mAbs that could be used to detect low expression of GPC3 in tumor cells for cancer therapy and diagnostics.

Filmus and colleagues developed the widely-used 1G12 mAb specific for the C-terminus of GPC3 and established an ELISA method to detect serum GPC3 in HCC patients (Capurro *et al.*, *Gastroenterology* 125:89-97, 2003). Hippo *et al.* developed mAbs specific for the N-terminus of GPC3 (Hippo *et al.*, *Cancer Res*, 64:2418-2423, 2004). While both studies detected soluble GPC3 protein in HCC culture supernatant or in the circulating blood of cancer patients, it is not clear whether the N-terminal or C-terminal subunit actually represents the soluble GPC3 format (Capurro and Filmus, *Cancer Res* 65:372, 2005). Prior studies indicate that concentrations of serum GPC3 are generally not high in patients and that none of the readily available mAbs can be used to measure serum GPC3, most likely due to low affinity of the mAb for GPC3.

SUMMARY

Disclosed herein is a panel of monoclonal antibodies that bind with high affinity to cell surface and soluble GPC3 proteins. The antibodies provided herein include immunoglobulin molecules, such as IgG antibodies, as well as antibody fragments and humanized monoclonal antibodies and fragments. Further provided are compositions including the antibodies that bind, for example specifically bind, to GPC3, nucleic acid molecules encoding these antibodies, expression vectors comprising the nucleic acid molecules, and isolated host cells that express the nucleic acid molecules. Also provided are immunoconjugates comprising the antibodies disclosed herein and an effector molecule, such as a toxin.

Further provided is an isolated monoclonal antibody that binds glypican-3 (GPC3), wherein:

(i) the heavy chain of the antibody comprises a complementarity determining region (CDR) 1, a CDR2 and a CDR3 respectively comprising amino acid residues 26-33, 51-60 and 99-106 of SEQ ID NO: 8, and the light chain of the antibody comprises a CDR1, a CDR2 and a CDR3 respectively comprising amino acid residues 27-38, 56-58 and 95-103 of SEQ ID NO: 10; or

(ii) the heavy chain of the antibody comprises a CDR1, a CDR2 and a CDR3 respectively comprising amino acid residues 31-35, 50-68 and 101-106 of SEQ ID NO: 8 and the light chain of the antibody comprises a CDR1, a CDR2 and a CDR3 respectively comprising amino acid residues 24-40, 56-62 and 95-103 of SEQ ID NO: 10.

Further provided is an isolated monoclonal antibody that binds glypican-3 (GPC3), wherein:

(i) the heavy chain of the antibody comprises amino acid residues 26-33, 51-60 and 99-106 of SEQ ID NO: 12, and the light chain of the antibody comprises amino acid residues 27-38, 56-58 and 95-103 of SEQ ID NO: 14;

(ii) the heavy chain of the antibody comprises amino acid residues 26-33, 51-60 and 99-106 of SEQ ID NO: 8, and the light chain of the antibody comprises amino acid residues 27-38, 56-58 and 95-103 of SEQ ID NO: 10;

(iii) the heavy chain of the antibody comprises amino acid residues 26-33, 51-60 and 99-106 of SEQ ID NO: 16, and the light chain of the antibody comprises amino acid residues 27-38, 56-58 and 95-103 of SEQ ID NO: 18; or

(iv) the heavy chain of the antibody comprises amino acid residues 26-33, 51-60 and 99-106 of SEQ ID NO: 20, and the light chain of the antibody comprises amino acid residues 27-38,

CLAIMS

1. An isolated monoclonal antibody that binds glypican-3 (GPC3), wherein:

(i) the heavy chain of the antibody comprises a complementarity determining region (CDR) 1, a CDR2 and a CDR3 respectively comprising amino acid residues 26-33, 51-60 and 99-106 of SEQ ID NO: 8, and the light chain of the antibody comprises a CDR1, a CDR2 and a CDR3 respectively comprising amino acid residues 27-38, 56-58 and 95-103 of SEQ ID NO: 10; or

(ii) the heavy chain of the antibody comprises a CDR1, a CDR2 and a CDR3 respectively comprising amino acid residues 31-35, 50-68 and 101-106 of SEQ ID NO: 8 and the light chain of the antibody comprises a CDR1, a CDR2 and a CDR3 respectively comprising amino acid residues 24-40, 56-62 and 95-103 of SEQ ID NO: 10.

2. The isolated monoclonal antibody of claim 1, wherein:

the amino acid sequence of the heavy chain is at least 90% or at least 95% identical to SEQ ID NO: 8; and the amino acid sequence of the light chain is at least 90% or at least 95% identical to SEQ ID NO: 10.

3. The isolated monoclonal antibody of claim 1 or claim 2, wherein:

the heavy chain of the antibody comprises SEQ ID NO: 8 and the light chain of the antibody comprises SEQ ID NO: 10.

4. The isolated monoclonal antibody of any one of claims 1-3, wherein the antibody is a Fab fragment, a Fab' fragment, a $F(ab)'_2$ fragment, a single chain variable fragment (scFv), or a disulfide stabilized variable fragment (dsFv).

5. The isolated monoclonal antibody of any one of claims 1-3, wherein the antibody is an IgG.

6. The isolated monoclonal antibody of any of claims 1-5, wherein the antibody is labeled.

7. The isolated monoclonal antibody of claim 6, wherein the label is a fluorescence, enzymatic, or radioactive label.

8. The isolated monoclonal antibody of any one of claims 1-7, wherein the monoclonal antibody is a humanized monoclonal antibody.

9. A composition comprising a therapeutically effective amount of the antibody of any one of claims 1-8 in a pharmaceutically acceptable carrier.

10. An isolated immunoconjugate comprising the monoclonal antibody of any of claims 1-8 and an effector molecule.

11. The isolated immunoconjugate of claim 10, wherein the effector molecule is a toxin.

12. The isolated immunoconjugate of claim 11, wherein the toxin is *Pseudomonas* exotoxin or a variant thereof.

13. The isolated immunoconjugate of claim 12, wherein the toxin is PE38 comprising the amino acid sequence of SEQ ID NO: 1.

14. The isolated immunoconjugate of any one of claims 10-13, comprising the amino acid sequence of residues residues 2-597 of SEQ ID NO: 28.

15. The isolated immunoconjugate of claim 10, wherein the effector molecule is a drug.

16. The isolated immunoconjugate of claim 10, wherein the effector molecule is a detectable label.

17. A chimeric antigen receptor (CAR), comprising the monoclonal antibody of any one of claims 1-8.

18. A bispecific antibody, comprising the monoclonal antibody of any one of claims 1-8.

19. A composition comprising a therapeutically effective amount of the isolated immunoconjugate of any one of claims 10-16, the CAR of claim 17 or the bispecific antibody of claim 18, in a pharmaceutically acceptable carrier.

20. Use of a therapeutically effective amount of the composition of claim 9 or claim 19 in the manufacture of a medicament for treating cancer in a subject with a cancer that expresses GPC3.

21. Use of a therapeutically effective amount of the composition of claim 9 or claim 19 in the manufacture of a medicament for inhibiting tumor growth or metastasis in a subject with a cancer that expresses GPC3.

22. A method of determining if a subject has cancer, or a method of confirming the diagnosis of cancer in a subject, comprising:

contacting a sample from the subject with the monoclonal antibody of any of claims 1-8; and

detecting binding of the antibody to the sample,

wherein an increase in binding of the antibody to the sample as compared to binding of the antibody to a control sample identifies the subject as having cancer, or confirms the diagnosis of cancer in the subject.

23. The method of claim 22, wherein the monoclonal antibody is directly labeled.

24. The method of claim 22, further comprising:

contacting a second antibody that specifically binds the monoclonal antibody with the sample, and

detecting the binding of the second antibody,

wherein an increase in binding of the second antibody to the sample as compared to binding of the second antibody to a control sample detects cancer in the subject or confirms the diagnosis of cancer in the subject.

25. The method of any one of claims 22-24, wherein the cancer is hepatocellular carcinoma (HCC), melanoma, squamous cell carcinoma of the lung or ovarian clear cell carcinoma.

26. An isolated nucleic acid molecule encoding the monoclonal antibody of any of claims 1-3.

27. The isolated nucleic acid molecule of claim 26, wherein:

the nucleotide sequence encoding the heavy chain of the monoclonal antibody comprises SEQ ID NO: 7 and the nucleotide sequence encoding the light chain of the monoclonal antibody comprises SEQ ID NO: 9.

28. An expression vector comprising the isolated nucleic acid molecule of claim 26 or claim 27.

29. An isolated host cell transformed with the nucleic acid molecule or expression vector of any one of claims 26-28.