complement complex/membrane attack complex (TCC/MAC) and an array of biologically active products.

Human cells and organs have a family of membrane-bound complement regulatory proteins to protect them from homologous complement-mediated lysis. These complement regulatory proteins include CD55 (decay-accelerating factor, DAF), CD46 (membrane cofactor protein, MCP), CD35 (complement receptor 1, CR1), and CD59 (membrane inhibitor of reactive lysis) (Carroll et al., 1988; Rey-Campos et al., 1988; Lublin et al., 1989; Morgan et al., 1994; Kim and Song, 2006).

CD55 is a glycosylphosphatidylinositol (GPI)-anchored protein and attaches to a cell plasma membrane through a glycolipid moiety (GPI anchor) at its C-terminus. The GPI-anchored proteins such as CD55 can be endocytosed and degraded or cleaved and released from cell plasma membrane (Censullo and Davitz, 1994a, 1994b; Turner 1994). For example, The GPI-anchored proteins including CD55 can be released from the cell surface by the action of GPI-specific phospholipases C and D (Turner 1994). These enzymatic activities likely control the catabolism of GPI-anchored proteins and regulate the cell surface expression of these proteins (Censullo and Davitz, 1994b).

SUMMARY

In one aspect, the present disclosure refers to a fusion protein comprising: (a) a CD55 peptide sequence, (b) a linker sequence C-terminal to the CD55 sequence, (c) a transmembrane domain C-terminal to the linker sequence, and (d) an intracellular domain C-terminal to the transmembrane domain; wherein the fusion protein does not contain a GPI anchor. In another aspect, the present disclosure refers to a nucleic acid encoding the protein as disclosed herein. In another aspect, the present disclosure refers to an expression vector comprising the nucleic acid as disclosed herein, operatively linked to a control sequence. In another aspect, the present disclosure refers to a cell line stably expressing the protein as disclosed herein on the cell surface. In yet another aspect, the present disclosure refers to enveloped virus incorporating the protein as disclosed herein

on the virus membrane. In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the virus of the present disclosure and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to the use of the virus of the present disclosure in the manufacture of a medicament for treating a neoplastic condition in a mammalian subject.

Also disclosed herein is a recombinant fusion protein comprising: (a) a CD55 peptide sequence, (b) a linker sequence C-terminal to the CD55 sequence, (c) a transmembrane domain C-terminal to the linker sequence, and (d) an intracellular domain C-terminal to the transmembrane domain, wherein the fusion protein does not contain a GPI anchor. This disclosure also provides nucleic acids and expression vectors encoding the protein, cells expressing the protein, enveloped viruses incorporating the protein on the viral membrane, pharmaceutical compositions comprising the protein-incorporating virus of this disclosure, as well as methods of treatment and uses of the virus.

This disclosure is based, in part, on the finding that virus expressing a fusion protein according to this disclosure was resistant to inactivation by normal human serum, as evidenced by a higher recovery rate compared to virus not expressing the fusion protein. The oncolytic enveloped virus produced by the engineered cells of the disclosure which incorporate complement inhibitor in the form of a recombinant fusion protein on the viral membrane is a better cancer therapeutic and affords better clinical outcomes for cancer patients as compared to the corresponding virus lacking a complement inhibitor on the viral membrane, due to its survival capability in the human serum circulation before it gets into a tumor. The benefits are three-fold: 1) the oncolytic virus can be produced in a cell culturing system in a bio-reactor; 2) fewer viral particles are needed to achieve the same therapeutic efficacy as compared to the parental oncolytic virus produced in chicken eggs; 3) infusion of fewer viral particles to a cancer patient may reduce side effects associated with large amounts of viral particles such as cytokine storm or impurity related effects.

Others who have studied the effects of the complement regulatory protein CD55 on the protection of Newcastle Disease Virus (NDV) (Biswas et al., 2012; Rangaswamy et al., 2016) used native unmodified CD55, which includes a glycosylphosphatidyl-inositol (GPI) anchor. In contrast the fusion protein of this disclosure omits the GPI anchor. Without wishing to be bound by theory it is believed that the omission of a GPI anchor changed the catabolism dynamics of CD55 on the cell surface. The fusion protein of this disclosure was able to withstand inactivation conditions more stringent than those utilized by Biswas and Rangaswamy. Biswas used 5 to 10% normal human serum and Rangaswamy used 0.3 to 5% normal human serum in their inactivation assays. The example below used 40% normal human serum to conduct the inactivation assay on NDV that has incorporated the recombinant fusion protein.

CLAIMS

What is claimed is:

1. A fusion protein comprising: (a) a CD55 peptide sequence, (b) a linker sequence C-terminal to the CD55 sequence, (c) a transmembrane domain C-terminal to the linker sequence, and (d) an intracellular domain C-terminal to the transmembrane domain; wherein the fusion protein does not contain a GPI anchor.

2. The protein of claim 1, wherein the CD55 peptide sequence is a human CD55 peptide sequence.

3. The protein of claim 1, wherein sequence (a) comprises four short consensus repeats (SCR) of CD55.

4. The protein of claim 1, wherein the linker is a (G4S1)3 linker.

5. The protein of claim 1, wherein transmembrane domain is a CD8 transmembrane domain.

6. The protein of claim 1, wherein the intracellular domain is a truncated CD8 intracellular domain.

7. The protein of claim 1, wherein peptide sequence (a) is covalently bonded to peptide sequence (b) by a single peptide bond.

8. The protein of claim 1, wherein peptide sequence (a) is covalently bonded to peptide sequence (b) by a spacer.

9. The protein of claim 1, wherein peptide sequence (b) is covalently bonded to peptide sequence (c) by a single peptide bond.

10. The protein of claim 1, wherein peptide sequence (b) is covalently bonded to peptide sequence (c) by a spacer.

11. The protein of claim 1, wherein peptide sequence (c) is covalently bonded to peptide sequence (d) by a single peptide bond.

12. The protein of claim 1, wherein peptide sequence (c) is covalently bonded to peptide sequence (d) by a spacer.

13. The protein of claim 1, wherein the fusion protein further comprises a secretory signal peptide N-terminal to sequence (a).

14. The protein of claim 13, wherein the secretory signal peptide is a secretory signal peptide of CD55.

15. The protein of claim 13, wherein the N-terminal secretory signal peptide is covalently bonded to sequence (a) by a single peptide.

16. The protein of claim 13, wherein the N-terminal secretory signal peptide is covalently bonded to sequence (a) by a spacer.

17. The protein of claim 1, having the sequence SEQ ID NO:2.

18. The protein of claim 1, having the sequence SEQ ID NO:3.

19. A nucleic acid encoding the protein of any one of claims 1 through 18.

20. The nucleic acid of claim 19, wherein the nucleic acid is DNA.

21. The nucleic acid of claim 20, further comprising one or more introns.

22. The nucleic acid of claim 19, encoding a protein having the sequence SEQ ID NO:2.

23. The nucleic acid of claim 22, having the sequence SEQ ID NO:1.

24. An expression vector comprising the nucleic acid of any one of claims 19 through 23, operatively linked to a control sequence.

25. A cell line stably expressing the protein of any one of claims 1 through 18 on the cell surface.

26. The cell line of claim 25, wherein the cell line is a mammalian cell line.

27. The cell line of claim 25, wherein the cell line is a DF-1 chicken embryonic fibroblast cell line.

28. An enveloped virus incorporating the protein of any one of claims 1 through 18 on the virus membrane.

29. The virus of claim 28, wherein the virus is an oncolytic virus.

30. The virus of claim 29, wherein the oncolytic virus is a Newcastle Disease Virus.

31. A pharmaceutical composition comprising the virus of claim 29 or 30 and a pharmaceutically acceptable carrier.

32. Use of the virus of claim 28 in the manufacture of a medicament for treating a neoplastic condition in a mammalian subject.

33. The use of 32, wherein the virus is to be administered intratumorally.

34. The use of claim 32, wherein the virus is to be administered intravenously.