siRNA consisting of one RNA strand consisting of nucleotide sequence of SEQ ID No: 25 and another RNA strand consisting of nucleotide sequence of SEQ ID No: 26, wherein the RNA strands are complementarily bound to each other.