

[0009] In one example, the disclosure provides an isolated nucleic acid encoding a chimeric adaptor (CAD) polypeptide, wherein the CAD polypeptide comprises a DAP10 domain and at least one costimulatory signaling domain, and wherein the CAD polypeptide specifically lacks an ectodomain comprising a functional extracellular receptor and/or ligand binding domain. In preferred examples, the DAP10 domain comprises a human DAP10 amino acid sequence.

[0009a] Thus, in one aspect, the disclosure provides an isolated nucleic acid encoding a chimeric adaptor (CAD) polypeptide, wherein the CAD polypeptide comprises a DAP10 domain comprising a mutated human DAP10 amino acid sequence and at least one costimulatory domain, wherein said CAD polypeptide specifically lacks an ectodomain comprising a functional extracellular receptor and/or ligand-binding domain, and wherein the mutated human DAP10 amino acid sequence comprises amino acid substitutions at positions corresponding to K84 and/or Y86.

[0010] In embodiments, the at least one costimulatory domain is selected from the group comprising or consisting of TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD3C, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD70, CD80, CD83, CD86, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), FcR, LAT, NKD2C, SLP76, TRIM, and ZAP70, or combinations thereof. In embodiments, the at least one costimulatory domain is 4-1BB. In embodiments, the at least one costimulatory domain is CD28.

[0011] In embodiments, the CAD polypeptide further comprises at least one intracellular signaling domain, wherein the at least one intracellular signaling domain is selected from the group comprising or consisting of CD3 ζ , DAP12, LFA-1, and CD3t, or combinations thereof. In embodiments, the at least one signaling domain is CD3 ζ . In embodiments, the at least one costimulatory domain is 4-1BB and the at least one intracellular signaling domain is CD3 ζ . In embodiments, the CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the 4-1BB costimulatory domain followed by the CD3 ζ intracellular signaling domain.

[0012] In embodiments, the CAD polypeptide comprises, N-terminus to C-terminus, the DAP10 domain, a 4-1BB costimulatory domain and a CD28 costimulatory domain. In embodiments, the CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the 4-1BB costimulatory domain followed by the CD28 costimulatory domain, followed in turn by a CD3 ζ intracellular signaling domain. In embodiments, the CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the 4-1BB costimulatory domain followed by the CD28 costimulatory domain, followed in turn by a CD3 ζ intracellular signaling domain.

[0013] In embodiments, the DAP10 domain comprises a human DAP10 amino acid sequence with an amino acid sequence having at least 90%, 95%, 97%, or 99% sequence identity to SEQ ID NO: 1. In examples, the human DAP10 amino acid sequence comprises a mutated human DAP10 amino acid sequence. In examples, the mutated human DAP10 amino acid sequence comprises amino acid substitutions at positions corresponding to K84 and/or Y86. In embodiments, the amino acid substitution at position K84 comprises a K84R substitution. In embodiments, the amino acid substitution at position Y86 comprises a Y86F substitution.

[0014] In embodiments, the isolated nucleic acid comprises a nucleic acid sequence set forth in SEQ ID NO: 60, or SEQ ID NO: 62, or SEQ ID NO: 64, or SEQ ID NO: 66, or SEQ ID NO: 68, or SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 82, or SEQ ID NO: 90.

[0015] In embodiments, the isolated nucleic acid is operably linked to a regulatable promoter. In embodiments, the isolated nucleic acid further encodes for a cytokine. In embodiments, the cytokine is selected from the group consisting of IL-2, IL-4, IL-7, IL-15, IL-21, IL-23. In embodiments, the isolated nucleic acid further encodes a marker protein. In embodiments, the marker protein is selected from the group consisting of truncated CD19, CD20 (Rituxumab recognition domain), truncated EGFR, and LNGFR.

[0016] In another aspect, the disclosure provides an expression vector comprising any of the foregoing isolated nucleic acids.

[0017] In another aspect, the disclosure provides a chimeric adaptor (CAD) polypeptide encoded by any of the foregoing isolated nucleic acids or expression vectors.

[0018] In another aspect, the disclosure provides a mammalian cell comprising any of the foregoing expression vectors or CAD polypeptides, wherein the mammalian cell expresses at least one receptor that associates with DAP10. In embodiments, the at least one receptor that associates with DAP10 is endogenous, exogenous, or over-expressed. In exemplary embodiments, the receptor is NKG2D. In embodiments, the mammalian cell is an immune cell, preferably wherein the immune cell is a cytotoxic cell.

[0019] In another aspect, the disclosure provides a method for activating an immune cell, comprising: expressing a CAD polypeptide according to the disclosure in the

immune cell, wherein the immune cell expresses at least one receptor that associates with DAP 10; and wherein the activating occurs responsive to the receptor engaging a corresponding target molecule. Hence, in one aspect, there is provided an *in vitro* method for activating an immune cell, comprising: expressing the CAD polypeptide as described herein in the immune cell, wherein the immune cell expresses at least one receptor that associated with DAP 10; and wherein the activating occurs responsive to the receptor engaging a corresponding target molecule. In embodiments, the receptor is endogenous, exogenous, or over-expressed. In exemplary embodiments, the receptor is NKG2D. In embodiments, the immune cell, or a plurality thereof, are introduced to a subject in need thereof; and the activating occurs in the subject.

[0020] In a further example, the disclosure provides a use of the mammalian cell as described herein, or a plurality thereof, in the preparation of a medicament for treating a subject with a condition for which the mammalian cell, or the plurality thereof, reduces at least one symptom or sign of said condition in the subject.

[0020a] In another aspect, there is provided a use of the mammalian cell as described herein, or a plurality thereof, in the manufacture of a medicament for treating cancer, an infectious disease, or an autoimmune disorder in a subject.

[0021] In a still further aspect, pharmaceutical compositions are provided comprising a pharmaceutically acceptable excipient and a plurality of mammalian cells as described herein.

[0021a] In a further aspect, the disclosure provides the CAD polypeptide as described herein or the mammalian cell, or a plurality thereof, as described herein for use in therapy.

[0022] Other features, objects, and advantages will be apparent from the disclosure that follows.

INCORPORATION BY REFERENCE

[0023] 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 DESCRIPTION OF THE DRAWINGS

[0024] FIGS. 1A-1D are schematic depictions of exemplary chimeric DAP 10 adaptor polypeptides of the present disclosure, in conjunction with a receptor with which they associate. In the depicted illustrations, the receptor is NKG2D. FIG. 1 A depicts a chimeric DAP10 adaptor polypeptide with K84R and Y86F modifications; FIG. 1B depicts a chimeric DAP 10 adaptor polypeptide with K84R and Y86F modifications, as well as a C-terminal fusion comprising CD3 ζ signaling domain; FIG. 1C depicts a chimeric DAP 10 adaptor polypeptide with K84R and Y86F modifications, as well as a C-terminal fusion comprising a 4-1BB costimulatory domain; and FIG. 1D depicts a chimeric DAP 10 adaptor polypeptide with K84R and Y86F modifications,

CLAIMS

1. An isolated nucleic acid encoding a chimeric adaptor (CAD) polypeptide, wherein the CAD polypeptide comprises a DAP10 domain comprising a mutated human DAP10 amino acid sequence and at least one costimulatory domain, wherein said CAD polypeptide specifically lacks an ectodomain comprising a functional extracellular receptor and/or ligand-binding domain, and wherein the mutated human DAP10 amino acid sequence comprises amino acid substitutions at positions corresponding to K84 and/or Y86.
2. The isolated nucleic acid according to claim 1, wherein the costimulatory domain is selected from TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, CARD11, CD2, CD3C, CD7, CD27, CD28, CD30, CD40, CD54 (ICAM), CD70, CD80, CD83, CD86, CD134 (OX40), CD137 (4-1BB), CD278 (ICOS), FcR, LAT, NKD2C, SLP76, TRIM, and ZAP70, or combinations thereof.
3. The isolated nucleic acid according to claim 2, wherein the at least one costimulatory domain is 4-1BB.
4. The isolated nucleic acid according to claim 2, wherein the at least one costimulatory domain is CD28.
5. The isolated nucleic acid according to any one of claims 1-4, wherein said CAD polypeptide further comprises at least one intracellular signaling domain, wherein the at least one intracellular signaling domain is selected from CD3 ζ , DAP12, LFA-1, and CD3t.
6. The isolated nucleic acid according to claim 5, wherein the at least one signaling domain is CD3 ζ , optionally wherein CD3 ζ has an amino acid sequence set forth as SEQ ID NO: 76.
7. The isolated nucleic acid according to any one of claims 1-6, wherein the at least one costimulatory domain is 4-1BB and the intracellular signaling domain is CD3 ζ .

8. The isolated nucleic acid according to claim 7, wherein said CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the 4-1BB costimulatory domain followed by the CD3 ζ intracellular signaling domain.
9. The isolated nucleic acid according to claim 1, wherein said CAD polypeptide comprises a 4-1BB costimulatory domain and a CD28 costimulatory domain.
10. The isolated nucleic acid according to claim 9, wherein said CAD polypeptide comprises, from N-terminus to C-terminus, the DAP10 domain, the 4-1BB costimulatory domain followed by the CD28 costimulatory domain, followed in turn by a CD3 ζ intracellular signaling domain, optionally wherein CD3 ζ has an amino acid sequence set forth as SEQ ID NO: 76.
11. The isolated nucleic acid according to claim 9, wherein said CAD polypeptide comprises, from N-terminus to C-terminus, the DAP 10 domain, the CD28 costimulatory domain followed by the 4-1BB costimulatory domain, followed in turn by a CD3 ζ signaling domain, optionally wherein CD3 ζ has an amino acid sequence set forth as SEQ ID NO: 76.
12. The isolated nucleic acid according to any one of claims 1-11, wherein the human DAP 10 amino acid sequence comprises an amino acid sequence having at least 90%, 95%, 97%, or 99% sequence identity to SEQ ID NO: 1.
13. The isolated nucleic acid according to claim 5 or 6, wherein the at least one costimulatory domain is a costimulatory domain of DAP10.
14. The isolated nucleic acid according to any one of claims 1-13, wherein the amino acid substitution at position K84 comprises a K84R substitution.
15. The isolated nucleic acid according to any one of claims 1-13, wherein the amino acid substitution at position Y86 comprises a Y86F substitution.
16. The isolated nucleic acid according to any one of claims 1-15, wherein the isolated nucleic acid is operably linked to a regulatable promoter.

17. The isolated nucleic acid according to any one of claims 1-16, wherein said isolated nucleic acid further encodes for a cytokine.
18. The isolated nucleic acid according to claim 17, wherein the cytokine is selected from the group consisting of IL-2, IL-4, IL-7, IL-15, IL-21, IL-23.
19. An expression vector comprising the isolated nucleic acid of any one of claims 1-18.
20. A chimeric adaptor (CAD) polypeptide encoded by the isolated nucleic acid according to any one of claims 1-17, or the expression vector according to claim 19.
21. A mammalian cell comprising the expression vector of claim 19, or the CAD polypeptide according to claim 19, wherein the mammalian cell expresses at least one receptor that associates with DAP 10.
22. The mammalian cell according to claim 21, wherein the at least one receptor that associates with DAP 10 is endogenous.
23. The mammalian cell according to claim 21, wherein the at least one receptor that associates with DAP 10 is over-expressed.
24. The mammalian cell according to claim 21, wherein the at least one receptor that associates with DAP 10 is exogenous.
25. The mammalian cell according to any one of claims 21-24, wherein the receptor is NKG2D.
26. The mammalian cell according to any one of claims 21-25, wherein the mammalian cell is an immune cell, preferably wherein said immune cell is a cytotoxic cell.
27. An *in vitro* method for activating an immune cell, comprising:
expressing the CAD polypeptide of claim 20 in the immune cell, wherein the immune cell expresses at least one receptor that associates with DAP 10; and
wherein the activating occurs responsive to the receptor engaging a corresponding target molecule.

28. The method according to claim 27, wherein the receptor is endogenous.
29. The method according to claim 27, wherein the receptor is exogenous.
30. The method according to claim 27, wherein the receptor is over-expressed.
31. The method according to any one of claims 27-30, wherein the receptor is NKG2D.
32. Use of the mammalian cell, or a plurality thereof, according to any one of claims 21-26, in the manufacture of a medicament for treating cancer, an infectious disease, or an autoimmune disorder in a subject.
33. A pharmaceutical composition comprising a pharmaceutically acceptable excipient and a plurality of mammalian cells according to any one of claims 21-26.
34. The CAD polypeptide of claim 20 or the mammalian cell, or a plurality thereof, according to any one of claims 21-26 for use in therapy.