TARGETING OF SRC-3 IN IMMUNE CELLS AS AN IMMUNOMODULATORY THERAPEUTIC FOR THE TREATMENT OF CANCER

[0001] This application claims priority to U.S. Provisional Application Serial No. 63/106,770, filed October 28, 2020, which is incorporated by reference herein in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under W81XWH-13-1-0285 awarded by the Department of Defense and under HD008188 awarded by the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] Embodiments of the disclosure concern at least the fields of cell biology, molecular biology, immunology, and medicine, including cancer medicine.

BACKGROUND

[0004] Steroid receptor coactivator-3 (SRC-3) functions as a key driver of breast cancer (BC) proliferation, metastasis and resistance to standard and endocrine-based cancer therapy. As an oncogene, SRC-3 acts as a pleiotropic coactivator for nuclear receptors and multiple other transcription factors that drive programs required for cancer cell proliferation and metastasis. Importantly, SRC-3 also plays an important role in regulation of the host immune system.

[0005] The present disclosure addresses a long-felt need in the art of providing effective cancer therapies by targeting SRC-3 in a unique manner.

~~BRIEF~~ SUMMARY

[0006] The present disclosure is directed to systems, methods, and compositions for treating, delaying progression of, delaying onset of, or reducing the risk of getting cancer of any kind. In particular ~~embodiment~~examples, the disclosure concerns adoptive cell transfer in which the cells being administered to an individual in need thereof are immune cells that (1) have disruption of expression of endogenous SRC-3; and/or (2) have had exposure to one or more agents that target SRC-3. In specific ~~embodiment~~examples, the immune cells are CD4+ cells, including T regulatory cells (Treg) that are CD4+.

[0007] Current Treg-targeting immune checkpoint inhibitors are mostly focused on surface proteins (receptors) that disrupt their cell surface interactions with other immune cells. The present disclosure distinguishes from this, because SRC-3 as a Treg gene target is a nuclear protein that functions to regulate Treg nuclear gene expression programs. As a consequence of its role as a transcriptional master regulator, it is shown herein that ablation of SRC-3 in a mouse genetic model can modulate Treg function in a way that promotes tumor eradication while avoiding the otherwise severe side effects that are frequently observed with established immune checkpoint inhibitors. In specific ~~embodiment~~examples, the present disclosure concerns a CRISPR-based approach to target the SRC-3 gene in immune cells, including for genetically modified Treg cells, to be used for adoptive transfer into individuals with cancer. Thus, the present disclosure provides a unique way to support immune-system based tumor eradication.

[0008] In one ~~embodiment~~aspect, the present disclosure refers to~~, there is~~ an engineered immune cell, comprising disruption of steroid receptor coactivator-3 (SRC-3). In specific ~~embodiment~~examples, the immune cell is a T cell, such as a T regulatory cell. The T cell may be CD4+, CD25+, and/or FOXP3+. In certain ~~embodiment~~examples, the disruption is further defined as the immune cell being genetically modified to have reduced level of expression of SRC-3 or having essentially no expression of SRC-3. In specific cases, the immune cell is engineered using one or more guide RNAs and a Cas9 enzyme. The immune cell may be autologous, allogeneic, or syngeneic with respect to an individual.

[0009] In ~~certain embodiments~~yet another aspect, the present disclosure refers to~~there is~~ a composition, comprising: (a) any immune cell encompassed herein; and (b) one or more agents that target SRC-3. In some cases, (a) and (b) are in different formulations, although they may be in the same formulation. In specific ~~embodiment~~examples, the agent that targets SRC-3 is a small molecule inhibitor, an antibody, a protein, a nucleic acid, or a combination thereof. The small molecule inhibitor of SRC-3 may be Bufalin, gossypol, Verrucarin A, SI-2, SI-10, SI-12, a functional derivative thereof, or a combination thereof. The antibody may be a monoclonal antibody or polyclonal antibody, and specific examples include 5E11, LS-C801929, PA1-845, AX15.3, PA5-29854, EPR4374(3), or a combination thereof.

[0010] In one ~~embodiment~~ example, there is a method of treating cancer in an individual, comprising the step of administering to the individual a therapeutically effective amount any cells encompassed herein. The cancer may be SRC-3+ cancer. The cancer may be breast, ovarian, endometrial, prostate, gastric, multiple myeloma, thyroid cancer, or pancreatic cancer.

[0010a] In another aspect, the present disclosure refers to the use of the cells as described herein in the manufacture of a medicament for the treatment of cancer in an individual.

[0011] In some cases, prior to the step of administering, the cells are exposed ex vivo to an effective amount of one or more agents that target SRC-3, such as a small molecule inhibitor (Bufalin, gossypol, Verrucarin A, SI-2, SI-10, SI-12, a functional derivative thereof, combination thereof), an antibody (monoclonal antibody or polyclonal or fragments, such as scFv), a protein, a nucleic acid, or a combination thereof. Specific antibodies include 5E11, LS-C801929, PA1-845, AX15.3, PA5-29854, EPR4374(3), or a combination thereof. In some cases, the individual is administered a therapeutically effective amount of an additional cancer therapy, such as surgery, radiation, chemotherapy, hormone therapy, drug therapy, protein therapy, immunotherapy, or a combination thereof. The additional cancer therapy may comprise one or more agents that target SRC-3. The cells and the additional cancer therapy may be administered to the individual at substantially the same time or at different times. The cells and the additional cancer therapy may or may not be in the same formulation. The cells may be administered intravenously, intraperitoneally, intraarterially, topically, by inhalation, intramuscularly, intrasternally, by intraarticular injection, or by infusion. The cells may be administered once or multiple times, and when they are administered multiple times, the duration between administrations is within 1-24 hours, 1-7 days, 1-4 weeks, or 1-12 months.

[0012] In one ~~embodiment~~ example, there is a method of treating cancer in an individual, comprising the step of administering to the individual a therapeutically effective amount of any composition encompassed herein, including one that comprises (a) any immune cell encompassed herein; and (b) one or more agents that target SRC-3. In such cases, (a) and (b) may or may not be administered to the individual in different formulations and/or at the same or different times. When administered at different times, the duration between administrations of (a) and (b) may be within 1-24 hours, 1-7 days, 1-4 weeks, or 1-12 months. The composition may be administered to the individual once or multiple times and when the composition is administered to the individual at multiple times, the duration between administrations is within 1-24 hours, 1-7 days, 1-4 weeks, or 1-12 months. The cancer may be SRC-3+. The cancer may be of the breast, ovarian, endometrial, prostate, gastric, multiple myeloma, thyroid, or pancreatic cancer. In specific cases, prior to the step of administering, the cells in (a) are exposed ex vivo to an effective amount of one or more agents that target SRC-3.

[0012a] In another aspect, the present disclosure refers to the use of the composition as described herein in the manufacture of a medicament for the treatment of cancer in an individual.

[0013] In one ~~embodiment~~aspect, the present disclosure refers to~~, there is~~ a method of producing any immune cells encompassed herein, comprising the step of disrupting expression of SRC-3 in the immune cells.

[0014] The immune cells may be T cells, including Tregs. In specific cases, the method is further defined as: (a) obtaining CD4+ T cells or CD4+ Tregs, respectively; and (b) exposing the CD4+ T cells or CD4+ Tregs, respectively, to one or more agents that disrupt expression of endogenous SRC-3 in the T cells or Tregs, respectively. In specific ~~embodiment~~examples, the T cells or Tregs are obtained from the spleen, bone marrow, blood, plasma, or a combination thereof. The method may further comprise the step of obtaining T regulatory cells from the CD4+ T cells. In specific cases, the T regulatory cells are CD25+ and/or Fox3p+. In specific cases, the one or more agents that disrupt expression of endogenous SRC-3 in the T cells comprises nucleic acid. In particular examples~~aspects~~, the one or more agents that disrupt expression of endogenous SRC-3 in the T cells comprises CRISPR reagents. The method may further comprise the step of exposing the immune cells ex vivo to an effective amount of one or more agents that target SRC-3.

[0015] The foregoing has outlined rather broadly the features and technical advantages of the present disclosure in order that the detailed description that follows may be better understood. Additional features and advantages will be described hereinafter which form the subject of the claims herein. It should be appreciated by those skilled in the art that the conception and specific ~~embodiment~~examples disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present designs. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope as set forth in the appended claims. The novel features which are believed to be characteristic of the designs disclosed herein, both as to the organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] For a more complete understanding of the present disclosure, reference is now made to the following descriptions taken in conjunction with the accompanying drawings.

[0017] FIG. 1: Comprehensive immunophenotyping of Src-3 gene knockout (KO) mice reveals broad lymphocytosis. Immunophenotyping of peripheral blood from aged (16-20 months) Src-3 KO mice (-/-~~-~~, n=6) and wild-type littermates (+/+, n=7). There was a significant elevation of T and B cells, as well as specific subtypes, in SRC-3 knockout mice. There was no

CLAIMS

What is claimed is:

1. An engineered immune cell, comprising disruption of steroid receptor coactivator-3 (SRC-3).

2. The immune cell of claim 1, wherein the immune cell is a T cell.

3. The immune cell of claim 2, wherein the T cell is a T regulatory cell.

4. The immune cell of claim 2 or 3, wherein the T cell is CD4+.

5. The immune cell of any one of claims 2-4, wherein the T cell is a CD25+ cell.

6. The immune cell of any one of claims 2-4, wherein the T cell is a FOXP3+ cell.

7. The immune cell of any one of claims 1-6, wherein the disruption is further defined as the immune cell being genetically modified to have reduced level of expression of SRC-3 or having essentially no expression of SRC-3.

8. The immune cell of any one of claims 1-7, wherein said immune cell is engineered using one or more guide RNAs and a Cas9 enzyme.

9. The immune cell of any one of claims 1-8, wherein the immune cell is autologous with respect to an individual.

10. The immune cell of any one of claims 1-8, wherein the immune cell is allogeneic with respect to an individual.

11. A composition, comprising:

(a) the immune cell of any one of claims 1-10; and

(b) one or more agents that target SRC-3.

12. The composition of claim 11, wherein (a) and (b) are in different formulations.

13. The composition of claim 11, wherein (a) and (b) are in the same formulation.

14. The composition of any one of claims 11-13, wherein the agent that targets SRC-3 is a small molecule inhibitor, an antibody, a protein, a nucleic acid, or a combination thereof.

15. The composition of claim 14, wherein the small molecule inhibitor of SRC-3 is Bufalin, gossypol, Verrucarin A, SI-2, SI-10, SI-12, a functional derivative thereof, or a combination thereof.

16. The composition of claim 14 or 15, wherein the antibody is a monoclonal antibody or polyclonal antibody.

17. The composition of any one of claims 14-16, wherein the antibody is 5E11, LS-C801929, PA1-845, AX15.3, PA5-29854, EPR4374(3), or a combination thereof.

18. ~~A method of treating~~ Use of the cells of any one of claims 1-10 in the manufacture of a medicament for the treatment of cancer in an individual~~, comprising the step of administering to the individual a therapeutically effective amount of the cells of any one of claims 1-10.~~

19. The ~~method~~ use of claim 18, wherein the cancer is SRC-3+ cancer.

20. The ~~method~~use of claim 18 or 19, wherein the cancer is breast, ovarian, endometrial, prostate, gastric, multiple myeloma, thyroid cancer, or pancreatic cancer.

21. The ~~method~~ use of any one of claims 18-20, wherein prior to ~~the step of administering~~ administration of the medicament, the cells are exposed *ex vivo* to an effective amount of one or more agents that target SRC-3.

22. The ~~method~~use of claim 21, wherein the agent that targets SRC-3 is a small molecule inhibitor, an antibody, a protein, a nucleic acid, or a combination thereof.

23. The ~~method~~ use of claim 22, wherein the small molecule inhibitor of SRC-3 is Bufalin, gossypol, Verrucarin A, SI-2, SI-10, SI-12, a functional derivative thereof, combination thereof.

24. The ~~method~~use of claim 22 or 23, wherein the antibody is a monoclonal antibody or polyclonal antibody.

25. The ~~method~~ use of any one of claims 22-24, wherein the antibody is 5E11, LS-C801929, PA1-845, AX15.3, PA5-29854, EPR4374(3), or a combination thereof.

26. The ~~method~~ use of any one of claims 18-25, wherein the individual is to be administered a therapeutically effective amount of an additional cancer therapy.

27. The ~~method~~ use of claim 26, wherein the additional cancer therapy comprises surgery, radiation, chemotherapy, hormone therapy, drug therapy, protein therapy, immunotherapy, or a combination thereof.

28. The ~~method~~ use of claim 26 or 27, wherein the additional cancer therapy comprises one or more agents that target SRC-3.

29. The ~~method~~ use of claim 28, wherein the agent that targets SRC-3 is a small molecule inhibitor, an antibody, a protein, a nucleic acid, or a combination thereof.

30. The ~~method~~ use of claim 29, wherein the small molecule inhibitor of SRC-3 is Bufalin, gossypol, Verrucarin A, SI-2, SI-10, SI-12, a functional derivative thereof, or a combination thereof.

31. The ~~method~~ use of claim 29 or 30, wherein the antibody is a monoclonal antibody or polyclonal antibody.

32. The ~~method~~ use of any one of claims 29-31, wherein the antibody is 5E11, LS-C801929, PA1-845, AX15.3, PA5-29854, EPR4374(3), or a combination thereof.

33. The ~~method~~ use of any one of claims 26-32, wherein the cells and the additional cancer therapy are to be administered to the individual at substantially the same time.

34. The ~~method~~use of any one of claims 26-32, wherein the cells and the additional cancer therapy are to be administered to the individual at different times.

35. The ~~method~~ use of any one of claims 26-34, wherein the cells and the additional cancer therapy are in the same formulation.

36. The ~~method~~use of any one of claims 26-34, wherein the cells and the additional cancer therapy are in different formulations.

37. The ~~method~~use of any one of claims 18-36, wherein the cells are to be administered intravenously, intraperitoneally, intraarterially, topically, by inhalation, intramuscularly, intrasternally, by intraarticular injection, or by infusion.

38. The ~~method~~ use of any one of claims 18-37, wherein the cells are to be administered once or multiple times.

39. The ~~method~~ use of claim 38, wherein when the cells are to be administered to the individual multiple times, the duration between administrations is within 1-24 hours, 1-7 days, 1-4 weeks, or 1-12 months.

40. ~~A method~~ Use of the composition of any one of claims 11-17 in the manufacture of a medicament for the treatment of ~~treating~~ cancer in an individual.~~, comprising the step of administering to the individual a therapeutically effective amount of the composition of any one of claims 11-17.~~

41. The ~~method~~ use of claim 40, wherein (a) and (b) are to be administered to the individual in different formulations.

42. The ~~method~~ use of claim 40, wherein (a) and (b) are to be administered to the individual in the same formulation.

43. The ~~method~~ use of claim 41, wherein (a) and (b) are to be administered to the individual at substantially the same time.

44. The ~~method~~ use of claim 41, wherein (a) and (b) are to be administered to the individual at different times.

45. The ~~method~~ use of claim 44, wherein when (a) and (b) are to be administered to the individual at different times, the duration between administrations of (a) and (b) is within 1-24 hours, 1-7 days, 1-4 weeks, or 1-12 months.

46. The ~~method~~ use of any one of claims 40-45, wherein the composition is to be administered to the individual once or different times.

47. The ~~method~~ use of claim 46, wherein when the composition is to be administered to the individual at different times, the duration between administrations is within 1-24 hours, 1-7 days, 1-4 weeks, or 1-12 months.

48. The ~~method~~ use of any one of claims 40-47, wherein the cancer is SRC-3+ cancer.

49. The ~~method~~ use of any one of claims 40-48, wherein the cancer is breast, ovarian, endometrial, prostate, gastric, multiple myeloma, thyroid, or pancreatic cancer.

50. The ~~method~~ use of any one of claims 40-49, wherein prior to ~~the step of administering~~administration of the medicament, the cells in (a) are exposed *ex vivo* to an effective amount of one or more agents that target SRC-3.

51. A method of producing the immune cells of any one of claims 1-10, comprising the step of disrupting expression of SRC-3 in the immune cells.

52. The method of claim 51, wherein the immune cells are T cells.

53. The method of claim 51 or 52, wherein the immune cells are Tregs.

54. The method of claim 52 or 53, wherein the method is further defined as:

(a) obtaining CD4+ T cells or CD4+ Tregs, respectively; and

(b) exposing the CD4+ T cells or CD4+ Tregs, respectively, to one or more agents that disrupt expression of endogenous SRC-3 in the T cells or Tregs, respectively.

55. The method of claim 54, wherein the T cells or Tregs are obtained from the spleen, bone marrow, blood, plasma, or a combination thereof.

56. The method of claim 54 or 55, further comprising the step of obtaining T regulatory cells from the CD4+ T cells.

57. The method of claim 56, wherein the T regulatory cells are CD25+ and/or Fox3p+.

58. The method of any one of claims 54-57, wherein the one or more agents that disrupt expression of endogenous SRC-3 in the T cells comprises nucleic acid.

59. The method of claim 58, wherein the one or more agents that disrupt expression of endogenous SRC-3 in the T cells comprises CRISPR reagents.

60. The method of any one of claims 51-59, further comprising the step of exposing the immune cells *ex vivo* to an effective amount of one or more agents that target SRC-3.