

[0007] This is different from other technologies in the field which express a single scFV but do not express a library. Moreover, other technologies are designed to re-direct vaccinia infection through the scFV for gene therapy and are not used for antibody discovery. Additionally, the current technology differs from the previous technology by using EEV instead of the IMV, and also by using different fusion proteins (e.g., A56R).

SUMMARY

[0007a] In one aspect, there is provided a fusion protein comprising:

- (a) a first polypeptide segment comprising a heavy chain constant region domain,
 - (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein,
 - (c) a third polypeptide segment comprising an immunoglobulin heavy chain variable region or fragment thereof, and
 - (d) a signal peptide for facilitating expression of the fusion polypeptides on the surface of EEV,
- wherein the fusion protein is expressed on the surface of a vaccinia virus and/or on a host cell surface, and
- wherein the fusion protein when combined with a light chain variable domain forms a functional antibody.

[0008] In certain embodiments, the disclosure is directed to fusion protein comprising (a) a first polypeptide segment comprising a heavy chain CHI domain and (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein.

[0009] In some embodiments, the fusion protein further comprising a third polypeptide segment comprising an immunoglobulin heavy chain variable region or fragment thereof. In another embodiment, the vaccinia EEV-specific membrane protein is A56R.

[0009a] In one aspect, there is provided a polynucleotide encoding the fusion protein as described herein.

[0010] In certain embodiments, the disclosure is directed to a polynucleotide encoding a fusion protein comprising (a) a first polypeptide segment comprising the human heavy chain CHI domain and (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein.

[0011a] In one aspect, there is provided a recombinant vaccinia virus comprising the polynucleotide as described herein.

[0012] In certain embodiments, the disclosure is directed to a recombinant vaccinia virus comprising a polynucleotide encoding a fusion protein comprising (a) a first polypeptide segment comprising the human heavy chain CHI domain and (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein. In another aspect, there is provided a host cell infected with the recombinant vaccinia virus as described herein.

[0012a] In one aspect, there is provided a recombinant vaccinia library comprising a first library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides, wherein the vaccinia virus vector comprises (a) a first polynucleotide encoding a first polypeptide segment comprising an entire or partial heavy chain constant region CH1 domain (b) a second polynucleotide encoding a second polypeptide segment comprising the transmembrane domain of a vaccinia virus EEV-specific membrane protein situated downstream of the CH1 domain, and (c) a third polynucleotide encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the CH1 domain.

[0013] In another embodiment, the disclosure is directed to recombinant vaccinia library comprising a first library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides, wherein the vaccinia virus vector comprises (a) a first polynucleotide encoding a first polypeptide segment comprising a heavy chain CHI domain (b) a second polynucleotide encoding a second polypeptide segment comprising the transmembrane domain of a vaccinia virus EEV-specific membrane protein situated downstream of the CHI domain, and (c) a third polynucleotide encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the CHI domain. In one embodiment, the first library further comprising a signal peptide for facilitating expression of the fusion polypeptides on the surface of EEV. In another embodiment, the EEV-specific membrane protein is A56R. In another embodiment, the vaccinia EEV-specific membrane protein is A56R. In another embodiment, the second polypeptide segment further comprises the extracellular domain of the EEV-specific membrane protein, or a portion thereof. In another embodiment, the second polypeptide segment further comprises the intracellular domain of the EEV-specific membrane protein, or a portion thereof. In certain embodiments, the fusion protein comprises amino acids of SEQ ID NO: 11 which correspond to the polypeptide sequence amino acids 108 to 314 of A56R from Western

Reserve Vaccinia virus strain. In certain embodiments, the fusion protein comprises amino acids 215 to 421 of SEQ ID NO: 11. In certain embodiments, the fusion protein comprises amino acids 215 to 421 of SEQ ID NO: 11, which is the polypeptide sequence amino acids 108 to 314 of A56R from Western Reserve Vaccinia virus strain.

[0013a] In one aspect, there is provided a method for selecting polynucleotides which encode an antigen-specific immunoglobulin heavy chain variable region or antigen-binding fragment thereof, comprising:

(a) introducing a recombinant vaccinia library comprising a first library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides as described herein into a population of host cells permissive for vaccinia virus infectivity;

(b) introducing one or more polynucleotides encoding an immunoglobulin light chain into the population of host cells,

wherein said immunoglobulin fusion polypeptides are capable of combining with an immunoglobulin light chain to form an antigen-binding domain of an immunoglobulin molecule;

- (c) permitting release of extracellular enveloped virus (EEV) from the host cells;
- (d) collecting the released EEV from the supernatant;
- (e) contacting the released EEV with an antigen; and
- (f) recovering the polynucleotides of the first library which encode the immunoglobulin fusion polypeptides expressed on the membrane surface of EEV and specific for the antigen.

[0014] In another embodiment, the disclosure is directed to methods for selecting polynucleotides which encode an antigen-specific immunoglobulin heavy chain variable region or antigen-binding fragment thereof, comprising: (a) introducing the first library of any one of claims 13 to 18 encoding immunoglobulin fusion proteins into a

[0007] This is different from other technologies in the field which express a single scFV but do not express a library. Moreover, other technologies are designed to re-direct vaccinia infection through the scFV for gene therapy and are not used for antibody discovery. Additionally, the current technology differs from the previous technology by using EEV instead of the IMV, and also by using different fusion proteins (e.g., A56R).

SUMMARY

[0007a] In one aspect, there is provided a fusion protein comprising an immunoglobulin heavy chain variable region domain fused to the N-terminus of (a) a polypeptide segment consisting of the CH1 domain of human IgG1 fused to amino acids 215 to 421 of SEQ ID NO: 11, or (b) a polypeptide segment consisting of the full-length constant region of human IgG1 fused to amino acids 215 to 421 of SEQ ID NO: 11; wherein amino acids 215 to 421 of SEQ ID NO: 11 is identical to amino acids 108 to 314 of A56R from the Western Reserve Vaccinia virus strain; wherein the fusion protein can be expressed on the surface of a vaccinia extracellular enveloped virus (EEV) virus particle, and wherein the fusion protein when combined with a light chain variable domain can form a functional antibody or antigen binding fragment thereof.

[0008] In certain embodiments, the disclosure is directed to fusion protein comprising (a) a first polypeptide segment comprising a heavy chain CHI domain and (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein.

[0009] In some embodiments, the fusion protein further comprising a third polypeptide segment comprising an immunoglobulin heavy chain variable region or fragment thereof. In another embodiment, the vaccinia EEV-specific membrane protein is A56R.

[0009a] In one aspect, there is provided a polynucleotide encoding the fusion protein as described herein.

[0010] In certain embodiments, the disclosure is directed to a polynucleotide encoding a fusion protein comprising (a) a first polypeptide segment comprising the human heavy chain CHI domain and (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein.

[0011a] In one aspect, there is provided a recombinant vaccinia virus comprising the polynucleotide as described herein.

[0012] In certain embodiments, the disclosure is directed to a recombinant vaccinia virus comprising a polynucleotide encoding a fusion protein comprising (a) a first polypeptide segment comprising the human heavy chain CHI domain and (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein. In another aspect, there is provided a host cell infected with the recombinant vaccinia virus as described herein.

[0012a] In one aspect, there is provided recombinant vaccinia library comprising a library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides, wherein the vaccinia virus vector comprises (a) a first polynucleotide encoding a first polypeptide segment comprising a heavy chain CHI domain (b) a second polynucleotide encoding a second polypeptide segment comprising the transmembrane domain of a vaccinia virus EEV-specific membrane protein situated downstream of the CHI domain, and (c) a third polynucleotide encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the CHI domain.

[0013] In another embodiment, the disclosure is directed to recombinant vaccinia library comprising a first library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides, wherein the vaccinia virus vector comprises (a) a first polynucleotide encoding a first polypeptide segment comprising a heavy chain CHI domain (b) a second polynucleotide encoding a second polypeptide segment comprising the transmembrane domain of a vaccinia virus EEV-specific membrane protein situated downstream of the CHI domain, and (c) a third polynucleotide encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the CHI domain. In one embodiment, the first library further comprising a signal peptide for facilitating expression of the fusion polypeptides on the surface of EEV. In another embodiment, the EEV-specific membrane protein is A56R. In another embodiment, the vaccinia EEV-specific membrane protein is A56R. In another embodiment, the second polypeptide segment further comprises the extracellular domain of the EEV-specific membrane protein, or a portion thereof. In another embodiment, the second polypeptide segment further comprises the intracellular domain of the EEV-specific membrane protein, or a portion thereof. In certain embodiments, the fusion protein comprises amino acids of SEQ ID NO: 11 which correspond to the polypeptide sequence amino acids 108 to 314 of A56R from Western

Reserve Vaccinia virus strain. In certain embodiments, the fusion protein comprises amino acids 215 to 421 of SEQ ID NO: 11. In certain embodiments, the fusion protein comprises amino acids 215 to 421 of SEQ ID NO: 11, which is the polypeptide sequence amino acids 108 to 314 of A56R from Western Reserve Vaccinia virus strain.

[0013a] In one aspect, there is provided a method for selecting polynucleotides which encode an antigen-specific immunoglobulin heavy chain variable region or antigen-binding fragment thereof, comprising:

(a) introducing a recombinant vaccinia library encoding immunoglobulin fusion polypeptides into a population of host cells permissive for vaccinia virus infectivity, the recombinant vaccinia library comprising a first library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides, wherein the vaccinia virus vector comprises (1) a first polynucleotide encoding a first polypeptide segment comprising a heavy chain CH1 domain (2) a second polynucleotide encoding a second polypeptide segment comprising the transmembrane domain of a vaccinia virus extracellular enveloped virus (EEV)-specific membrane protein situated downstream of the CH1 domain, and (3) a third polynucleotide encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the CH1 domain;

(b) introducing one or more polynucleotides encoding an immunoglobulin light chain into the population of host cells,

wherein an immunoglobulin fusion protein is capable of combining with an immunoglobulin light chain to form an antigen-binding domain of an immunoglobulin molecule;

(c) permitting release of EEV from the host cells;

(d) collecting the released EEV from the supernatant;

(e) contacting the released EEV with an antigen; and

(f) recovering the polynucleotides of the first library which encode the immunoglobulin fusion polypeptides expressed on the membrane surface of EEV and specific for the antigen.

[0014] In another embodiment, the disclosure is directed to methods for selecting polynucleotides which encode an antigen-specific immunoglobulin heavy chain variable region or antigen-binding fragment thereof, comprising: (a) introducing the first library of any one of claims 13 to 18 encoding immunoglobulin fusion proteins into a

WHAT IS CLAIMED IS:

1. A fusion protein comprising:
 - (a) a first polypeptide segment comprising a heavy chain constant region domain,
 - (b) a second polypeptide segment comprising the transmembrane domain of a vaccinia extracellular enveloped virus (EEV)-specific membrane protein,
 - (c) a third polypeptide segment comprising an immunoglobulin heavy chain variable region or fragment thereof, and
 - (d) a signal peptide for facilitating expression of the fusion polypeptides on the surface of EEV,wherein the fusion protein is expressed on the surface of a vaccinia virus and/or on a host cell surface, and
wherein the fusion protein when combined with a light chain variable domain forms a functional antibody.
2. The fusion protein of claim 1, wherein the vaccinia EEV-specific membrane protein is A56R.
3. The fusion protein of claim 1, wherein the second polypeptide segment further comprises the extracellular domain of the EEV-specific membrane protein, or a portion thereof.
4. The fusion protein of claim 1, wherein the second polypeptide segment further comprises the intracellular domain of the EEV-specific membrane protein, or a portion thereof.
5. The fusion protein of claim 1, wherein the constant region comprises a CH1 domain, or a portion thereof.

6. The fusion protein of claim 1, wherein the constant region comprises a IgG-gamma heavy chain.

7. The fusion protein of claim 1, wherein the fusion protein comprises amino acids 215 to 421 of SEQ ID NO: 11.

8. A polynucleotide encoding the fusion protein of claim 1.

9. The polynucleotide of claim 8 comprising the nucleotides of SEQ ID NO: 10 which encodes amino acids 108 to 314 of A56R from Western Reserve Vaccinia virus strain or amino acids 215 to 421 of SEQ ID NO:11.

10. A vector comprising the polynucleotide of claim 8.

11. A recombinant vaccinia virus comprising the polynucleotide of claim 8.

12. A host cell infected with the recombinant vaccinia virus of claim 11.

13. A recombinant vaccinia library comprising a first library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides, wherein the vaccinia virus vector comprises (a) a first polynucleotide encoding a first polypeptide segment comprising an entire or partial heavy chain constant region CH1 domain (b) a second polynucleotide encoding a second polypeptide segment comprising the transmembrane domain of a vaccinia virus EEV-specific membrane protein situated downstream of the CH1 domain, and (c) a third polynucleotide

encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the CH1 domain.

14. The first library of claim 13, comprising a signal peptide for facilitating expression of the fusion polypeptides on the surface of EEV.

15. The first library of claim 13, wherein the EEV-specific membrane protein is A56R.

16. The first library of claim 13, wherein the vaccinia EEV-specific membrane protein is A56R.

17. The first library of claim 13, wherein the second polypeptide segment further comprises the extracellular domain of the EEV-specific membrane protein, or a portion thereof.

18. The first library of claim 13, wherein the second polypeptide segment further comprises the intracellular domain of the EEV-specific membrane protein, or a portion thereof.

19. The first library of claim 13, wherein the constant region comprises a CHI domain.

20. The first library of claim 13, wherein the constant region comprises a full length IgG.

21. The fusion protein of claim 1 or first library of claim 13, wherein the first polypeptide segment comprises amino acids 215 to 421 of SEQ ID NO:11.

22. The fusion protein of claim 1 or first library of claim 13, wherein the second polypeptide segment comprises amino acids 215 to 421 of SEQ ID NO:11.

23. A method for selecting polynucleotides which encode an antigen-specific immunoglobulin heavy chain variable region or antigen-binding fragment thereof, comprising:

(a) introducing a recombinant vaccinia library comprising a first library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides as in claim 1 into a population of host cells permissive for vaccinia virus infectivity;

(b) introducing one or more polynucleotides encoding an immunoglobulin light chain into the population of host cells,

wherein said immunoglobulin fusion polypeptides are capable of combining with an immunoglobulin light chain to form an antigen-binding domain of an immunoglobulin molecule;

(c) permitting release of extracellular enveloped virus (EEV) from the host cells;

(d) collecting the released EEV from the supernatant;

(e) contacting the released EEV with an antigen; and

(f) recovering the polynucleotides of the first library which encode the immunoglobulin fusion polypeptides expressed on the membrane surface of EEV and specific for the antigen.

24. The method of claim 23, further comprising:

(g) introducing the polynucleotides recovered in (f) into a second population of host cells permissive for vaccinia virus infectivity;

(h) introducing one or more polynucleotides encoding an immunoglobulin light chain into the population of host cells;

(i) permitting release of extracellular enveloped virus (EEV) from the host cells;

(j) collecting the released EEV from the supernatant;
(k) contacting the released EEV with an antigen; and
(l) recovering the polynucleotides of the first library which encode the immunoglobulin fusion polypeptides expressed on the membrane surface of EEV and specific for the antigen.

25. The method of claim 24, further comprising repeating steps (g)-(l) one or more times, thereby enriching for polynucleotides of the first library which encode immunoglobulin heavy chain variable regions or antigen-specific fragments thereof, as part of an immunoglobulin fusion polypeptide that specifically binds the antigen.

26. The method of claim 23, further comprising isolating the polynucleotides recovered from the first library.

WHAT IS CLAIMED IS:

1. A method for selecting polynucleotides which encode an antigen-specific immunoglobulin heavy chain variable region or antigen-binding fragment thereof, comprising:

(a) introducing a recombinant vaccinia library encoding immunoglobulin fusion polypeptides into a population of host cells permissive for vaccinia virus infectivity, the recombinant vaccinia library comprising a first library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides, wherein the vaccinia virus vector comprises (1) a first polynucleotide encoding a first polypeptide segment comprising a heavy chain CH1 domain (2) a second polynucleotide encoding a second polypeptide segment comprising the transmembrane domain of a vaccinia virus extracellular enveloped virus (EEV)-specific membrane protein situated downstream of the CH1 domain, and (3) a third polynucleotide encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the CH1 domain;

(b) introducing one or more polynucleotides encoding an immunoglobulin light chain into the population of host cells,

wherein an immunoglobulin fusion protein is capable of combining with an immunoglobulin light chain to form an antigen-binding domain of an immunoglobulin molecule;

(c) permitting release of EEV from the host cells;

(d) collecting the released EEV from the supernatant;

(e) contacting the released EEV with an antigen; and

(f) recovering the polynucleotides of the first library which encode the immunoglobulin fusion polypeptides expressed on the membrane surface of EEV and specific for the antigen.

2. The method of claim 1, further comprising:

(g) introducing the polynucleotides recovered in (f) into a second population of host cells permissive for vaccinia virus infectivity;

(h) introducing one or more polynucleotides encoding an immunoglobulin light chain into the population of host cells;

(i) permitting release of extracellular enveloped virus (EEV) from the host cells;

(j) collecting the released EEV from the supernatant;

(k) contacting the released EEV with an antigen; and

(1) recovering the polynucleotides of the first library which encode the immunoglobulin fusion polypeptides expressed on the membrane surface of EEV and specific for the antigen.

3. The method of claim 2, further comprising repeating steps (g)-(l) one or more times, thereby enriching for polynucleotides of the first library which encode immunoglobulin heavy chain variable regions or antigen-specific fragments thereof, as part of an immunoglobulin fusion polypeptide that specifically binds the antigen.

4. The method of claim 1, further comprising isolating the polynucleotides recovered from the first library.

5. The method of any one of claims 1 to 4, wherein the first polypeptide segment further comprises a heavy chain CH2 domain, a heavy chain CH3 domain, or a combination thereof.

6. The method of claim 5, wherein the first polypeptide segment comprises a human IgG constant region, or portion thereof.

7. The method of any one of claims 1 to 4, wherein the second polypeptide segment comprises the transmembrane domain of the vaccinia EEV-specific membrane protein A33R, A34R, A56R, A36R, F13L, or B5R.

8. The method of claim 7, wherein the vaccinia EEV-specific membrane protein is A56R.

9. The method of claim 8, wherein the second polypeptide segment comprises the stalk region, the transmembrane domain, and the intracellular domain of the A56R protein.

10. The method of claim 9, wherein the third polypeptide segment comprises amino acids 215 to 421 of SEQ ID NO: 11 or amino acids 447 to 653 of SEQ ID NO: 30.

11. The method of any one of claims 1 to 4, wherein the one or more polynucleotides encoding an immunoglobulin light chain of (b) are each contained on a vaccinia virus vector.

12. The method of any one of claims 1 to 4, wherein the one or more polynucleotides encoding an immunoglobulin light chain of (b) are contained on a plasmid vector.

13. A fusion protein comprising an immunoglobulin heavy chain variable region domain fused to the N-terminus of (a) a polypeptide segment consisting of the CH1 domain of human IgG1 fused to amino acids 215 to 421 of SEQ ID NO: 11, or (b) a polypeptide segment consisting of the full-length constant region of human IgG1 fused to amino acids 215 to 421 of SEQ ID NO: 11; wherein amino acids 215 to 421 of SEQ ID NO: 11 is identical to amino acids 108 to 314 of A56R from the Western Reserve Vaccinia virus strain; wherein the fusion

protein can be expressed on the surface of a vaccinia extracellular enveloped virus (EEV) virus particle, and wherein the fusion protein when combined with a light chain variable domain can form a functional antibody or antigen binding fragment thereof.

14. The fusion protein of claim 13, further comprising a signal peptide for facilitating expression of the fusion polypeptides on the surface of EEV.

15. A polynucleotide encoding the fusion protein of claim 13 or claim 14.

16. A vector comprising the polynucleotide of claim 15.

17. A recombinant vaccinia virus comprising the polynucleotide of claim 15 or the vector of claim 16.

18. A host cell infected with the recombinant vaccinia virus of claim 17.

19. A recombinant vaccinia library comprising a library of polynucleotides constructed in a vaccinia virus vector encoding a plurality of immunoglobulin fusion polypeptides, wherein the vaccinia virus vector comprises (a) a first polynucleotide encoding a first polypeptide segment comprising a heavy chain CH1 domain (b) a second polynucleotide encoding a second polypeptide segment comprising the transmembrane domain of a vaccinia virus EEV-specific membrane protein situated downstream of the CH1 domain, and (c) a third polynucleotide encoding an immunoglobulin heavy chain variable region or fragment thereof situated upstream of the CH1 domain.

20. The recombinant vaccinia library of claim 19, wherein each immunoglobulin fusion polypeptide further comprises a signal peptide for facilitating expression of the plurality of immunoglobulin fusion polypeptides on the surface of EEV.

21. The recombinant vaccinia library of claim 19 or claim 20, wherein the first polypeptide segment further comprises a heavy chain CH2 domain, a heavy chain CH3 domain, or a combination thereof.

22. The recombinant vaccinia library of claim 21, wherein the first polypeptide segment comprises a human IgG constant region, or portion thereof.

23. The recombinant vaccinia library of claim 19 or claim 20, wherein the second polypeptide segment comprises the transmembrane domain of the vaccinia EEV-specific membrane protein A33R, A34R, A56R, A36R, F13L, or B5R.

24. The recombinant vaccinia library of claim 23, wherein the vaccinia EEV-specific membrane protein is A56R.

25. The recombinant vaccinia library of claim 24, wherein the second polypeptide segment comprises the stalk region, the transmembrane domain, and the intracellular domain of the A56R protein.

26. The recombinant vaccinia library of claim 25, wherein the third polypeptide segment comprises amino acids 215 to 421 of SEQ ID NO: 11 or amino acids 447 to 653 of SEQ ID NO: 30.