mouse fibroblasts to mouse hepatocyte-like cells, the same methodologies may not necessarily be successful when applied to human cells. Scientific literature has indicated that factors and processes necessary and sufficient for altering the differentiation state of mouse cells may be distinct from that necessary and sufficient for altering the differentiation state of human cells. (See, *e.g.*, Yi et al., (2012) Cell Research 22:616-619; Gonzalez *et al.*, (2011) Nature Reviews Genetics 12:231-242; Pang *et al.*, (2011) Nature 476:220-223; Vierbuchen & Wernig (2011) Nature Biotechnology 29:892-907.)

The generation of human induced hepatocytes from non-hepatocyte cells, without any risk of genomic alteration, offers great promise as a means for treating disease through cell transplantation. Human induced hepatocytes generated from human non-hepatocyte cells obtained from individual patients may enable development of patient-specific therapies which do not have a risk of immune rejection, thus eliminating the need for immunosuppressive procedures. Additionally, generation of human induced hepatocytes from disease-specific non-hepatocyte cells could provide a means to model and study specific disease states and develop therapeutics useful for the treatment of these diseases.

Therefore, there exists a need in the art to identify the key transcription factors necessary and sufficient to reprogram human non-hepatocyte cells into human induced hepatocytes. The present invention meets this need by providing methods and compositions useful for generating human induced hepatocytes from human non-hepatocyte cells.

SUMMARY

The present disclosure provides, in part, methods and compositions for reprogramming non-hepatocyte cells to induced hepatocytes, populations of induced hepatocytes, compositions comprising induced hepatocytes, and uses thereof. In some embodiments, the non-hepatocyte cells are non-hepatocyte somatic cells. In other embodiments, the compositions and methods provided herein are useful for producing human induced hepatocytes by reprogramming human non-hepatocyte cells.

In one aspect, there is provided a method for producing a human induced hepatocyte from a human fibroblast cell, the method comprising providing a human fibroblast cell, introducing into the human fibroblast cell a nucleic acid or protein preparation which encodes or provides a combination of reprogramming factors, wherein the combination of reprogramming factors is selected from the group consisting of a combination comprising FOXA1, FOXA3, and HNF1A; a combination comprising FOXA1, FOXA2, and HNF1A; a combination comprising FOXA2, FOXA3, and HNF1A; a combination comprising FOXA1, HNF1A, and HNF4A; a combination comprising FOXA2, HNF1A, and HNF4A; and a combination comprising FOXA3, HNF1A, and HNF4A, and culturing the human fibroblast cell under conditions suitable for reprogramming a human fibroblast cell to a human induced hepatocyte, thereby producing a human induced hepatocyte from the human fibroblast cell.

~~In one aspect, there is provided~~Disclosed is a method for producing an induced hepatocyte from a non-hepatocyte cell, the method comprising providing a non-hepatocyte cell, contacting the non-hepatocyte with an agent which increases or induces the expression or activity of one or more

reprogramming factors, and culturing the non-hepatocyte cell under conditions suitable for reprogramming a non-hepatocyte cell to an induced hepatocyte, thereby producing an induced hepatocyte from the non-hepatocyte cell.

In another aspect, there is provided a method for producing a human induced hepatocyte from a human fibroblast cell, the method comprising providing a human fibroblast cell, introducing into the human fibroblast cell a nucleic acid or protein preparation which encodes or provides a combination of reprogramming factors, wherein the combination of reprogramming factors comprise FOXA1, FOXA2, FOXA3, HNF1A, and HNF4A, and culturing the human fibroblast cell under conditions suitable for reprogramming a human fibroblast cell to a human induced hepatocyte, thereby producing a human induced hepatocyte from the human fibroblast cell.

~~In one aspect, there is provided~~Disclosed is a method for producing an induced hepatocyte from a non-hepatocyte cell, the method comprising providing a non-hepatocyte cell, introducing into the non-hepatocyte cell a nucleic acid or protein preparation which encodes or provides one or more reprogramming factors, and culturing the non-hepatocyte cell under conditions suitable for reprogramming a non-hepatocyte cell to an induced hepatocyte, thereby producing an induced hepatocyte from the non-hepatocyte cell.

In another aspect, there is provided a cell population comprising human induced hepatocytes obtained by any of the methods described herein.

~~In one aspect, there is provided~~Disclosed is a cell population comprising induced hepatocytes obtained by the methods as described herein.

In another aspect, there is provided an isolated human induced hepatocyte obtained by any of the methods described herein.

~~In one aspect, there is provided~~Disclosed is an isolated induced hepatocyte obtained by the methods as described herein.

In another aspect, there is provided a composition comprising a nucleic acid preparation, wherein the composition is capable of reprogramming a human fibroblast cell to a human induced hepatocyte when the composition is introduced into the human fibroblast cell, wherein the nucleic acid preparation comprises nucleic acid molecules encoding a combination of reprogramming factors, wherein the combination of reprogramming factors is selected from the group consisting of a combination comprising FOXA1, FOXA3, and HNF1A; a combination comprising FOXA1, FOXA2, and HNF1A; a combination comprising FOXA2, FOXA3, and HNF1A; a combination comprising FOXA1, HNF1A, and HNF4A; a combination comprising FOXA2, HNF1A, and HNF4A; and a combination comprising FOXA3, HNF1A, and HNF4A.

In another aspect, there is provided a composition comprising a nucleic acid preparation, wherein the composition is capable of reprogramming a human fibroblast cell to a human induced hepatocyte when the composition is introduced into a human fibroblast cell, wherein the nucleic acid preparation comprises nucleic acid molecules encoding a combination of reprogramming factors, where the combination of reprogramming factors comprises FOXA1, FOXA2, FOXA3, HNF1A, and HNF4A.

~~In one aspect, there is provided~~Disclosed is a composition comprising a nucleic acid preparation, wherein the composition is capable of reprogramming a non-hepatocyte cell to an induced hepatocyte when the composition is introduced into a non-hepatocyte cell.

In another aspect, there is provided a method of screening a test compound, the method comprising contacting the human induced hepatocyte obtained by any of the methods described herein with a test compound, and determining the effect of the test compound on the human induced hepatocyte.

~~In one aspect, there is provided~~Disclosed is a method of screening a test compound, the method comprising contacting an induced hepatocyte obtained by the methods as described herein with a test compound, and determining the effect of the test compound on the induced hepatocyte.

The methods and compositions provided herein are useful for reprogramming a starting cell or starting cell population (*e.g.*, a non-hepatocyte cell or non-hepatocyte cell population) to an induced hepatocyte or a population of induced hepatocytes with high efficiency. In some

WHAT IS CLAIMED IS:

1. A method for producing a human induced hepatocyte from a human fibroblast cell, the method comprising providing a human fibroblast cell, introducing into the human fibroblast cell a nucleic acid or protein preparation which encodes or provides a combination of reprogramming factors, wherein the combination of reprogramming factors is selected from the group consisting of a combination comprising FOXA1, FOXA3, and HNF1A; a combination comprising FOXA1, FOXA2, and HNF1A; a combination comprising FOXA2, FOXA3, and HNF1A; a combination comprising FOXA1, HNF1A, and HNF4A; a combination comprising FOXA2, HNF1A, and HNF4A; and a combination comprising FOXA3, HNF1A, and HNF4A, and culturing the human fibroblast cell under conditions suitable for reprogramming a human fibroblast cell to a human induced hepatocyte, thereby producing a human induced hepatocyte from the human fibroblast cell.

2. The method of claim 1, wherein the combination of reprogramming factors is the combination comprising FOXA1, FOXA3, and HNF1A; the combination comprising FOXA1, FOXA2, and HNF1A; or the combination comprising FOXA2, FOXA3, and HNF1A, the combination further comprising HNF4A.

3. The method of claim 1 or claim 2, wherein the one or more reprogramming factors is selected from the group consisting of FOXA1, FOXA2, FOXA3, HNFIA, HNF4A, and GATA4.

4. The method of claim 1 or claim 2, wherein the reprogramming factors comprise CEBPA, GATA6, HHEX, HNFIB, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNFIA, and HNF4A.

5. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA1, FOXA2, FOXA3, HNFIA, HNF4A, and GATA4.

6. A method for producing a human induced hepatocyte from a human fibroblast cell, the method comprising providing a human fibroblast cell, introducing into the human fibroblast cell a nucleic acid or protein preparation which encodes or provides a combination of reprogramming factors, wherein the combination of reprogramming factors comprise FOXA1, FOXA2, FOXA3, HNF1A, and HNF4A, and culturing the human fibroblast cell under conditions suitable for reprogramming a human fibroblast cell to a human induced hepatocyte, thereby producing a human induced hepatocyte from the human fibroblast cell.

7. The method of claim 6, wherein the combination of reprogramming factors further comprises GATA4.

8. A cell population comprising human induced hepatocytes obtained by the method of any of claims 1 to claim 7.

9. An isolated human induced hepatocyte obtained by the method of any of claims 1 to claim 7.

10. A composition comprising a nucleic acid preparation, wherein the composition is capable of reprogramming a human fibroblast cell to a human induced hepatocyte when the composition is introduced into the human fibroblast cell, wherein the nucleic acid preparation comprises nucleic acid molecules encoding a combination of reprogramming factors, wherein the combination of reprogramming factors is selected from the group consisting of a combination comprising FOXA1, FOXA3, and HNF1A; a combination comprising FOXA1, FOXA2, and HNF1A; a combination comprising FOXA2, FOXA3, and HNF1A; a combination comprising FOXA1, HNF1A, and HNF4A; a combination comprising FOXA2, HNF1A, and HNF4A; and a combination comprising FOXA3, HNF1A, and HNF4A.

11. The composition of claim 10, wherein when the nucleic acid preparation comprises nucleic acid molecules encoding the combination of reprogramming factors comprising FOXA1, FOXA3, and HNF1A; the combination comprising FOXA1, FOXA2, and HNF1A; or the combination comprising FOXA2, FOXA3, and HNF1A, the combination further comprises nucleic acid molecules encoding HNF4A.

12. A composition comprising a nucleic acid preparation, wherein the composition is capable of reprogramming a human fibroblast cell to a human induced hepatocyte when the composition is introduced into a human fibroblast cell, wherein the nucleic acid preparation comprises nucleic acid molecules encoding a combination of reprogramming factors, where the combination of reprogramming factors comprises FOXA1, FOXA2, FOXA3, HNF1A, and HNF4A.

13. The composition of claim 12, wherein the composition further comprises nucleic acid molecules encoding GATA4.

14. A method of screening a test compound, the method comprising contacting the human induced hepatocyte obtained by the method of any of claims 1 to 7 with a test compound, and determining the effect of the test compound on the human induced hepatocyte.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for producing an induced hepatocyte from a non-hepatocyte cell, the method comprising providing a non-hepatocyte cell, contacting the non-hepatocyte with an agent which increases or induces the expression or activity of one or more reprogramming factors, and culturing the non-hepatocyte cell under conditions suitable for reprogramming a non-hepatocyte cell to an induced hepatocyte, thereby producing an induced hepatocyte from the non-hepatocyte cell.

2. A method for producing an induced hepatocyte from a non-hepatocyte cell, the method comprising providing a non-hepatocyte cell, introducing into the non-hepatocyte cell a nucleic acid or protein preparation which encodes or provides one or more reprogramming factors, and culturing the non-hepatocyte cell under conditions suitable for reprogramming a non-hepatocyte cell to an induced hepatocyte, thereby producing an induced hepatocyte from the non-hepatocyte cell.

3. The method of claim 1 or claim 2, wherein the one or more reprogramming factors is selected from the group consisting of CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A.

4. The method of claim 1 or claim 2, wherein the one or more reprogramming factors is selected from the group consisting of FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4.

5. The method of claim 1 or claim 2, wherein the reprogramming factors comprise CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A.

6. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4.

7. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA1, FOXA2, FOXA3, HNF1A, and HNF4A.

8. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA1, FOXA3, HNF1A, and HNF4A.

9. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA1, FOXA2, HNF1A, and HNF4A.

10. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA2, FOXA3, HNF1A, and HNF4A.

11. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA1, HNF1A, and HNF4A.

12. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA3, HNF1A, and HNF4A.

13. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA2, HNF1A, and HNF4A.

14. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA1, FOXA3, and HNF1A.

15. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA1, FOXA2, and HNF1A.

16. The method of claim 1 or claim 2, wherein the reprogramming factors comprise FOXA2, FOXA3, and HNF1A.

17. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ

ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, a nucleic acid sequence of SEQ ID NO:41, and a nucleic acid sequence comprising SEQ ID NO:43.

18. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

19. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

20. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

21. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

22. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

23. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

24. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

25. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:37, and a nucleic acid sequence comprising SEQ ID NO:39.

26. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, and a nucleic acid sequence comprising SEQ ID NO:39.

27. The method of claim 2, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, and a nucleic acid sequence comprising SEQ ID NO:39.

28. A cell population comprising induced hepatocytes obtained by the method of claim 1 or claim 2.

29. An isolated induced hepatocyte obtained by the method of claim 1 or claim 2.

30. A composition comprising a nucleic acid preparation, wherein the composition is capable of reprogramming a non-hepatocyte cell to an induced hepatocyte when the composition is introduced into a non-hepatocyte cell.

31. The composition of claim 30, wherein the non-hepatocyte cell is a human non-hepatocyte cell and the induced hepatocyte is a human induced hepatocyte.

32. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding one or more reprogramming factors selected from the group consisting of CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A.

33. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding one or more reprogramming factors selected from the group consisting of FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4.

34. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding CEBPA, GATA6, HHEX, HNF1B, HNF6A, FOXA1, FOXA2, FOXA3, GATA4, HNF1A, and HNF4A.

35. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA2, FOXA3, HNF1A, HNF4A, and GATA4.

36. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA2, FOXA3, HNF1A, and HNF4A.

37. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA3, HNF1A, and HNF4A.

38. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA2, HNF1A, and HNF4A.

39. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA2, FOXA3, HNF1A, and HNF4A.

40. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, HNF1A, and HNF4A.

41. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA3, HNF1A, and HNF4A.

42. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA2, HNF1A, and HNF4A.

43. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA3, and HNF1A.

44. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA1, FOXA2, and HNF1A.

45. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules encoding FOXA2, FOXA3, and HNF1A.

46. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, a nucleic acid sequence of SEQ ID NO:41, and a nucleic acid sequence comprising SEQ ID NO:43.

47. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

48. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

49. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a

nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

50. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

51. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

52. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:37, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

53. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:39, and a nucleic acid sequence of SEQ ID NO:41.

54. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:37, and a nucleic acid sequence comprising SEQ ID NO:39.

55. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:33, a nucleic acid sequence comprising SEQ ID NO:35, and a nucleic acid sequence comprising SEQ ID NO:39.

56. The composition of claim 30, wherein the nucleic acid preparation comprises nucleic acid molecules comprising a nucleic acid sequence comprising SEQ ID NO:35, a nucleic acid sequence comprising SEQ ID NO:37, and a nucleic acid sequence comprising SEQ ID NO:39.

57. A method of screening a test compound, the method comprising contacting an induced hepatocyte obtained by the method of claim 1 or claim 2 with a test compound, and determining the effect of the test compound on the induced hepatocyte.