binding to the lipids of mitochondrial membrane, the release of cytochrome C, SMAC/Diablo protein and AIF factor into the cytoplasm occurs, which causes proapoptotic caspase activation in the affected cell. Degradation of the mitochondrial membranes leads also to the activation of caspase-9, resulting in the induction of apoptosis.

Summary of Disclosure

In one embodiment, there is provided a fusion protein comprising domain (a) which is a functional fragment of the sequence of soluble hTRAIL protein, which fragment begins with an amino acid in the range from hTRAIL95 to hTRAIL121, inclusive, and ends with the amino acid at the position hTRAIL281, or a homolog of said functional fragment having at least 70% sequence identity, preferably 85% identity, and at least one domain (b) which is the sequence of a cytolytic effector peptide with an amphipatic alpha-helix conformation forming pores in the cell membrane, wherein the sequence of the domain (b) is attached at the C-terminus and/or N-terminus of domain (a).

In one embodiment, there is provided a polynucleotide, coding the fusion protein as defined herein.

In one embodiment, there is provided an expression vector comprising polynucleotide as defined herein.

In one embodiment, there is provided a non-human host cell comprising the expression vector as defined herein.

In one embodiment, there is provided a pharmaceutical composition comprising as an active ingredient the fusion protein as defined herein, in combination with a pharmaceutically acceptable carrier.

Description of Figures.

Fig. 1 presents tumor volume changes (% of initial stage) in Cby.Cg-foxn1(nu)/J mice burdened with lung cancer A549 treated with fusion protein of the invention of Ex. 11^a compared to rhTRAIL114-281;

Fig. 2 presents tumor growth inhibition values (%TGI) in Cby.Cg-foxn1(nu)/J mice burdened with lung cancer A549 treated with fusion protein of the invention of Ex. 11^a compared to rhTRAIL114-281;

Fig. 3 presents tumor volume changes (% of initial stage) in Crl:SHO-PrkdcscidHrhr mice burdened with lung cancer A549 treated with fusion protein of the invention of Ex. 11^a compared to rhTRAIL114-281;

Fig. 4 presents tumor growth inhibition values (%TGI) in Crl:SHO-PrkdcscidHrhr mice burdened with lung cancer A549 treated with fusion protein of the invention of Ex. 11^a compared to rhTRAIL114-281;

Fig. 5 presents tumor volume changes (% of initial stage) in Crl:SHO-PrkdcscidHrhr mice nu burdened with lung cancer NCI-H460-Luc2 treated with fusion protein of the invention of Ex. 11^a compared to rhTRAIL114-281;

Fig. 6 presents tumor growth inhibition values (%TGI) in Crl:SHO-PrkdcscidHrhr mice burdened with lung cancer NCI-H460-Luc2 treated with fusion protein of the invention of Ex. 11^a compared to rhTRAIL114-281;

Fig. 7 presents tumor volume changes (% of initial stage) in Cby.Cg-foxn1(nu)/J mice burdened with prostate cancer PC3 treated with fusion protein of the invention of Ex. 11^a compared to rhTRAIL114-281;

Fig. 8 presents tumor growth inhibition values (%TGI) in Cby.Cg-foxn1(nu)/J mice burdened with prostate cancer PC3 treated with fusion protein of the invention of Ex. 11^a compared to rhTRAIL114-281;

Fig. 9 presents tumor volume changes (% of initial stage) in Crl:SHO-PrkdcscidHrhr mice burdened with pancreas cancer PANC-1 treated with fusion protein of the invention of Ex. 11^a compared to rhTRAIL114-281;

Claims

1. A fusion protein comprising:

- domain (a) which is a functional fragment of the sequence of soluble hTRAIL protein, which fragment begins with an amino acid at a position not lower than hTRAIL95 and ends with the amino acid at the position hTRAIL281, or a homolog of said functional fragment having at least 70% sequence identity, preferably 85% identity, and
- at least one domain (b) which is the sequence of a cytolytic effector peptide forming pores in the cell membrane,

wherein the sequence of the domain (b) is attached at the C-terminus and/or N-terminus of domain (a),

2. The fusion protein according to claim 1, wherein domain (a) comprises a fragment of soluble hTRAIL (SEQ. No. 90) protein sequence, which begins with an amino acid in the range from hTRAIL95 to hTRAIL121, inclusive, and ends with the amino acid hTRAIL281.

3. The fusion protein according to claim 1 or 2, wherein domain (a) is selected from the group consisting of hTRAIL95-281, hTRAIL114-281, hTRAIL115-281, hTRAIL116-281, hTRAIL119-281, and hTRAIL121-281.

4. The fusion protein according to claim 1 to 3, wherein domain (b) is selected from the group consisting of:

- active form of human granulysin of SEQ. No. 34,
- 15-amino acids synthetic lytic peptide of SEQ. No. 35,
- pilosulin-1 of SEQ. No. 36,
- pilosulin-5 of SEQ. No. 37,
- peptide from tachyplesin of SEQ. No. 38,
- fusion peptide bombesin-magainin 2 of SEQ. No. 39,
- magainin-2 of SEQ. No. 40,

- 14-amino acids synthetic lytic peptide of SEQ. No. 41,
- 26-amino acids hybride peptide cecropin-melittin of SEQ. No. 42,
- 27-amino acids peptide FFhCAP18 of SEQ. No. 43,
- BAMP-28peptide of SEQ. No. 44,
- analogue of isoform C of lytic peptide from *Entamoeba histolytica* of SEQ. No. 45,
- analogue of isoform A of lytic peptide from *Entamoeba histolytica* SEQ. No. 46,
- analogue of isoform B of lytic peptide from *Entamoeba histolytica* of SEQ. No. 47,
- fragment of HA2 domain of influenza virus haemagglutinin of SEQ. No. 48,
- N-terminal domain of alpha-toxin from *Clostridium perfringens* with phospholipase C activity of SEQ. No. 49,
- listeriolysin O of SEQ. No. 50,
- phospholipase PC-PLC of SEQ. No. 51
- equinatoxin EqTx-II of SEQ. No. 52
- viscotoxin A3 (VtA3) of SEQ. No. 53
- active fragment of human perforin of SEQ. No. 54,
- parasporin-2 z *Bacillus thuringensis* of SEQ. No. 55, i
- fusion peptide comprising an EGF inhibitor and synthetic lytic peptide of SEQ. No. 56,
- fusion protein comprising synthetic lytic peptide with KLLK motif and a peptide being antagonist of PDGF receptor of SEQ. No. 125,
- pleurocidin analogue of SEQ. No. 126,
- pleurocidin analogue of SEQ. No. 127,
- synthetic lytic peptide of SEQ. No. 128,
- fusion peptide comprising bombesin and B27 peptide of SEQ. No. 129,
- 17-amino acids synthetic B27peptide of SEQ. No. 130,
- fusion peptide comprising bombesin and B28 peptide of SEQ. No. 131, and

- melittin peptide of SEQ. No. 132.

5. The fusion protein according to claim 1 to 4, in which domain (b) is a peptide with a strong positive charge selected from the group consisting of:

- active form of human granulysin of SEQ. No. 34,
- 15-amino acids synthetic lytic peptide of SEQ. No. 35,
- peptide from tachyplesin of SEQ. No. 38,
- fusion peptide bombesin-magainin 2 of SEQ. No. 39,
- magainin-2 of SEQ. No. 40,
- 26-amino acids hybride peptide cecropin-melittin of SEQ. No. 42,
- viscotoxin A3 (VtA3) of SEQ. No. 53,
- fusion peptide comprising an EGF inhibitor and synthetic lytic peptide of SEQ. No. 56,
- a fusion peptide comprising bombesin and B27 peptide of SEQ. No. 129,
- 17-amino acids synthetic B27peptide of SEQ. No. 130,
- a fusion peptide comprising bombesin and B28 peptide of SEQ. No. 131, and
- melittin peptide of SEQ. No. 132.

6. The fusion protein according to claim 1 to 5, in which domain (b) is a peptide with amphipathic alpha-helix selected from the group consisting of:

- pilosulin-1 of SEQ. No. 36,
- pilosulin-5 of SEQ. No. 37,
- 14-amino acids synthetic lytic peptide of SEQ. No. 41,
- 27-amino acids peptide FFhCAP18 of SEQ. No. 43,
- BAMP-28 peptide of SEQ. No. 44,
- analogue of isoform C of lytic peptide from *Entamoeba histolytica* of SEQ. No. 45,
- analogue of isoform A of lytic peptide from *Entamoeba histolytica* SEQ. No. 46,

- analogue of isoform B of lytic peptide from *Entamoeba histolytica* of SEQ. No. 47,

- fragment of HA2 domain of influenza virus haemagglutinin of SEQ. No. 48,

- active fragment of human perforin of SEQ. No. 54,

- parasporin-2 z *Bacillus thuringensis* of SEQ. No. 55,

- fusion protein comprising synthetic lytic peptide with KLLK motif and a peptide being antagonist of PDGF receptor of SEQ. No. 125,

- pleurocidin analogue of SEQ. No. 126,

- pleurocidin analogue of SEQ. No. 127, and

- synthetic lytic peptide of SEQ. No. 128.

7. The fusion protein according to claim 1 to 6, in which domain (b) is a peptide with enzymatic activity selected from the group of phospholipase, hemolysin and/or cytolysin, preferably selected from the group consisting of:

- N-terminal domain of alpha-toxin from *Clostridium perfringens* with phospholipase C activity of SEQ. No. 49,

- listeriolysin O of SEQ. No. 50,

- phospholipase PC-PLC of SEQ. No. 51, and

- equinatoxin EqTx-II of SEQ. No. 52.

8. The fusion protein according to any of the claims 1 to 7, which between domain (a) and domain (b) or between domains (b) contains domain (c) containing protease cleavage site, selected from a sequence recognized by metalloprotease MMP, a sequence recognized by urokinase uPA, and sequence recognized by furin and a sequence recognized by native furin.

9. The fusion protein according to claim 8, in which a sequence recognized by metalloprotease MMP is Pro Leu Gly Leu Ala Gly, a sequence recognized by urokinase uPA is Arg Val Val Arg, a sequence recognized by furin is Arg Lys Lys Arg, and a sequence recognized by native furin is Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu or His Arg Gln Pro Arg Gly Trp Glu Gln.

10. The fusion protein according to claim 8 or 9, in which domain (c) is a combination of sequence recognized by metalloprotease MMP and a sequence recognized by urokinase uPA located next to each other.

11. The fusion protein according to any of preceding claims, in which effector peptide of domain (b) is additionally connected with transporting domain (d), selected from the group consisting of:

- (d1) polyhistidine sequence transporting through the cell membrane comprising 6, 7 , 8, 9, 10 or 11 His residues, and

(d2) polyarginine sequence transporting through a cell membrane, consisting of 6, 7, 8, 9, 10 or 11 Arg residues,

(d3) PD4 transporting sequence (protein transduction domain 4) Tyr Ala Arg Ala Ala Ala Arg Gln Ala Arg Ala,

(d4) a transporting sequence consisting of transferrin receptor binding sequence Thr His Arg Pro Pro Met Trp Ser Pro Val Trp Pro, and

(d5) PD5 transporting sequence (protein transduction domain 5, TAT protein) Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg,
and combinations thereof.

12. The fusion protein according to claim 11, wherein sequence (d) is located at the C-terminus or N-terminus of the effector peptide domain (b).

13. The fusion protein according to claim 11, wherein transporting domain (d) is located between domain (b) and domain (c), or between domain (a) and domain (c), or between two domains (c).

14. The fusion protein according to claim 11, wherein sequence (d) is located at the C-terminus of the fusion protein.

15. The fusion protein according to any one of claims 8 to 14, which between two (c) domains contains domain (d) which is a linker for attachment of PEG molecule, selected from Ala Ser Gly Cys Gly Pro Glu Gly and Ala Ser Gly Cys Gly Pro Glu.

16. The fusion protein according to any one of claims from 8 to 15, which additionally comprises a flexible steric linker between domains (a), (b) and/or (c).

17. The fusion protein according to claim 16, wherein the steric linker is selected from Gly Gly, Gly Gly Gly, Gly Ser Gly, Gly Gly Gly Gly Ser, Gly Gly Gly Gly Gly Ser, Gly Gly Ser Gly Gly, Gly Gly Gly Ser Gly Gly Gly, Gly Gly Gly Gly Ser Gly, Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser, Gly Gly Gly Gly Ser Gly Gly Gly Gly, Gly Ser Gly Gly Gly Ser Gly Gly Gly, Cys Ala Ala Cys Ala Ala Ala Cys, Cys Ala Ala Ala Cys Ala Ala Cys, Ser Gly Gly, single glycine residue Gly, and single cysteine residue Cys, and combinations thereof.

18. The fusion protein according to claim 1, having the amino acid sequence selected from the group consisting of SEQ. No. 1; SEQ. No. 2; SEQ. No. 3; SEQ. No. 4; SEQ. No. 5; SEQ. No. 6; SEQ. No. 7; SEQ. No. 8; SEQ. No. 9; SEQ. No. 10; SEQ. No. 11; SEQ. No. 12; SEQ. No. 13; SEQ. No. 14; SEQ. No. 15; SEQ. No. 16; SEQ. No. 17; SEQ. No. 18; SEQ. No. 19; SEQ. No. 20; SEQ. No. 21; SEQ. No. 22; SEQ. No. 23; SEQ. No. 24; SEQ. No. 25; SEQ. No. 26, SEQ. No. 27; SEQ. No. 28; SEQ. No. 29; SEQ. No. 30; SEQ. No. 31; SEQ. No. 32, SEQ. No. 33; SEQ. No. 91; SEQ. No. 92; SEQ. No. 93; SEQ. No. 94; SEQ. No. 95; SEQ. No. 96; SEQ. No. 97, SEQ. No. 98; SEQ. No. 99; SEQ. No. 100; SEQ. No. 101; SEQ. No. 102;SEQ. No. 103, SEQ. No. 104; SEQ. No. 105; SEQ. No. 106, and SEQ. No. 107.

19. The fusion protein according to any one of the preceding claims, which is a recombinant protein.

20. A polynucleotide sequence, coding the fusion protein as defined in any one of claims 1 to 18.

21. The polynucleotide sequence according to claim 20, optimized for genetic expression in *E. coli*.

22. A sequence according to claim 21, selected from the group consisting of SEQ. No. 57; SEQ. No. 58; SEQ. No. 59; SEQ. No. 60; SEQ. No. 61; SEQ. No. 62; SEQ. No. 63; SEQ. No. 64; SEQ. No. 65; SEQ. No. 66; SEQ. No. 67; SEQ. No. 68; SEQ. No. 69; SEQ. No. 70; SEQ. No. 71; SEQ. No. 72; SEQ. No. 73; SEQ. No. 74; SEQ. No. 75; SEQ. No. 76; SEQ. No. 77; SEQ. No. 78; SEQ. No. 79; SEQ. No. 80; SEQ. No. 81; SEQ. No. 82, SEQ. No. 83; SEQ. No. 84; SEQ. No. 85; SEQ. No. 86; SEQ. No. 87;SEQ. No. 88, SEQ. No. 89, SEQ. No. 108; SEQ. No. 109; SEQ. No. 110; SEQ. No. 111; SEQ. No. 112; SEQ. No. 113; SEQ. No. 114, SEQ. No. 115; SEQ. No. 116; SEQ. No. 117; SEQ. No. 118; SEQ. No. 119; SEQ. No. 120; SEQ. No. 121; SEQ. No. 122; SEQ. No. 123, and SEQ. No. 124.

23. An expression vector comprising polynucleotide sequence according to any one of claims 20 to 22.

24. A host cell comprising the expression vector as defined in claim 23.

25. The host cell according to claim 24, which is an *E. coli* cell.

26. A pharmaceutical composition comprising as an active ingredient the fusion protein as defined in any one of claims 1 to 19, in combination with a pharmaceutically acceptable carrier.

27. The pharmaceutical composition according to claim 26 in a form for parenteral administration.

28. The fusion protein as defined in any one of claims 1 to 19 for use in the treatment of neoplastic diseases in mammals, including humans.

Claims

1. A fusion protein comprising:

domain (a) which is a functional fragment of the sequence of soluble hTRAIL protein, which fragment begins with an amino acid in the range from hTRAIL95 to hTRAIL121, inclusive, and ends with the amino acid at the position hTRAIL281, or a homolog of said functional fragment having at least 70% sequence identity, preferably 85% identity, and

at least one domain (b) which is the sequence of a cytolytic effector peptide with an amphipatic alpha-helix conformation forming pores in the cell membrane,

wherein the sequence of the domain (b) is attached at the C-terminus and/or N-terminus of domain (a).

2. The fusion protein according to claim 1, wherein domain (a) is selected from the group consisting of hTRAIL95-281, hTRAIL114-281, hTRAIL115-281, hTRAIL116-281, hTRAIL119-281, and hTRAIL121-281.

3. The fusion protein according to claim 1 to 2, in which domain (b) is selected from the group consisting of:

- pilosulin-1 of SEQ. No. 36,
- pilosulin-5 of SEQ. No. 37,
- 14-amino acids synthetic lytic peptide of SEQ. No. 41,
- 27-amino acids peptide FFhCAP18 of SEQ. No. 43,
- BAMP-28 peptide of SEQ. No. 44,
- analogue of isoform C of lytic peptide from Entamoeba histolytica of SEQ. No. 45,
- analogue of isoform A of lytic peptide from Entamoeba histolytica SEQ. No. 46,
- analogue of isoform B of lytic peptide from Entamoeba histolytica of SEQ. No. 47,

- fragment of HA2 domain of influenza virus haemagglutinin of SEQ. No. 48,

- active fragment of human perforin of SEQ. No. 54,

- parasporin-2 z Bacillus thuringensis of SEQ. No. 55,

- fusion protein comprising synthetic lytic peptide with KLLK motif and a peptide being antagonist of PDGF receptor of SEQ. No. 125,

- pleurocidin analogue of SEQ. No. 126,

- pleurocidin analogue of SEQ. No. 127, and

- synthetic lytic peptide of SEQ. No. 128.

4. The fusion protein according to any of the claims 1 to 3, which between domain (a) and domain (b) or between domains (b) contains domain (c) containing protease cleavage site, selected from a sequence recognized by metalloprotease MMP, a sequence recognized by urokinase uPA, and sequence recognized by furin and a sequence recognized by native furin.

5. The fusion protein according to claim 4, in which a sequence recognized by metalloprotease MMP is Pro Leu Gly Leu Ala Gly, a sequence recognized by urokinase uPA is Arg Val Val Arg, a sequence recognized by furin is Arg Lys Lys Arg, and a sequence recognized by native furin is Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu or His Arg Gln Pro Arg Gly Trp Glu Gln.

6. The fusion protein according to claim 4 or 5, in which domain (c) is a combination of sequence recognized by metalloprotease MMP and a sequence recognized by urokinase uPA located next to each other.

7. The fusion protein according to any of preceding claims, in which effector peptide of domain (b) is additionally connected with transporting domain (d), selected from the group consisting of:

- (d1) polyhistidine sequence transporting through the cell membrane comprising 6, 7 , 8, 9, 10 or 11 His residues, and

(d2) polyarginine sequence transporting through a cell membrane, consisting of 6, 7, 8, 9, 10 or 11 Arg residues,

(d3) PD4 transporting sequence (protein transduction domain 4) Tyr Ala Arg Ala Ala Ala Arg Gln Ala Arg Ala,

(d4) a transporting sequence consisting of transferrin receptor binding sequence Thr His Arg Pro Pro Met Trp Ser Pro Val Trp Pro, and

(d5) PD5 transporting sequence (protein transduction domain 5, TAT protein) Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg,
and combinations thereof.

8. The fusion protein according to claim 7, wherein sequence (d) is located at the C-terminus or N-terminus of the effector peptide domain (b).

9. The fusion protein according to claim 7, wherein transporting domain (d) is located between domain (b) and domain (c), or between domain (a) and domain (c), or between two domains (c).

10. The fusion protein according to claim 7, wherein sequence (d) is located at the C-terminus of the fusion protein.

11. The fusion protein according to any one of claims 4 to 10, which between two (c) domains contains domain (e) which is a linker for attachment of PEG molecule, selected from Ala Ser Gly Cys Gly Pro Glu Gly and Ala Ser Gly Cys Gly Pro Glu.

12. The fusion protein according to any one of claims from 4 to 11, which additionally comprises a flexible steric linker between domains (a), (b) and/or (c).

13. The fusion protein according to claim 12, wherein the steric linker is selected from Gly Gly, Gly Gly Gly, Gly Ser Gly, Gly Gly Gly Gly Ser,
Gly Gly Gly Gly Gly Ser, Gly Gly Ser Gly Gly, Gly Gly Gly Ser Gly Gly Gly,
Gly Gly Gly Gly Ser Gly, Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser,
Gly Gly Gly Gly Ser Gly Gly Gly Gly, Gly Ser Gly Gly Gly Ser Gly Gly Gly,
Cys Ala Ala Cys Ala Ala Ala Cys, Cys Ala Ala Ala Cys Ala Ala Cys, Ser Gly Gly, single glycine residue Gly, and single cysteine residue Cys, and combinations thereof.

14. The fusion protein according to claim 1, having the amino acid sequence selected from the group consisting of SEQ. No. 4; SEQ. No. 5; SEQ. No. 6; SEQ. No. 11; SEQ. No. 12; SEQ. No. 13; SEQ. No. 14; SEQ. No. 15; SEQ. No. 16; SEQ. No. 18; SEQ. No. 19; SEQ. No. 20; SEQ. No. 21; SEQ. No. 22; SEQ. No. 23; SEQ. No. 30;

SEQ. No. 31; SEQ. No. 32; SEQ. No. 91; SEQ. No. 92; SEQ. No. 93; SEQ. No. 94; SEQ. No. 95; SEQ. No. 96; SEQ. No. 97; SEQ. No. 98; and SEQ. No. 102.

15. The fusion protein according to any one of the preceding claims, which is a recombinant protein.

16. A polynucleotide, coding the fusion protein as defined in any one of claims 1 to 14.

17. The polynucleotide according to claim 16, optimized for genetic expression in E. coli.

18. The polynucleotide according to claim 17, selected from the group consisting of SEQ. No. 60; SEQ. No. 61; SEQ. No. 62; SEQ. No. 67; SEQ. No. 68; SEQ. No. 69; SEQ. No. 70; SEQ. No. 71; SEQ. No. 72; SEQ. No. 74; SEQ. No. 75; SEQ. No. 76; SEQ. No. 77; SEQ. No. 78; SEQ. No. 79; SEQ. No. 86; SEQ. No. 87;SEQ. No. 88; SEQ. No. 108; SEQ. No. 109; SEQ. No. 110; SEQ. No. 111; SEQ. No. 112; SEQ. No. 113; SEQ. No. 114, SEQ. No. 115; and SEQ. No. 119.

19. An expression vector comprising polynucleotide according to any one of claims 16 to 18.

20. A non-human host cell comprising the expression vector as defined in claim 19.

21. The host cell according to claim 20, which is an E. coli cell.

22. A pharmaceutical composition comprising as an active ingredient the fusion protein as defined in any one of claims 1 to 15, in combination with a pharmaceutically acceptable carrier.

23. The pharmaceutical composition according to claim 22 in a form for parenteral administration.

24. The fusion protein as defined in any one of claims 1 to 15 for use in the treatment of neoplastic diseases in mammals, including humans.