THERAPEUTIC COMPOSITIONS AND METHODS INVOLVING mRNA TRANSFECTION

GOVERNMENT FUNDING

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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 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 a method comprising: introducing into a mucosal squamous epithelial cell a composition comprising (i) an mRNA that comprises a synthetic 5' cap and that encodes a polypeptide involved in innate immunity selected from cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a β-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme and (ii) a pharmaceutically acceptable *in vivo* delivery vehicle formulated for topical application; 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 squamous mucosal epithelial cell being infected by a pathogen; wherein the medicament is to be introduced into the cell in a pharmaceutically acceptable *in vivo* deliver vehicle formulated for topical application, 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, wherein the polypeptide comprises cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a B-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme, and wherein the mRNA comprises a synthetic 5' cap.

In another 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 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 comprises a synthetic 5' cap and that encodes a polypeptide involved in innate immunity selected from cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a β-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme; and a pharmaceutically acceptable in vivo delivery vehicle formulated for topical application.

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

What is claimed is:

1. A method comprising:

   introducing into a mucosal squamous epithelial cell a composition comprising (i) an mRNA that comprises a synthetic 5' cap and that encodes a polypeptide involved in innate immunity selected from cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a β-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme and (ii) a pharmaceutically acceptable *in vivo* delivery vehicle formulated for topical application; 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 5' cap is anti-reverse cap analogue (ARCA).

3. The method of claim 1 wherein the mRNA further comprises a 3' extension stabilizing moiety.

4. The method of any one of claims 1-3 further comprising permitting the cell to be exposed to the one or more pathogens.

5. The method of any one of claims 1-4 wherein the one or more pathogens comprises a bacterium.

6. The method of claim 5 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.

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

8. The method of claim 7 wherein the fungus comprises fungi *Candida albicans*, *Acinetobacter baumannii*, or an *Aspergillus* spp.

9. The method of any one of claims 1-8 wherein the cell is a cell of a subject having or at risk of having a condition caused by infection by the pathogen.

10. 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.

11. The method of claim 10 wherein the mucosal fluid comprises saliva.

12. A composition comprising:
an mRNA that comprises a synthetic 5' cap and that encodes a polypeptide involved in innate immunity selected from cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a β-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme; and
a pharmaceutically acceptable *in vivo* delivery vehicle formulated for topical application.

13. The composition of claim 12 wherein the 5' cap is anti-reverse cap analogue (ARCA).

14. The composition of claim 12 wherein the mRNA further comprises a 3' extension stabilizing moiety.

15. Use of an mRNA encoding a polypeptide involved in innate immunity in the manufacture of a medicament for decreasing the likelihood of a squamous mucosal epithelial cell being infected by a pathogen; wherein the medicament is to be introduced into the cell in a pharmaceutically acceptable *in vivo* deliver vehicle formulated for topical application, 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, wherein the polypeptide comprises cathelicin antimicrobial protein (CAMP), calprotectin, S100A8, S100A9, a B-defensin, S100A7, secretory leukocyte inhibitor, lipocalin 2, or lysozyme, and wherein the mRNA comprises a synthetic 5' cap.

16. The use of claim 15 wherein the 5' cap is anti-reverse cap analogue (ARCA).

17. The use of claim 15 wherein the mRNA further comprises a 3' extension stabilizing moiety.

18. The use of any one of claims 15-17 wherein the cell is further permitted to be exposed to the pathogen.

19. The use of any one of claims 15-18 wherein the pathogen comprises a bacterium.

20. The use of claim 19 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.

21. The use of any one of claims 15-18 wherein the pathogen comprises a fungus.

22. The use of claim 21 wherein the fungus comprises fungi *Candida albicans*, *Acinetobacter baumanni*, or an *Aspergillus* spp.

23. The use of any one of claims 15-22 wherein the cell is a cell of a subject having or at risk of having a condition caused by infection by the pathogen.

24. 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.

25. The use of claim 24, wherein the mucosal fluid comprises saliva.