

advantageous for detecting and quantifying GPC-1 levels in persons undergoing diagnostic and/or prognostic tests.

Summary

The present inventors have determined that a series of epitopes within the GPC-1 protein are preferably targeted by binding entities including, but not limited to, antibodies. In one aspect, the present invention relates to an isolated epitope or epitope segment for an anti-glypican 1 (GPC-1) antibody located within a portion of the GPC-1 flexible loop defined by an amino acid sequence KVN PQGPGPEEK (**SEQ ID NO: 1**), wherein the isolated epitope or epitope segment consists of: (i) KVN PQGPGPEEK (**SEQ ID NO: 1**); or (ii) a fragment of KVN PQGPGPEEK (**SEQ ID NO: 1**) consisting of VNP QGPGPEEK (**SEQ ID NO: 2**), VNP QGPGPEE (**SEQ ID NO: 3**), NP QGPGPEE (**SEQ ID NO: 4**), KVN PQGPGPE (**SEQ ID NO: 5**) or KVN PQGPGP (**SEQ ID NO: 6**).

In another aspect, the present invention discloses an isolated epitope consisting of a first epitope segment as disclosed herein and a second segment, wherein: (i) the first segment is: the fragment of KVNPGPGPEEK (**SEQ ID NO: 1**) consisting of VNPQGPPEEK (**SEQ ID NO: 2**), KVNPGPGPE (**SEQ ID NO: 5**) or KVNPGPGP (**SEQ ID NO: 6**); the variant of KVNPGPGPE (**SEQ ID NO: 5**); or the variant of KVNPGPGP (**SEQ ID NO: 6**); and(ii) the second segment consists of an amino acid sequence TQNARA (**SEQ ID NO: 8**).

In yet another aspect, the present invention refers to an isolated epitope consisting of a first epitope segment as disclosed herein and a second segment, wherein: (i) the first segment is: the fragment of KVNPGPGPEEK (**SEQ ID NO: 1**) consisting of VNPQGPPEEK (**SEQ ID NO: 2**), KVNPGPGPE (**SEQ ID NO: 5**) or KVNPGPGP (**SEQ ID NO: 6**); the variant of VNPQGPPEEK (**SEQ ID NO: 2**); the variant of KVNPGPGPE (**SEQ ID NO: 5**); or the variant of KVNPGPGP (**SEQ ID NO: 6**); and (ii) the second segment consists of an amino acid sequence TQNARAFRD (**SEQ ID NO: 7**).

In yet another aspect, the present invention refers to an isolated epitope consisting of a first epitope segment as disclosed herein and a second segment, wherein: (i) the first segment is: the fragment of KVNPGPGPEEK (**SEQ ID NO: 1**) consisting of NPQGPPEE (**SEQ ID NO: 4**); or the variant of NPQGPPEE (**SEQ ID NO: 4**); and (ii) the second segment consists of an amino acid sequence ALSTASDDR (**SEQ ID NO: 9**).

In yet another aspect, the present invention refers to an isolated epitope consisting of a first epitope segment as disclosed herein and a second segment, wherein: (i) the first segment is: the fragment of KVNPGPGPEEK (**SEQ ID NO: 1**) consisting of VNPQGPPEE (**SEQ ID NO: 3**); and (ii) the epitope second segment consists of: an amino acid sequence PRERPP (**SEQ ID NO: 10**) or an amino acid sequence QDASDDGSGS (**SEQ ID NO: 11**).

In yet another aspect, the present invention refers to an isolated epitope consisting of a first epitope segment according to claim 1, a second segment, and a third segment wherein: (i) the first segment is: the fragment of KVNPGPGPEEK (**SEQ ID NO: 1**) consisting of VNPQGPPEE (**SEQ ID NO: 3**); and (ii)the second segment consists of an amino acid sequence PRERPP (**SEQ ID NO: 10**), and the third segment consists of an amino acid sequence QDASDDGSGS (**SEQ ID NO: 11**).

In yet another aspect, the present invention refers to an isolated epitope comprising the epitope segment as disclosed herein and consisting of the amino acid sequence CGELYTQNARAFRDLGCGNPKVNPGPGPEEKRRRGC (**SEQ ID NO: 12**).

In yet another aspect, the present invention refers to an isolated epitope or epitope segment for an anti-glypican 1 (GPC-1) antibody consisting of an amino acid sequence selected from any one or a plurality of: TQNARA (**SEQ ID NO: 8**), ALSTASDDR (**SEQ ID NO: 9**), PRERPP (**SEQ ID NO: 10**), QDASDDGSGS (**SEQ ID NO: 11**), LGPECSRAVMK (**SEQ ID NO: 13**), and TQNARAFRD (**SEQ ID NO: 7**).

In yet another aspect, the present invention refers to an arrangement of epitopes comprising a combination of two or more distinct epitopes, wherein each said distinct epitope is an epitope as disclosed herein.

In yet another aspect, the present invention refers to an arrangement of epitopes comprising a combination of two or more distinct epitopes, wherein the arrangement comprises a first epitope as disclosed herein, and a second epitope as disclosed herein.

In yet another aspect, the present invention refers to a composition comprising an epitope as disclosed herein, or an arrangement of epitopes as disclosed herein, and a pharmaceutically acceptable carrier or excipient.

In yet another aspect, the present invention refers to an assembly comprising an epitope as disclosed herein, or an arrangement of epitopes as disclosed herein, bound to one or more soluble or insoluble supports.

In yet another aspect, the present invention refers to a nucleic acid encoding the epitope as disclosed herein.

In yet another aspect, the present invention refers to a vector comprising the nucleic acid as disclosed herein.

In yet another aspect, the present invention refers to a host cell comprising the vector as disclosed herein.

In yet another aspect, the present invention refers to an isolated binding entity capable of specifically binding to an epitope as disclosed herein, wherein the binding entity is not an antibody.

In yet another aspect, the present invention refers to an isolated binding entity capable of specifically binding to an epitope as disclosed herein, wherein the binding entity is an antibody and with the proviso that the antibody is not a: MIL38 antibody (CBA20140026), rabbit anti-GPC-1 polyclonal antibody (ab137604, abcam), mouse anti-glypican monoclonal antibody 2600 clone 4D1 (Millipore), or goat anti-glypican 1 antibody (AA 24-530).

In yet another aspect, the present invention refers to a fusion protein comprising the epitope as disclosed herein.

In yet another aspect, the present invention refers to use of the epitope as disclosed herein, the arrangement of epitopes as disclosed herein, or the fusion protein as disclosed herein, as a positive control element in a method for detecting GPC-1.

Accordingly, the present disclosure relates to at least the following examples :

Claims

1. An isolated epitope or epitope segment for an anti-glypican 1 (GPC-1) antibody located within a portion of the GPC-1 flexible loop defined by an amino acid sequence KVNPPQGGPGEK (**SEQ ID NO: 1**), wherein the isolated epitope or epitope segment consists of:

- (i) KVNPPQGGPGEK (**SEQ ID NO: 1**); or
- (ii) a fragment of KVNPPQGGPGEK (**SEQ ID NO: 1**) consisting of VNPQGGPGEK (**SEQ ID NO: 2**), VNPQGGPGE (**SEQ ID NO: 3**), NPQGGPGE (**SEQ ID NO: 4**), KVNPPQGGPE (**SEQ ID NO: 5**) or KVNPPQGGP (**SEQ ID NO: 6**).

2. An isolated epitope consisting of a first epitope segment according to claim 1 and a second segment, wherein:

- (i) the first segment is: the fragment of KVNPPQGGPGEK (**SEQ ID NO: 1**) consisting of VNPQGGPGEK (**SEQ ID NO: 2**), KVNPPQGGPE (**SEQ ID NO: 5**) or KVNPPQGGP (**SEQ ID NO: 6**); the variant of VNPQGGPGEK (**SEQ ID NO: 2**); the variant of KVNPPQGGPE (**SEQ ID NO: 5**); or the variant of KVNPPQGGP (**SEQ ID NO: 6**); and
- (ii) the second segment consists of an amino acid sequence TQNARA (**SEQ ID NO: 8**).

3. An isolated epitope consisting of a first epitope segment according to claim 1 and a second segment, wherein:

- (i) the first segment is: the fragment of KVNPPQGGPGEK (**SEQ ID NO: 1**) consisting of VNPQGGPGEK (**SEQ ID NO: 2**), KVNPPQGGPE (**SEQ ID NO: 5**) or KVNPPQGGP (**SEQ ID NO: 6**); the variant of VNPQGGPGEK (**SEQ ID NO: 2**); the variant of KVNPPQGGPE (**SEQ ID NO: 5**); or the variant of KVNPPQGGP (**SEQ ID NO: 6**); and
- (ii) the second segment consists of an amino acid sequence TQNARAFRD (**SEQ ID NO: 7**).

4. An isolated epitope consisting of a first epitope segment according to claim 1 and a second segment, wherein:

- (i) the first segment is: the fragment of KVNPPQGGPGEK (**SEQ ID NO: 1**) consisting of NPQGGPGE (**SEQ ID NO: 4**); or the variant of NPQGGPGE (**SEQ ID NO: 4**); and

(ii) the second segment consists of an amino acid sequence ALSTASDDR (**SEQ ID NO: 9**).

5. An isolated epitope consisting of a first epitope segment according to claim 1 and a second segment, wherein:

(i) the first segment is: the fragment of KVNPGPGPEEK (**SEQ ID NO: 1**) consisting of VNPQGPPEE (**SEQ ID NO: 3**); and

(ii) the epitope second segment consists of:
an amino acid sequence PRERPP (**SEQ ID NO: 10**) or
an amino acid sequence QDASDDGSGS (**SEQ ID NO: 11**).

6. An isolated epitope consisting of a first epitope segment according to claim 1, a second segment, and a third segment wherein:

(i) the first segment is: the fragment of KVNPGPGPEEK (**SEQ ID NO: 1**) consisting of VNPQGPPEE (**SEQ ID NO: 3**); and

(ii) the second segment consists of an amino acid sequence PRERPP (**SEQ ID NO: 10**), and the third segment consists of an amino acid sequence QDASDDGSGS (**SEQ ID NO: 11**).

7. An isolated epitope comprising the epitope segment according to claim 1 and consisting of the amino acid sequence CGELYTQNARAFRDL CGNPKVNPGPGPEEKRRRGC (**SEQ ID NO: 12**).

8. The epitope according to claim 1, wherein the epitope is a linear epitope.

9. The epitope according to claim 6, wherein the second segment and the third segment of the epitope are discontinuous.

10. The epitope according to any one of claims 2 to 5 or 9, wherein the first segment and the second segment of the epitope are discontinuous.

11. An isolated epitope or epitope segment for an anti-glypican 1 (GPC-1) antibody consisting of an amino acid sequence selected from any one or a plurality of: TQNARA (**SEQ ID NO: 8**), ALSTASDDR (**SEQ ID NO: 9**), PRERPP (**SEQ ID NO: 10**), QDASDDGSGS (**SEQ ID NO: 11**), LGPECSRAVMK (**SEQ ID NO: 13**), and TQNARAFRD (**SEQ ID NO: 7**).

12. The epitope or epitope segment according to any one of claims 1 to 11, wherein the epitope is a synthetic polypeptide.

13. An arrangement of epitopes comprising a combination of two or more distinct epitopes, wherein each said distinct epitope is an epitope according to any one of claims 1 to 12.

14. An arrangement of epitopes comprising a combination of two or more distinct epitopes, wherein the arrangement comprises

- a first epitope according to claim 2, and
- a second epitope according to claim 3.

15. An arrangement of epitopes comprising a combination of two or more distinct epitopes, wherein the arrangement comprises

- a first epitope according to claim 2, and
- a second epitope according to claim 4 or claim 5.

16. A composition comprising an epitope according to any one of claims 1 to 12, or an arrangement of epitopes according to any one of claims 13 to 15, and a pharmaceutically acceptable carrier or excipient.

17. An assembly comprising an epitope according to any one of claims 1 to 12, or an arrangement of epitopes according to any one of claims 13 to 15, bound to one or more soluble or insoluble supports.

18. The assembly of claim 17, wherein the assembly is a component of an enzyme-linked immunosorbent assay (ELISA).

19. A nucleic acid encoding the epitope according to any one of claims 1 to 12.

20. A vector comprising the nucleic acid according to claim 19.

21. A host cell comprising the vector according to claim 20.

22. An isolated binding entity capable of specifically binding to an epitope according to any one of claims 1 to 12, wherein the binding entity is not an antibody.

23. An isolated binding entity capable of specifically binding to an epitope according to any one of claims 1 to 12, wherein the binding entity is an antibody and with the proviso that the antibody is not a: MIL38 antibody (CBA20140026), rabbit anti-GPC-1 polyclonal antibody (ab137604, abcam), mouse anti-glypican monoclonal antibody 2600 clone 4D1 (Millipore), or goat anti-glypican 1 antibody (AA 24-530).

24. A fusion protein comprising the epitope according to any one of claims 1 to 12.

25. Use of the epitope according to any one of claims 1 to 12, the arrangement of epitopes according to any one of claims 13 to 15, or the fusion protein according to claim 24, as a positive control element in a method for detecting GPC-1.