VARIANT NUCLEIC ACID LIBRARIES FOR GLP1 RECEPTOR

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/810,377 filed on February 26, 2019; U.S. Provisional Patent Application No. 62/830,316 filed on April 5, 2019; U.S. Provisional Patent Application No. 62/855,836 filed on May 31, 2019; U.S. Provisional Patent Application No. 62/904,563 filed on September 23, 2019; U.S. Provisional Patent Application No. 62/945,049 filed on December 6, 2019; and U.S. Provisional Patent Application No. 62/961,104 filed on January 14, 2020, each of which is incorporated by reference in its entirety.

BACKGROUND

[0002] G protein-coupled receptors (GPCRs) are implicated in a wide variety of diseases. Raising antibodies to GPCRs has been difficult due to problems in obtaining suitable antigen because GPCRs are often expressed at low levels in cells and are very unstable when purified. Thus, there is a need for improved agents for therapeutic intervention which target GPCRs.

INCORPORATION BY REFERENCE

[0003] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF SUMMARY

[0003a] In one aspect, the present disclosure provides a nucleic acid library, comprising: a plurality of nucleic acids, wherein each of the nucleic acids encodes for a sequence that when translated encodes for a GLP1R binding immunoglobulin, wherein the GLP1R binding immunoglobulin comprises a ~~variant of a~~ GLP1R binding domain, wherein the GLP1R binding domain is a ligand for the GLP1R, wherein the GLP1R binding domain is placed in a CDR-H3 region, and wherein the nucleic acid library comprises at least 10,000 variant sequences that when translated encode for immunoglobulin heavy chains and at least 10,000 variant sequences that when translated encode for immunoglobulin light chains.

[0003b] In another aspect, the present disclosure provides a nucleic acid library comprising: a plurality of nucleic acids, wherein each of the nucleic acids encodes for a sequence that when translated encodes for a GLP1R single domain antibody~~,~~; wherein a GLP1R binding domain is placed in a CDR-H3 region of the GLP1R single domain antibody, wherein the GLP1R binding domain is a ligand for the GLP1R, and ~~each sequence of the plurality of sequences comprises a~~

~~variant sequence encoding for at least one of a CDR1, CDR2, and CDR3 on a heavy chain;~~ wherein the library comprises at least 30,000 variant sequences~~; and wherein the antibody or antibody fragments bind to its antigen with a KD of less than 100 nM~~.

[0003c] In another aspect, the present disclosure provides an antibody or antibody fragment that binds GLP1R, wherein the antibody or antibody fragment comprises a GLP1R binding domain, wherein the GLP1R binding domain is a ligand for the GLP1R, wherein the GLP1R binding domain is placed in a CDR-H3 region, wherein the antibody or antibody fragment comprises ~~comprising~~ an immunoglobulin heavy chain and an immunoglobulin light chain: a. wherein the immunoglobulin heavy chain comprises an amino acid sequence ~~at least about 90% identical to that~~as set forth in any one of SEQ ID NO: 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2317, 2318, 2319, 2320, ~~or~~ and 2321; and b. wherein the immunoglobulin light chain comprises an amino acid sequence ~~at least about 90% identical to that~~as set forth in any one of SEQ ID NO: 2310, 2311, 2312, 2313, 2314, 2315, ~~or~~ and 2316.

~~**[0003d]** In another aspect, the present disclosure provides an antagonist of GLP1R comprising SEQ ID NO: 2279 or 2320.~~

~~**[0003e]** In yet another aspect, the present disclosure provides an agonist of GLP1R comprising SEQ ID NO: 2317.~~

[0004] Provided herein are antibodies or antibody fragments thereof that binds GLP1R, comprising an immunoglobulin heavy chain and an immunoglobulin light chain: (a) wherein the immunoglobulin heavy chain comprises an amino acid sequence at least about 90%, 95%, 97%, 99%, or 100% identical to that set forth in SEQ ID NO: 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2317, 2318, 2319, 2320, or 2321; and (b) wherein the immunoglobulin light chain comprises an amino acid sequence at least about 90%, 95%, 97%, 99%, or 100% identical to that set forth in SEQ ID NO: 2310, 2311, 2312, 2313, 2314, 2315, or 2316. Further provided herein are antibodies or antibody fragments thereof that binds GLP1R, wherein the immunoglobulin heavy chain comprises an amino acid sequence at least about 90%, 95%, 97%, 99%, or 100% identical to that set forth in SEQ ID NO: 2303; and wherein the immunoglobulin light chain comprises an amino acid sequence at least about 90%, 95%, 97%, 99%, or 100% identical to that set forth in SEQ ID NO: 2310. Further provided herein are antibodies or antibody fragments thereof that binds GLP1R, wherein the immunoglobulin heavy chain comprises an amino acid sequence at least about

CLAIMS

WHAT IS CLAIMED IS:

1. A nucleic acid library, comprising: a plurality of nucleic acids, wherein each of the nucleic acids encodes for a sequence that when translated encodes for a GLP1R binding immunoglobulin, wherein the GLP1R binding immunoglobulin comprises a ~~variant of a~~ GLP1R binding domain, wherein the GLP1R binding domain is a ligand for the GLP1R, wherein the GLP1R binding domain is placed in a CDR-H3 region, and wherein the nucleic acid library comprises at least 10,000 variant sequences that when translated encode for immunoglobulin heavy chains and at least 10,000 variant sequences that when translated encode for immunoglobulin light chains.

2. The nucleic acid library of claim 1, wherein the nucleic acid library comprises at least 50,000 variant sequences that when translated encode for immunoglobulin heavy chains and at least 50,000 variant sequences that when translated encode for immunoglobulin light chains.

3. The nucleic acid library of claim 1, wherein the nucleic acid library comprises at least 10^5 non-identical nucleic acids.

4. The nucleic acid library of claim 1, wherein a length of the immunoglobulin heavy chains~~chain when translated~~ is ~~about~~ 90 to ~~about~~ 100 amino acids.

5. The nucleic acid library of claim 1, wherein a length of the immunoglobulin heavy chains~~chain when translated~~ is ~~about~~ 100 to ~~about~~ 400 amino acids.

6. The nucleic acid library of claim 1, wherein the nucleic acid library comprises at least one nucleic acid encoding for an~~variant~~ immunoglobulin heavy chain comprising the amino acid~~when translated comprises at least 90%~~ sequence as set forth in any one of~~identity to~~ SEQ ID NO: 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2317, 2318, 2319, 2320, and~~or~~ 2321.

7. The nucleic acid library of claim 1, wherein the nucleic acid library comprises at least one nucleic acid encoding for an~~variant~~ immunoglobulin light chain comprising the amino acid~~when translated comprises at least 90%~~ sequence as set forth in any one of~~identity to~~ SEQ ID NO: 2310, 2311, 2312, 2313, 2314, 2315, and~~or~~ 2316.

8. A nucleic acid library comprising: a plurality of nucleic acids, wherein each of the nucleic acids encodes for a sequence that when translated encodes for a GLP1R single domain antibody~~,~~;; wherein a GLP1R binding domain is placed in a CDR-H3 region of the GLP1R single domain antibody, wherein the GLP1R binding domain is a ligand for the GLP1R, and ~~each sequence of the plurality of sequences comprises a variant sequence encoding for at least one of a CDR1, CDR2, and CDR3 on a heavy chain;~~ wherein the library comprises at least 30,000 variant sequences~~; and wherein the antibody or antibody fragments bind to its antigen with a K_D of less than 100 nM~~.

9. The nucleic acid library of claim 8, wherein the nucleic acid library comprises at least 10^5 non-identical nucleic acids.

10. The nucleic acid library of claim 8, wherein a length of a~~the~~ heavy chain of the GLP1R single domain antibody~~when translated~~ is ~~about~~ 90 to ~~about~~ 100 amino acids.

11. The nucleic acid library of claim 8, wherein a length of a~~the~~ heavy chain of the GLP1R single domain antibody~~when translated~~ is ~~about~~ 100 to ~~about~~ 400 amino acids.

12. The nucleic acid library of claim 8, wherein the library~~variant heavy chain when translated~~ comprises at least one GLP1R single domain antibody comprising the amino acid~~90%~~ sequence as set forth in any one of~~identity to~~ SEQ ID NO: 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2317, 2318, 2319, 2320, and~~or~~ 2321.

13. An antibody or antibody fragment that binds GLP1R, wherein the antibody or antibody fragment comprises a GLP1R binding domain, wherein the GLP1R binding domain is a ligand for the GLP1R, wherein the GLP1R binding domain is placed in a CDR-H3 region, wherein the antibody or antibody fragment comprises~~comprising~~ an immunoglobulin heavy chain and an immunoglobulin light chain:

a. wherein the immunoglobulin heavy chain comprises an amino acid sequence as~~at least about 90% identical to that~~ set forth in any one of SEQ ID NO: 2303, 2304, 2305, 2306, 2307, 2308, 2309, 2317, 2318, 2319, 2320, and~~or~~ 2321; and

b. wherein the immunoglobulin light chain comprises an amino acid sequence as~~at least about 90% identical to that~~ set forth in any one of SEQ ID NO: 2310, 2311, 2312, 2313, 2314, 2315, and~~or~~ 2316.

~~14. The antibody or antibody fragment of claim 13, wherein the antibody is a monoclonal antibody, a polyclonal antibody, a bi-specific antibody, a multispecific antibody, a grafted antibody, a human antibody, a humanized antibody, a synthetic antibody, a chimeric antibody, a camelized antibody, a single-chain Fvs (scFv), a single chain antibody, a Fab fragment, a F(ab')2 fragment, a Fd fragment, a Fv fragment, a single-domain antibody, an isolated complementarity determining region (CDR), a diabody, a fragment comprised of only a single monomeric variable domain, disulfide-linked Fvs (sdFv), an intrabody, an anti-idiotypic (anti-Id) antibody, or ab antigen-binding fragments thereof.~~

~~15~~14. The antibody or antibody fragment of claim 13, wherein the antibody has an EC50 less than about 25 nanomolar in a cAMP assay.

~~16~~15. The antibody or antibody fragment of claim 13, wherein the antibody or antibody fragment comprises at least a CDR-H3 region comprising a sequence of any one of SEQ ID NOS: 2277,

2278, 2281, 2282, 2283, 2284, 2285, 2286, 2289, 2290, 2291, 2292, 2294, 2295, 2296, 2297, 2298, 2299, 2300, 2301, or 2302 or a sequence set forth in Table 27.

~~17~~16. The antibody or antibody fragment of claim 13, wherein the antibody or antibody fragment is an ~~An~~ antagonist of GLP1R and comprises ~~comprising~~ SEQ ID NO: 2279 or 2320.

~~18~~17. The antibody or antibody fragment~~antagonist~~ of claim 16~~17~~, wherein ~~the antagonist comprises an~~ EC50 of the antagonist is no more than 1.5 nM.

~~19~~18. ~~An~~ The antibody or antibody fragment of claim 13, wherein the antibody or antibody fragment is an agonist of GLP1R and comprises ~~comprising~~ SEQ ID NO: 2317.

~~20~~19. The antibody or antibody fragment~~agonist~~ of claim 18~~19~~, wherein ~~the agonist comprises an~~ EC50 of the agonist is no more than 1.5 nM.