

nNIF AND nNIF-RELATED PEPTIDES AND RELATED METHODS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of United States Provisional Application No. 62/492,019, filed April 28, 2017, and United States Provisional Application No. 62/383,243, filed September 2, 2016, each of which is hereby incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0001a] This invention was made with government support under Grant Numbers K08 HD049699, R01 HL044525 and R01 HL066277 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

[0002] The present disclosure is directed to neonatal Neutrophil Inhibitory Factor (nNIF) and nNIF-Related Peptides (NRPs). The present disclosure is also directed to methods of using nNIF and NRPs for the inhibition of neutrophil extracellular trap (NET) formation. Furthermore, nNIF and NRPs can be used for the treatment of and the prophylaxis against inflammatory disorders and cancer.

BACKGROUND

[0003] Formation of neutrophil extracellular traps (NETs) can be an important component in the defensive armamentarium of neutrophils (polymorphonuclear leukocytes; PMNs) that allows them to capture, immobilize, and putatively kill microbes in the extracellular space (see Sorensen OE, *et al.*, *Journal of clinical investigation*. 2016;126 (5):1612-20; Brinkmann V, *et al.*, *J Cell Biol.* 2012;198 (5):773-83; Yipp BG, *et al.*, *Blood*. 2013;122 (16):2784-94; and Brinkmann V, *et al.*, *Science*. 2004;303 (5663):1532-5). NET formation occurs by a novel cell death process often called NETosis, although "vital" NETosis, in which the neutrophils do not immediately die, has also been described (see Yipp BG, *et al.*, *Blood*. 2013;122 (16):2784-94 and Yipp BG, *et al.*, *Nature medicine*. 2012;18 (9):1386-93). The molecular mechanisms leading to NET formation have not been completely dissected and may depend in part on the stimulus (see Sorensen OE, *et al.*, *Journal of clinical investigation*. 2016;126 (5):1612-20; Brinkmann V, *et al.*, *J Cell Biol.* 2012;198 (5):773-83; Yipp BG, *et al.*, *Blood*. 2013;122 (16):2784-94; and Papayannopoulos V, *et al.*, *J Cell Biol.* 2010;191 (3):677-91). Nevertheless, decondensation of chromatin and extrusion of DNA together with histones and granule contents are central events (see Sorensen OE, *et al.*, *Journal of clinical investigation*. 2016;126 (5):1612-20; Brinkmann V, *et al.*, *J Cell Biol.* 2012;198 (5):773-83; Yipp BG, *et al.*, *Blood*. 2013;122 (16):2784-94; Yipp BG, *et al.*, *Nature medicine*. 2012;18 (9):1386-93; Papayannopoulos V, *et al.*, *J Cell Biol.* 2010;191

maladaptive activity of neutrophils (see Sorensen OE, *et al.*, *Journal of clinical investigation*. 2016;126 (5):1612-20) if it is triggered inappropriately or is unregulated in infection and inflammation.

[0005] Human neonates have unique and complicated immune regulation, susceptibility to infection, and inflammatory pathology. Although the infant is in a sterile environment *in utero*, it can be challenged by pathogens and their products before or during labor (see McDonagh S, *et al.*, *Journal of infectious diseases*. 2004;190 (4):826-34). Furthermore, newborns are rapidly colonized with bacteria after delivery, a process associated with increases in circulating and bone marrow neutrophils (see Palmer C, *et al.* *PLoS biology*. 2007;5 (7):e177; Jost T, *et al.*, *PloS one*. 2012;7 (8):e44595; and Deshmukh HS, *et al.*, *Nat Med*. 2014;20 (5):524-30). Complex adaptations appear to have evolved that prevent excessive, injurious inflammation in the perinatal period and in the abrupt neonatal transition from the protected intrauterine environment to continuous microbial colonization and exposure (see Dowling DJ, *et al.* *Trends in immunology*. 2014;35 (7):299310; Adkins B., *Immunologic research*. 2013;57 (1-3):246-57; and Elahi S, *et al.*, *Nature*. 2013;504 (7478):158-62). These adaptations may, however, be accompanied by increased susceptibility to infection (see Adkins B., *Immunologic research*. 2013;57 (1-3):246-57 and Elahi S, *et al.*, *Nature*. 2013;504 (7478):158-62). It has been found that PMNs isolated from umbilical cord blood of preterm and term infants do not form NETs when stimulated and have a defect in NET-mediated bacterial killing, suggesting such an adaptation (see Yost CC, *et al.*, *Blood*. 2009;113 (25):6419-27). Other investigators subsequently reported temporally delayed NET formation when isolated neonatal neutrophils were stimulated *in vitro* (see Marcos V, *et al.* *Blood*. 2009;114 (23):4908-11, author reply 11-2).

SUMMARY OF INVENTION

[0005a] In one aspect, there is provided a use of an effective amount of a pharmaceutical composition comprising a NET-Inhibitory Peptide (NIP) and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating a patient having, or at risk of developing, a cancer, wherein the medicament reduces a pathological effect or symptom of the cancer, or reduces the risk of developing the cancer.

[0005b] In one aspect, there is provided a use of an effective amount of a pharmaceutical composition comprising a NET-Inhibitory Peptide (NIP) and a

pharmaceutically acceptable carrier in the manufacture of a medicament for inhibiting metastasis in a patient having cancer, wherein the medicament reduces a risk of metastasis in the patient.

[0005c] In one aspect, there is provided a method of diagnosing a patient having cancer who would benefit from treatment with a NET-Inhibitory Peptide (NIP), the method comprising: detecting whether NET formation is present in a sample of cancerous cells obtained from the patient; and diagnosing the patient as a patient who would benefit from treatment with a NIP when the presence of NET formation in the sample of cancerous cells is detected.

[0005d] In one aspect, there is provided a use of an effective amount of a pharmaceutical composition comprising a NIP in the manufacture of a medicament for treating a patient diagnosed as having cancer who would benefit from treatment with a NET-Inhibitory Peptide (NIP), wherein the patient is diagnosed by: detecting whether NET formation is present in a sample of cancerous cells obtained from the patient; diagnosing the patient as a patient who would benefit from treatment with a NIP when the presence of NET formation in the sample of cancerous cells is detected, and wherein the medicament is to be administered to the diagnosed patient.

[0005e] In one aspect, there is provided a use of an effective amount of a pharmaceutical composition comprising at least one of a neonatal NIF-Related Peptide (NRP), a pharmaceutically acceptable salt of a NRP, a NRP analog, or a pharmaceutically acceptable salt of a NRP analog and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating a patient having, or at risk of developing, an inflammatory disorder, wherein the medicament reduces a pathological effect or symptom of the inflammatory disorder, or reduces the risk of developing the inflammatory disorder.

[0005f] In one aspect, there is provided a use of an effective amount of a pharmaceutical composition comprising a neonatal NIF-Related Peptide (NRP), a pharmaceutically acceptable salt of a NRP, a NRP analog, or a pharmaceutically acceptable salt of a NRP analog and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating a patient having, or at risk of developing, a complication of prematurity, wherein the medicament reduces a pathological effect or symptom of the complication of prematurity, or reduces the risk of developing the complication of prematurity.

[0005g] In one aspect, there is provided a pharmaceutical composition, comprising: a neonatal NIF-Related Peptide (NRP); and a pharmaceutically acceptable carrier.

[0005h] In one aspect, there is provided a nNIF-Related Peptide (NRP) comprising six or more contiguous amino acids of one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

[0005i] In one aspect, there is provided a nNIF-Related Peptide (NRP), wherein a sequence of the NRP is at least 20% identical to one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

BRIEF DESCRIPTION OF THE DRAWINGS

[0006] The embodiments disclosed herein will become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings. These drawings depict only typical embodiments, which will be described with additional specificity and detail through use of the accompanying drawings in which:

[0007] FIG. 1A is a series of images depicting neutrophils from seven preterm neonates that were longitudinally examined over the first 28 days after birth for NET formation in response to lipopolysaccharide (LPS) (100 ng/mL, 1 hour) assessed by

Claims:

1. Use of an effective amount of a pharmaceutical composition comprising a NET-Inhibitory Peptide (NIP) and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating a patient having, or at risk of developing, a cancer, wherein the medicament reduces a pathological effect or symptom of the cancer, or reduces the risk of developing the cancer.
2. The use of claim 1, wherein the NIP is one of neonatal NET-Inhibitory Factor (nNIF), a pharmaceutically acceptable salt of nNIF, a nNIF analog, a pharmaceutically acceptable salt of a nNIF analog, a nNIF-Related Peptide (NRP), a pharmaceutically acceptable salt of a NRP, a NRP analog, or a pharmaceutically acceptable salt of a NRP analog.
3. The use of claim 1, wherein the cancer is at least one of melanoma, ovarian cancer, stomach cancer, or lung cancer.
4. The use of claim 1, wherein the medicament substantially inhibits neutrophil extracellular trap (NET) formation.
5. The use of claim 1, wherein the patient is human.
6. Use of an effective amount of a pharmaceutical composition comprising a NET-Inhibitory Peptide (NIP) and a pharmaceutically acceptable carrier in the manufacture of a medicament for inhibiting metastasis in a patient having cancer, wherein the medicament reduces a risk of metastasis in the patient.
7. The use of claim 6, wherein the NIP is one of neonatal NET-Inhibitory Factor (nNIF), a pharmaceutically acceptable salt of nNIF, a nNIF analog, a pharmaceutically acceptable salt of a nNIF analog, a nNIF-Related Peptide (NRP), a pharmaceutically acceptable salt of a NRP, a NRP analog, or a pharmaceutically acceptable salt of a NRP analog.
8. The use of claim 6, wherein the cancer is at least one of melanoma, ovarian cancer, stomach cancer, or lung cancer.
9. The use of claim 6, wherein the medicament substantially inhibits neutrophil extracellular trap (NET) formation.
10. The use of claim 6, wherein the patient is human.
11. A method of diagnosing a patient having cancer who would benefit from treatment with a NET-Inhibitory Peptide (NIP), the method comprising:

detecting whether NET formation is present in a sample of cancerous cells obtained from the patient; and

diagnosing the patient as a patient who would benefit from treatment with a NIP when the presence of NET formation in the sample of cancerous cells is detected.

12. The method of claim 11, wherein the cancerous cells include at least one of melanocytes, ovarian cells, stomach cells, or lung cells.

13. Use of an effective amount of a pharmaceutical composition comprising a NIP in the manufacture of a medicament for treating a patient diagnosed as having cancer who would benefit from treatment with a NET-Inhibitory Peptide (NIP), wherein the patient is diagnosed by

detecting whether NET formation is present in a sample of cancerous cells obtained from the patient;

diagnosing the patient as a patient who would benefit from treatment with a NIP when the presence of NET formation in the sample of cancerous cells is detected; and

wherein the medicament is to be administered to the diagnosed patient.

14. The use of claim 13, wherein the NIP is one of neonatal NET-Inhibitory Factor (nNIF), a pharmaceutically acceptable salt of nNIF, a nNIF analog, a pharmaceutically acceptable salt of a nNIF analog, a nNIF-Related Peptide (NRP), a pharmaceutically acceptable salt of a NRP, a NRP analog, or a pharmaceutically acceptable salt of a NRP analog.

15. The use of claim 13, wherein the cancerous cells include at least one of melanocytes, ovarian cells, stomach cells, or lung cells.

16. The use of claim 13, wherein the medicament substantially inhibits NET formation.

17. The use of claim 13, wherein the patient is human.

18. Use of an effective amount of a pharmaceutical composition comprising at least one of a neonatal NIF-Related Peptide (NRP), a pharmaceutically acceptable salt of a NRP, a NRP analog, or a pharmaceutically acceptable salt of a NRP analog and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating a patient having, or at risk of developing, an inflammatory disorder, wherein the medicament reduces a pathological effect or

symptom of the inflammatory disorder, or reduces the risk of developing the inflammatory disorder.

19. The use of claim 18, wherein the NRP is at least one of a Cancer-Associated SCM-Recognition, Immune Defense Suppression, and Serine Protease Protection Peptide (CRISPP); a 44 amino acid carboxy terminus cleavage fragment of A1AT (A1ATm³⁵⁸); or a high temperature requirement A1 (HTRA1) cleavage fragment of A1AT (HTRA1-CF).

20. The use of claim 18, wherein the inflammatory disorder is selected from at least one of an acute inflammatory disorder, a chronic inflammatory disorder, or an immune disorder.

21. The use of claim 18, wherein the inflammatory disorder is an autoimmunity disorder.

22. The use of claim 18, wherein the inflammatory disorder is selected from at least one of acute respiratory distress syndrome (ARDS), bronchopulmonary dysplasia (BPD), chronic obstructive pulmonary disease (COPD), cystic fibrosis, inflammation in cancer, inflammatory bowel disease (IBD), inflammatory lung disease (ILD), influenza-induced pneumonitis, necrotizing enterocolitis (NEC), neonatal chronic lung disease (CLD), periodontitis, pre-eclampsia, retinopathy of prematurity (ROP), sepsis, systemic inflammatory response syndrome (SIRS), thrombosis, transfusion-related acute lung injury (TRALI), vasculitis, rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Wegener's granulomatosis (WG), or a disorder of nonresolved inflammation.

23. The use of claim 22, wherein the thrombosis is venous thrombosis.

24. The use of claim 22, wherein the thrombosis is arterial thrombosis.

25. The use of claim 18, wherein the medicament substantially inhibits neutrophil extracellular trap (NET)-mediated inflammatory tissue damage.

26. The use of claim 18, wherein the patient is human.

27. Use of an effective amount of a pharmaceutical composition comprising a neonatal NIF-Related Peptide (NRP), a pharmaceutically acceptable salt of a NRP, a NRP analog, or a pharmaceutically acceptable salt of a NRP analog and a pharmaceutically acceptable carrier in the manufacture of a medicament for treating a patient having, or at risk of developing, a complication of prematurity, wherein the medicament reduces a pathological effect or symptom of the

complication of prematurity, or reduces the risk of developing the complication of prematurity.

28. The use of claim 27, wherein the NRP is at least one of a Cancer-Associated SCM-Recognition, Immune Defense Suppression, and Serine Protease Protection Peptide (CRISPP); a 44 amino acid carboxy terminus cleavage fragment of A1AT (A1ATm³⁵⁸); or a high temperature requirement A1 (HTRA1) cleavage fragment of A1AT (HTRA1-CF).

29. The use of claim 27, wherein the complication of prematurity is selected from at least one of necrotizing enterocolitis (NEC), respiratory distress syndrome (RDS), pneumonia, bronchopulmonary dysplasia (BPD), neonatal chronic lung disease (CLD), neurodevelopmental delay, retinopathy of prematurity (ROP), or sepsis.

30. The use of claim 27, wherein the medicament substantially inhibits neutrophil extracellular trap (NET)-mediated inflammatory tissue damage.

31. The use of claim 27, wherein the patient is human.

32. A pharmaceutical composition, comprising:

a neonatal NIF-Related Peptide (NRP); and

a pharmaceutically acceptable carrier.

33. The pharmaceutical composition of claim 32, wherein the NRP is at least one of a Cancer-Associated SCM-Recognition, Immune Defense Suppression, and Serine Protease Protection Peptide (CRISPP); a 44 amino acid carboxy terminus cleavage fragment of A1AT (A1ATm³⁵⁸); or a high temperature requirement A1 (HTRA1) cleavage fragment of A1AT (HTRA1-CF).

34. The pharmaceutical composition of claim 33, wherein the NRP is a CRISPP, or a salt thereof, and wherein the amino acid sequence of the CRISPP, or the salt thereof, is at least 80% identical to the amino acid sequence of SEQ ID NO:2.

35. The pharmaceutical composition of claim 33, wherein the NRP is an A1ATm³⁵⁸, or a salt thereof, and wherein the amino acid sequence of the A1ATm³⁵⁸, or the salt thereof, is at least 80% identical to the amino acid sequence of SEQ ID NO:3.

36. The pharmaceutical composition of claim 33, wherein the NRP is a HTRA1-CF, or a salt thereof, and wherein the amino acid sequence of the HTRA1-

CF, or the salt thereof, is at least 80% identical to the amino acid sequence of SEQ ID NO:4.

37. The pharmaceutical composition of claim 33, wherein at least one amino acid of the CRISPP, the A1AT³⁵⁸, or the HTRA1-CF is bound to a chemical modifier, and wherein the chemical modifier is selected from at least one of a lipid, a polyethylene glycol (PEG), or a saccharide.

38. The pharmaceutical composition of claim 33, wherein the CRISPP, the A1AT³⁵⁸, or the HTRA1-CF is present in an amount effective to substantially inhibit damage selected from at least one of inflammatory tissue injury or inflammatory vascular injury.

39. The pharmaceutical composition of claim 33, wherein the CRISPP, the A1AT³⁵⁸, or the HTRA1-CF does not globally depress polymorphonuclear leukocyte (PMN) function.

40. The pharmaceutical composition of claim 33, wherein the CRISPP, the A1AT³⁵⁸, or the HTRA1-CF does not substantially inhibit one or more activities of a polymorphonuclear leukocyte (PMN) selected from at least one of chemotaxis, chemokine synthesis and secretion, cytokine synthesis and secretion, extracellular bacterial killing, intracellular bacterial killing, phagocytosis, and reactive oxygen species (ROS) generation.

41. The pharmaceutical composition of claim 33, wherein the CRISPP, the A1AT³⁵⁸, or the HTRA1-CF is not naturally occurring.

42. The pharmaceutical composition of claim 33, wherein the CRISPP, the A1AT³⁵⁸, or the HTRA1-CF is present in an amount effective to substantially inhibit neutrophil extracellular trap (NET) formation.

43. The pharmaceutical composition of claim 42, wherein the NET formation is stimulated by at least one of a bacterium, a fungus, a parasite, or a virus.

44. The pharmaceutical composition of claim 43, wherein the virus is at least one of a hemorrhagic fever virus, a filovirus, an arenavirus, a hantavirus, or a flavivirus.

45. The pharmaceutical composition of claim 43, wherein the bacterium is at least one of a *Bacillus* species, an *Escherichia* species, a *Francisella* species, a *Staphylococcus* species, a *Streptococcus* species, or a *Yersinia* species.

46. The pharmaceutical composition of claim 42, wherein the NET formation is stimulated by at least one of beta-defensin 1, HIV-1, lipopolysaccharide (LPS), phorbol myristate acetate (PMA), or *Staphylococcus aureus* alpha-toxin.

47. A nNIF-Related Peptide (NRP) comprising six or more contiguous amino acids of one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

48. The NRP of claim 47 comprising at least 12 contiguous amino acids of one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

49. The NRP of claim 47 comprising at least 24 contiguous amino acids of one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

50. The NRP of claim 47 comprising one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

51. A nNIF-Related Peptide (NRP), wherein a sequence of the NRP is at least 20% identical to one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

52. The NRP of claim 51, wherein the sequence of the NRP is at least 40% identical to one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

53. The NRP of claim 51, wherein the sequence of the NRP is at least 60% identical to one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.

54. The NRP of claim 51, wherein the sequence of the NRP is at least 80% identical to one of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, or SEQ ID NO:4.