Cas9-knock-in mouse models, or other engineered murine genetic models (*e.g.* knockout (KO), inducible cell deletion, reporter).

SUMMARY

[0005] The instant disclosure generally relates to strategies for gene perturbation in primary myeloid cells, *e.g.* of human and murine origin. Using transfection techniques (e.g., electroporation/nucleofection) to deliver ribonuclear protein complexes (RNPs) including guide-RNAs non-covalently associated with recombinant Cas (e.g. Cas9), near population-level genetic knockout of single and multiple targets can be achieved in a range of cell types without the need for selection or enrichment of genetically modified cells. Cellular fitness and response to immunological stimuli of cells modified according to the methods described herein are not significantly affected by the gene editing process. Such advances enable pathway discovery and drug target validation across species in the field of innate immunity.

[0006] Delivery via transfection, e.g., nucleofection of Cas-RNP complexes to non-granulocytic cells (*i.e.* monocytes, macrophages and dendritic cells) in accordance with the methods described herein can generate up to at least about 90% knockout of single or multiple target genes without the need further selection of genetically modified cells. This can be achieved in both differentiated primary myeloid cell populations and freshly isolated cells. Provided herein are methods that enable rapid loss-of-function gene assessment within donor cell populations without substantially affecting normal cell function, including, for example, responsiveness to stimulation.

[0007] In an example, provided herein are methods for genetic modification of a myeloid cell including transfecting the myeloid cell with a gene editing reagent targeting a genetic site of interest, where the myeloid cell is not transduced with a viral vector. In one aspect, the present disclosure refers to a method for genetic modification of a myeloid cell, the method comprising transfecting the myeloid cell by electroporation with an electroporation enhancer and a CRIPSR-Cas system comprising a Cas protein and a guide RNA targeting a genetic site of interest comprising a target sequence, wherein the myeloid cell is not transduced with a viral vector. In examples, a gene editing reagent suitable for use with the methods described herein can include an RNP including a guide RNA non-covalently contacted with a Cas protein, such as a Cas9 protein.

[0008] In an example, provided herein are methods for genetic modification of a plurality of myeloid cells, including transfecting the plurality of myeloid cells with a gene editing reagent targeting a site of interest, wherein the myeloid cells are not transduced with a viral vector or viral delivery system. In one aspect, the present disclosure refers to a method for genetic modification of a plurality of myeloid cells, the method comprising transfecting by electroporation the plurality of myeloid cells in the presence of an electroporation enhancer and a CRIPSR-Cas system comprising a Cas protein and a guide RNA targeting a site of interest comprising a target sequence, wherein the myeloid cells are not transduced with a viral vector.

[0009] In an example, provided herein are methods of genetically modifying a plurality of myeloid cells, including transfecting the myeloid cells with a gene editing reagent, wherein the myeloid cells are not transduced with a viral vector, and wherein the method does not include a selection step or enrichment step following myeloid cell transfection. In one aspect, the present disclosure refers to a method of genetically modifying a plurality of myeloid cells, comprising transfecting by electroporation the myeloid cells in the presence of an electroporation enhancer and a CRIPSR-Cas system comprising a Cas protein and a guide RNA targeting a site of interest comprising a target sequence, wherein the myeloid cells are not transduced with a viral vector, and wherein the method does not comprise a selection step or enrichment step following myeloid cell transfection.

[0010] In an example, provided herein are systems for genetically modifying a myeloid cell in the absence of a viral vector. In examples, the systems include electroporation systems. In examples, the systems include nucleofection systems. In examples, the system includes a chamber compatible with electroporation or nucleofection system, multiple myeloid cells within the chamber in a media compatible with electroporation/nucleofection, and at least one gene editing system designed to target at least one site of interest in the genome of myeloid cells. In one aspect, the present disclosure refers to a system for genetically modifying a myeloid cell in the absence of a viral vector, the system comprising a chamber compatible with a transfection system, multiple myeloid cells within the chamber in a media compatible with electroporation, an electroporation enhancer, and at least one one CRIPSR-Cas system comprising a Cas protein and a guide RNA designed to target at least one site of interest comprising a target sequence in the genome of myeloid cells.

[0011] In an example, provided herein are methods of treating a disease treatable with a myeloid cell provided herein including examples thereof. The methods include providing a genetically modified myeloid cell that has not been transformed with a virus, where the myeloid cell has been transfected with a gene editing reagent; and administering the myeloid cell to a patient in need thereof.

[0012] In an example, provided herein is a genetically modified myeloid cell made by a method provided herein including examples thereof. In one aspect, the present disclosure refers to a genetically modified myeloid cell made by any one of the methods disclosed herein, wherein the genetically modified myeloid cell comprises a modification at the genetic site of interest comprising a target sequence, wherein the modification comprises an insertion, deletion, modification, or replacement at the genetic site of interest relative to the myeloid cell prior to genetic modification.

[0013] In an example, provided herein is an assay for drug discovery comprising screening the effect of one or more compounds on a genetically modified myeloid cell provided herein including examples thereof. In one aspect, the present disclosure refers to an assay for drug discovery comprising screening the effect of one or more compounds on the myeloid cell disclosed herein.

[0014] In an example, provided herein is a method for target validation of a compound, including contacting a genetically modified myeloid cell provided herein including examples thereof with the compound and monitoring an effect on the cell. In one aspect, the present disclosure refers to a method for target validation of a compound, comprising contacting a myeloid cell disclosed herein with the compound and monitoring an effect on the cell.

[0015] In an example, provided herein are compositions including a plurality of myeloid cells in contact with a gene editing reagent, a transfection buffer, and an electroporation enhancer, where the composition does not comprise a viral vector. In one aspect, the present disclosure refers to a composition comprising a plurality of myeloid cells, a CRIPSR-Cas system comprising a Cas protein and a guide RNA, a transfection buffer, and an electroporation enhancer, wherein the composition does not comprise a viral vector.

WHAT IS CLAIMED IS:

1. A method for genetic modification of a myeloid cell, the method comprising transfecting the myeloid cell by electroporation with an electroporation enhancer and a CRIPSR-Cas system comprising a Cas protein and a guide RNA targeting a genetic site of interest comprising a target sequence, wherein the myeloid cell is not transduced with a viral vector.

2. The method of claim 1, wherein the myeloid cell is a primary myeloid cell.

3. The method of claim 1 or 2, wherein the myeloid cell is a monocyte, macrophage, neutrophil, basophil, eosinophil, erythrocyte, dendritic cell, or megakaryocyte.

4. The method of any one of claims 1-3, wherein the cell is not subjected to a selection step and/or an enrichment step after transfection.

5. The method of any one of claims 1-4, wherein the CRISPR-Cas system comprises a Cas9 or a Cas9 variant.

6. The method of any one of claims 1-4, wherein the CRISPR-Cas system comprises a Cas12, a Cascade, a Cas13, or a variant of each thereof.

7. The method of any one of claims 1-4, wherein the CRISPR-Cas system further comprises a donor DNA.

8. The method of claim 7, wherein the Cas protein and guide RNA are non-covalently associated.

9. The method of claim 7, wherein the Cas protein, guide RNA and donor DNA are non-covalently associated.

10. The method of any one of claims 1-9, wherein the electroporation enhancer is selected from a carrier DNA, a single stranded DNA, a combination of single stranded and double stranded DNA, a polymeric additive, and an oligonucleotide.

11. The method of claim 10, wherein the carrier DNA is a single-stranded DNA oligonucleotide.

12. The method of claim 10, wherein the carrier DNA is non-homologous to human, mouse, and/or rat genomes.

13. The method of any one of claims 1-12, wherein the myeloid cell is differentiated prior to electroporation.

14. The method of claim 13, wherein the myeloid cell is differentiated into a dendritic cell.

15. The method of claim 13, wherein the myeloid cell is differentiated into a macrophage.

16. The method of any one of claims 1-15, wherein the myeloid cell is not activated prior to or during genetic modification.

17. The method of any one of claims 1-16, wherein the guide RNA comprises two or more distinct crispr RNAs (crRNA) to the site of interest and wherein the two or more distinct crRNA are introduced into the myeloid cell.

18. The method of claim 17, wherein introducing the crRNAs into the myeloid cell comprises transfecting the cell with the crRNAs.

19. The method of claim 17, wherein the crRNAs are contacted with tracrRNAs.

20. The method of claim 19, wherein the crRNAs are annealed to the tracrRNAs.

21. The method of claim 17, wherein the crRNAs are single guide RNAs (sgRNAs).

22. The method of any one of claims 1-21, wherein multiple genetic sites of interest are targeted.

23. The method of claim 22, wherein two or more distinct crRNAs to each site of interest are introduced into the myeloid cell.

24. The method of any one of claims 1-23, wherein the myeloid cell is a human cell.

25. A method for genetic modification of a plurality of myeloid cells, the method comprising transfecting by electroporation the plurality of myeloid cells in the presence of an electroporation enhancer and a CRIPSR-Cas system comprising a Cas protein and a guide RNA targeting a site of interest comprising a target sequence, wherein the myeloid cells are not transduced with a viral vector.

26. The method of claim 25, wherein the myeloid cells are cultured myeloid cells.

27. The method of claim 25 or 26, wherein the site of interest is modified in at least 70% of the plurality of myeloid cells.

28. The method of claim 27, wherein the site of interest is modified in at least 80% of the plurality of myeloid cells.

29. The method of claim 27 or 28, wherein the site of interest is modified in at least 85% of the plurality of myeloid cells.

30. The method of any one of claims 27-29, wherein the site of interest is modified in at least 90% of the plurality of myeloid cells.

31. The method of claim 25 or 26, wherein the plurality of myeloid cells comprises dendritic cells (DCs) and the site of interest is modified in at least 50% of the DCs.

32. The method of any one of claims 25-31, wherein the viability of the plurality of myeloid cells after electroporation is at least 80%.

33. The method of claim 32, wherein the viability of the plurality of myeloid cells after electroporation is at least 90%.

34. The method of any one of claims 25-33, wherein the CRISPR-Cas system comprises a Cas9 or a Cas9 variant.

35. The method of any one of claims 25-33, wherein the CRISPR-Cas system comprises a Cas12, a Cascade, a Cas13, or a variant of each thereof.

36. The method of any one of claims 25-33, wherein the CRISPR-Cas system further comprises a donor DNA.

37. The method of claim 36, wherein the Cas protein and guide RNA are non-covalently associated.

38. The method of claim 36, wherein the Cas protein, guide RNA and donor DNA are non-covalently associated.

39. The method of any one of claims 1-6 or 25-35, wherein the ratio of guide RNA to Cas protein is between 100:1 and 1:100.

40. The method of claim 39, wherein the ratio of guide RNA to Cas protein is less than or equal to 3:1.

41. The method of claim 39 or 40, wherein the ratio of guide RNA to Cas protein is less than or equal to 2:1.

42. The method of any one of claims 39-40, wherein the ratio of guide RNA to Cas protein is about 3:1.

43. The method of any one of claims 39-41, wherein the ratio of guide RNA to Cas protein is about 2:1.

44. The method of any one of claims 25-43, wherein the method does not comprise a selection step or enrichment step following myeloid cell transfection.

45. A method of genetically modifying a plurality of myeloid cells, comprising transfecting by electroporation the myeloid cells in the presence of an electroporation enhancer and a CRIPSR-Cas system comprising a Cas protein and a guide RNA targeting a site of interest comprising a target sequence, wherein the myeloid cells are not transduced with a viral vector, and wherein the method does not comprise a selection step or enrichment step following myeloid cell transfection.

46. The method of claim 45, wherein the site of interest is modified in at least 70% of the plurality of myeloid cells.

47. The method of claim 46, wherein the site of interest is modified in at least 80% of the plurality of myeloid cells.

48. The method of claim 46 or 47, wherein the site of interest is modified in at least 85% of the plurality of myeloid cells.

49. The method of any one of claims 46-48, wherein the site of interest is modified in at least 90% of the plurality of myeloid cells.

50. The method of claim 45, wherein the plurality of myeloid cells comprises dendritic cells (DCs) and the site of interest is modified in at least 50% of the DCs.

51. The method of any one of claims 45-50, wherein the viability of the plurality of myeloid cells after electroporation is at least 80%.

52. The method of claim 51, wherein the viability of the plurality of myeloid cells after electroporation is at least 90%.

53. The method of any one of claims 45-52, wherein the CRISPR-Cas system comprises a Cas9 or a Cas9 variant.

54. The method of any one of claims 45-52, wherein the CRISPR-Cas system comprises a Cas12, a Cascade, a Cas13, or a variant of each thereof.

55. The method of any one of claims 45-52, wherein the CRISPR-Cas system further comprises a donor DNA.

56. The method of claim 55, wherein the Cas protein and guide RNA are non-covalently associated.

57. The method of claim 55, wherein the Cas protein, guide RNA and donor DNA are non-covalently associated.

58. A system for genetically modifying a myeloid cell in the absence of a viral vector, the system comprising a chamber compatible with a transfection system, multiple myeloid cells within the chamber in a media compatible with electroporation, an electroporation enhancer, and at least one CRIPSR-Cas system comprising a Cas protein and

a guide RNA designed to target at least one site of interest comprising a target sequence in the genome of myeloid cells.

59. A composition comprising a plurality of myeloid cells, a CRIPSR-Cas system comprising a Cas protein and a guide RNA, a transfection buffer, and an electroporation enhancer, wherein the composition does not comprise a viral vector.

60. A genetically modified myeloid cell made by the method of any one of claims 1-57, wherein the genetically modified myeloid cell comprises a modification at the genetic site of interest comprising a target sequence, wherein the modification comprises an insertion, deletion, modification, or replacement at the genetic site of interest relative to the myeloid cell prior to genetic modification.

61. An assay for drug discovery comprising screening the effect of one or more compounds on the myeloid cell of claim 60.

62. A method for target validation of a compound, comprising contacting a myeloid cell of claim 60 with the compound and monitoring an effect on the cell.

63. The method of claim 1, wherein one or more of CD64, CD169, or HLA-DR expression is decreased or absent in the myeloid cell after genetic modification