THERAPEUTIC COMPOSITIONS AND METHODS INVOLVING mRNA TRANSFECTION

GOVERNMENT FUNDING

The present invention was made with government support under Grant No. 1R01DE021206, awarded by the NIH/NIDCR. The Government has certain rights in this invention.

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application Serial No. 61/751,504, filed January 11, 2013, which is incorporated herein by reference.

SUMMARY

This disclosure describes, in one ~~aspect~~embodiment, a method of suppressing cell proliferation. Generally the method includes introducing into an epithelial cell an mRNA encoding a polypeptide involved in suppressing cell proliferation and permitting the cell to express the polypeptide in an amount effective to decrease the likelihood that the cell proliferates in an anchorage-independent environment. In some embodiments, the polypeptide can include calprotectin (S100A8 complexed with S100A9; S100A8/A9), S100A8, or S100A9.

In one aspect, there is provided an in vitro method comprising: introducing into a mucosal epithelial cell in a sample an mRNA encoding a polypeptide involved in innate immunity; and permitting the cell to express the polypeptide in an amount effective to decrease the likelihood that the cell is infected by one or more pathogens.

In one aspect, there is provided the use of an mRNA encoding a polypeptide involved in innate immunity in the manufacture of a medicament for decreasing the likelihood of a cell being infected by a pathogen; wherein the medicament is to be introduced into the cell, wherein the cell is permitted to express the polypeptide in an amount effective to decrease the likelihood that the cell is infected by the pathogen.

In another ~~aspect~~embodiment, this disclosure describes a method of inhibiting infection of a cell. Generally, the method includes introducing into a cell an mRNA encoding a polypeptide involved in innate immunity and permitting the cell to express the polypeptide in an amount effective to decrease the likelihood that the cell is infected by a pathogen. In some embodiments, the polypeptide can include cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a β-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme.

In some embodiments ~~of either aspect~~ summarized above, the mRNA can include a stabilizing moiety such as, for example, a 5' cap or a 3' extension. In some embodiments, the cell can be a mucosal epithelial cell.

In one aspect, there is provided a composition comprising:an mRNA that encodes a polypeptide involved in innate immunity; and an in vivo delivery vehicle.

In another ~~aspect~~embodiment, this disclosure describes a composition that generally includes an mRNA that encodes a polypeptide and an *in vivo* delivery vehicle.

In some embodiments, the polypeptide can suppress epithelial cell proliferation. In some of these embodiments, the polypeptide can include calprotectin, S100A8, or S100A9.

In other embodiments, the polypeptide can be involved in innate immunity. In some of these embodiments, the polypeptide can include cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a β-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme. In some embodiments, the mRNA can include a stabilizing moiety such as, for example, a 5' cap or a 3' extension.

The above summary is not intended to describe each disclosed embodiment or every implementation of the present ~~invention~~disclosure. The description that follows more particularly exemplifies illustrative embodiments. In several places throughout the application, guidance is provided through lists of examples, which examples can be used in various combinations. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. CAMP protein expression by KB cells after transfection with ARCA-capped *CAMP* mRNA or CE capped *CAMP* mRNA. (A) CAMP protein expression after KB cells were transfected with ARCA, CE cap0, or cap1-capped *CAMP* mRNA. Upper, representative Western blot. Lower, quantitative interpretation, with ARCA cap expression at eight hours set to 100. After analysis using Quantity One analysis as described in the Materials and Methods, protein expression after transfection with the several capping methods was presented relative to 100. (B) CAMP protein expression over time after transfection with ARCA-capped *CAMP*. As in panel (A), CAMP protein expression after ARCA-capped *CAMP* mRNA transfection was detected using Western blot (upper panel) and presented in the lower panel relative to the level at eight hours, which is set to 100. In panels (A) and (B), the error bars show the means ± SD of three to six independent experiments.

FIG. 2. S100A8 and S100A9 protein expression by KB cells after transfection with ARCA-capped *S100A8*, *S100A9*, *A8-IRES-A9*, and *A8-nIRES-A9* mRNA. (A) S100A8 protein expression after KB cells were transfected with *S100A8* mRNA as detected using Western blot (representative image shown) and quantitated as in FIG. 1. Identical experiments were performed to determine the time course of expression of (B) S100A9 protein; (C) S100A8 and S100A9 protein after KB cells were cotransfected with *S100A8*/*S100A9* mRNA; (D) S100A8

What is claimed is:

1. An in vitro method comprising:

   introducing into a mucosal epithelial cell in a sample an mRNA encoding a polypeptide involved in innate immunity; and

   permitting the cell to express the polypeptide in an amount effective to decrease the likelihood that the cell is infected by one or more pathogens.

2. The method of claim 1 wherein the polypeptide comprises cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a β-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme.

3. The method of any preceding claim wherein the mRNA comprises a stabilizing moiety.

4. The method of claim 3 wherein the stabilizing moiety comprises a 5' cap.

5. The method of claim 3 wherein the stabilizing moiety comprises a 3' extension.

6. The method of any one of claims 1-5 further comprising permitting the cell to be exposed to the one or more pathogens.

7. The method of any one of claims 1-6 wherein the one or more pathogens comprises a bacterium.

8. The method of claim 7 wherein the bacterium comprises *Capnocytophaga sputigena*, *Escherichia coli*, a *Staphylococcus* spp., a *Streptococcus* spp., *Listeria monocytogenes*, *Salmonella typhimurium*, *Borrelia burgdorferi*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Pseudomonas aeruginosa*, a *Chlamydia* spp., a *Neisseria* spp., a *Gardnerella* spp., or a *Trichomonas* spp.

9. The method of any one of claims 1-8 wherein the one or more pathogens comprises a fungus.

10. The method of claim 9 wherein the fungus comprises fungi *Candida albicans*, *Acinetobacter baumannii*, or an *Aspergillus* spp.

11. The method of any one of claims 1-10 wherein the cell is a cell of a subject having or at risk of having a condition caused by infection by the pathogen.

12. The method of claim 11 wherein the condition comprises a carcinoma.

13. The method of any preceding claim further comprising washing the cell to remove mucosal fluid from the cell prior to introducing the mRNA into the cell.

14. The method of claim 13 wherein the mucosal fluid comprises saliva.

15. A composition comprising:
an mRNA that encodes a polypeptide involved in innate immunity; and
an *in vivo* delivery vehicle.

16. The composition of claim 15 wherein the polypeptide comprises cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a β-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme.

17. The composition of any one of claims 15-16 wherein the mRNA comprises a stabilizing moiety.

18. The composition of claim 17 wherein the stabilizing moiety comprises a 5' cap.

19. The composition of claim 17 wherein the stabilizing moiety comprises a 3' extension.

20. Use of an mRNA encoding a polypeptide involved in innate immunity in the manufacture of a medicament for decreasing the likelihood of a cell being infected by a pathogen; wherein the medicament is to be introduced into the cell, wherein the cell is permitted to express the polypeptide in an amount effective to decrease the likelihood that the cell is infected by the pathogen.

21. The use of claim 20 wherein the polypeptide comprises cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a B-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme.

22. The use of any preceding claim wherein the mRNA comprises a stabilizing moiety.

23. The use of claim 22 wherein the stabilizing moiety comprises a 5' cap.

24. The use of claim 22 wherein the stabilizing moiety comprises a 3' extension.

25. The use of any preceding claim wherein the cell is a mucosal epithelial cell.

26. The use of any one of claims 20-25 wherein the cell is further permitted to be exposed to the pathogen.

27. The use of any one of claims 20-26 wherein the pathogen comprises a bacterium.

28. The use of claim 27 wherein the bacterium comprises *Capnocytophaga sputigena*, *Escherichia coli*, a *Staphylococcus* spp., a *Streptococcus* spp., *Listeria monocytogenes*, *Salmonella typhimurium*, *Borrelia burgdorferi*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponeam denticola*, *Pseudomonas aeruginosa*, a *Chlamydia* spp., a *Neisseria* spp., a *Gardnerella* spp., or a *Trichomonas* spp.

29. The use of any one of claims 20-26 wherein the pathogen comprises a fungus.

30. The use of claim 29 wherein the fungus comprises fungi *Candida albicans*, *Acinetobacter baumanni*, or an *Aspergillus* spp.

31. The use of any one of claims 20-30 wherein the cell is a cell of a subject having or at risk of having a condition caused by infection by the pathogen.

32. The use of claim 31 wherein the condition comprises a carcinoma.

33. The use of any preceding claim wherein the cell is further washed to remove mucosal fluid from the cell prior to introducing the mRNA into the cell.

34. The use of claim 33, wherein the mucosal fluid comprises saliva.

~~What is claimed is:~~

1. A method comprising:

introducing into an epithelial cell an mRNA encoding a polypeptide involved in suppressing cell proliferation; and

permitting the cell to express the polypeptide in an amount effective to decrease the likelihood that the cell proliferates in an anchorage-independent environment.

2. The method of claim 1 wherein the polypeptide comprises calprotectin, S100A8, or S100A9.

3. A method comprising:

introducing into a cell an mRNA encoding a polypeptide involved in innate immunity; and

permitting the cell to express the polypeptide in an amount effective to decrease the likelihood that the cell is infected by a pathogen.

4. The method of claim 3 wherein the polypeptide comprises cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a β-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme.

5. The method of any preceding claim wherein the mRNA comprises a stabilizing moiety.

6. The method of claim 5 wherein the stabilizing moiety comprises a 5' cap.

7. The method of claim 5 wherein the stabilizing moiety comprises a 3' extension.

8. The method of any preceding claim wherein the cell is a mucosal epithelial cell.

9. The method of any one of claims 3-8 further comprising permitting the cell to be exposed to the pathogen.

10. The method of any one of claims 3-9 wherein the pathogen comprises a bacterium.

~~11. The method of claim 10 wherein the bacterium comprises *Capnocytophaga sputigena*, *Escherichia coli*, a *Staphylococcus* spp., a *Streptococcus* spp., *Listeria monocytogenes*, *Salmonella typhimurium*, *Borrelia burgdorferi*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Pseudomonas aeruginosa*, a *Chlamydia* spp., a *Neisseria* spp., a *Gardnerella* spp., or a *Trichomonas* spp.~~

12. The method of any one of claims 3-9 wherein the pathogen comprises a fungus.

13. The method of claim 12 wherein the fungus comprises fungi *Candida albicans*, *Acinetobacter baumannii*, or an *Aspergillus* spp.

14. The method of any one of claims 3-13 wherein the cell is a cell of a subject having or at risk of having a condition caused by infection by the pathogen.

15. The method of claim 14 wherein the condition comprises a carcinoma.

16. The method of any preceding claim further comprising washing the cell to remove mucosal fluid from the cell prior to introducing the mRNA into the cell.

17. The method of claim 16 wherein the mucosal fluid comprises saliva.

18. A composition comprising:

an mRNA that encodes a polypeptide that suppresses cell proliferation in an epithelial cell; and

an *in vivo* delivery vehicle.

19. The composition of claim 18 wherein the polypeptide comprises calprotectin, S100A8, or S100A9.

20. The composition of claim 18 or claim 19 wherein the polypeptide suppresses cell proliferation of the epithelial cell when the epithelial cell is grown in an anchorage-independent environment.

21. A composition comprising:
an mRNA that encodes a polypeptide involved in innate immunity; and
an *in vivo* delivery vehicle.

22. The composition of claim 21 wherein the polypeptide comprises cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a β-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme.

23. The composition of any one of claims 18-22 wherein the mRNA comprises a stabilizing moiety.

24. The composition of claim 23 wherein the stabilizing moiety comprises a 5' cap.

25. The composition of claim 23 wherein the stabilizing moiety comprises a 3' extension.