type II endothelial cell (EC) activation with leukocyte recruitment, (iii) thrombotic microangiopathy consisting of intravascular thrombosis with platelet consumption and EC activation, fibrin deposition, and thrombosis due to lack of thromboregulation, and (iv) chronic vasculopathy. These adverse events are due, at least in part, to molecular incompatibilities between the donor and the recipient, particularly with regard to genes involved in complement, coagulation, inflammatory, and immune response systems. The clinical use of xeno-organs (e.g., porcine) has been hindered by these immunological incompatibilities, which have thus far prevented the use of porcine cells, tissue, and vascularized porcine organs in clinical xenotransplantation.

[0004] Over the last two decades, several genetic modifications that diminish inter-species incompatibility between porcine and humans have been identified. However, these previously identified genetic modifications have not achieved long-term xenograft survival. Moreover, technical limitations with large-scale genome engineering have hindered the integration of these modifications in a single animal.

SUMMARY

[0004a] In one aspect, provided herein is an engineered porcine cell that comprises three polycistronic cassettes arranged in a nucleic acid molecule and expressed from a single genomic locus, wherein each of the three polycistronic cassettes comprises at least two human transgenes operably linked to a promoter, wherein the three polycistronic cassettes comprise:

(I) one or more complement response transgenes of cluster of differentiation 46 (CD46), CD55, or CD59;

(II) one or more inflammatory response transgenes of CD47, TNFα-induced protein 3 (A20), or heme oxygenase (HO-1); and

(III) one or more coagulation response transgenes of thrombomodulin (THBD), CD39, or tissue factor pathway inhibitor (TFPI); and

wherein the engineered porcine cell has inactivated glycoprotein α-galactosyltransferase 1 (GGTA), β1,4 N-acetylgalactosaminyltransferase 2 (B4GalNT2), and cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) genes.

[0004b] In another aspect, provided herein is a porcine tissue comprising the engineered porcine cell as described herein.

[0004c] In another aspect, provided herein is a porcine organ comprising the engineered porcine cell as described herein.

[0004d] In another aspect, provided herein is a genetically modified pig comprising the engineered porcine cell as described herein, the porcine tissue as described herein, or the porcine organ as described herein.

[0004e] In another aspect, provided herein is use of the engineered porcine cell of as described herein in the manufacture of a medicament for the treatment of a disease selected from the group consisting of fire burn, acid burn, autoimmune disease, heart disease, diabetes Type I or Type II, hepatitis, cystic fibrosis, cirrhosis, organ failure, lupus, scleroderma, IgA nephropathy, polycystic kidney disease, myocardial infarction, emphysema, chronic bronchitis, bronchiolitis obliterans, pulmonary hypertension, congenital diaphragmatic hernia, congenital surfactant protein B deficiency, congenital cystic emphysematous lung disease, primary biliary cholangitis, sclerosing cholangitis, biliary atresia, alcoholism, Wilson's disease, hemochromatosis, and alpha-1 antitrypsin deficiency.

[0004f] In another aspect, provided herein is use of the engineered porcine cell as described herein in the manufacture of a medicament for the treatment of liver failure or kidney failure.

[0004g] In yet another aspect, provided herein is method of generating the engineered porcine cell as described herein *in vitro*, the method comprising incorporating the nucleic acid molecule into the single genomic locus of a porcine cell *in vitro*.

[0004h] In another aspect, provided herein is the engineered porcine cell as described herein for use in therapy.

[0005] There is a need for developing porcine cells, tissues, organs, and/or porcine animals having a novel combination of gene modifications for use in xenotransplantation and for developing associated methods.

[0006] Accordingly, the present disclosure provides cells, tissues, organs, and animals comprising genetic modifications that result in enhanced immunological compatibility, as well as vectors and methods for use in generating these cells, tissues, organs, and animals, and the use of these cells, tissues, organs, and animals in xenotransplantation. In certain embodiments, the genetic modifications giving rise to enhanced immunological compatibility include one or more complement response genes (interchangeably referred to herein as complement toxicity genes), coagulation response genes (interchangeably referred to herein as coagulation genes), inflammatory response genes (interchangeably referred to herein as apoptosis/inflammation genes), immune response genes (interchangeably referred to herein as cellular toxicity genes), and/or immunomodulator genes.

Claims

1. An engineered porcine cell that comprises three polycistronic cassettes arranged in a nucleic acid molecule and expressed from a single genomic locus,

wherein each of the three polycistronic cassettes comprises at least two human transgenes operably linked to a promoter,

wherein the three polycistronic cassettes comprise:

(I) one or more complement response transgenes of cluster of differentiation 46 (CD46), CD55, or CD59;

(II) one or more inflammatory response transgenes of CD47, TNFα-induced protein 3 (A20), or heme oxygenase (HO-1); and

(III) one or more coagulation response transgenes of thrombomodulin (THBD), CD39, or tissue factor pathway inhibitor (TFPI); and

wherein the engineered porcine cell has inactivated glycoprotein α-galactosyltransferase 1 (GGTA), β1,4 N-acetylgalactosaminyltransferase 2 (B4GalNT2), and cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH) genes.

2. The engineered porcine cell of claim 1, wherein the three polycistronic cassettes further comprise: (IV) one or more immune response transgenes of human leukocyte antigen-E (HLA-E) or beta-2 microglobulin (B2M).

3. The engineered porcine cell of claim 1 or 2, wherein the engineered porcine cell further comprises a Fas ligand (FasL) transgene, an EPCR transgene, a tumor necrosis factor receptor 1 immunoglobulin (TNFR1-Ig) transgene, a PD-1 transgene, a cytotoxic T-lymphocyte-associated immunoglobulin (CTLA-Ig) transgene, an ecto-5' nucleotidase (CD73) transgene, a superoxide dismutase 3 (SOD3) transgene, a CXCL12 transgene, a C-X-C motif chemokine receptor 3 (CXCR3) transgene, a CD39L1 transgene, a glucagon like peptide 1 receptor (GLP-1R) transgene, a M3R transgene, an interleukin 35 (IL35) transgene, an IL12A transgene, an EB13 transgene, a transgene encoding a fusion of a FK506 binding protein, or a hCaspase8 transgene.

4. The engineered porcine cell of any one of the preceding claims, wherein a polycistronic cassette of the three polycistronic cassettes comprises three human transgenes.

5. The engineered porcine cell of any one of the preceding claims, wherein a polycistronic cassette of the three polycistronic cassettes comprises a human transgene separated from another human transgene by a coding sequence for a 2A peptide.

6. The engineered porcine cell of any one of the preceding claims, wherein a polycistronic cassette of the three polycistronic cassettes is operably linked to a ubiquitous promoter.

7. The engineered porcine cell of claim 6, wherein the ubiquitous promoter is a CAG promoter.

8. The engineered porcine cell of claim 6, wherein the ubiquitous promoter is an eF1α promoter.

9. The engineered porcine cell of any one of the preceding claims, wherein a polycistronic cassette of the three polycistronic cassettes is operably linked to a tissue-specific promoter.

10. The engineered porcine cell of any one of the preceding claims, wherein two polycistronic cassettes of the three polycistronic cassettes are operably linked to divergent promoters.

11. The engineered porcine cell of any one of the preceding claims, wherein the nucleic acid molecule comprises a ubiquitous chromatin opening element.

12. The engineered porcine cell of claim 11, wherein two polycistronic cassettes of the three polycistronic cassettes are separated by a ubiquitous chromatin opening element.

13. The engineered porcine cell of any one of the preceding claims, wherein the nucleic acid molecule further comprises an insulator or a terminator.

14. The engineered porcine cell of any one of the preceding claims, wherein the engineered porcine cell is substantially free of production of xenotropic porcine endogenous retrovirus (PERV) virions.

15. The engineered porcine cell of any one of the preceding claims, wherein the single genomic locus is an adeno-associated virus integration site 1 (AAVS1) locus.

16. A porcine tissue comprising the engineered porcine cell of any one of claims 1-15.

17. A porcine organ comprising the engineered porcine cell of any one of claims 1-15.

18. The porcine organ of claim 17, wherein the porcine organ is a heart.

19. The porcine organ of claim 17, wherein the porcine organ is a liver.

20. The porcine organ of claim 17, wherein the porcine organ is a kidney.

21. A genetically modified pig comprising the engineered porcine cell of any one of claims 1-15, the porcine tissue of claim 16, or the porcine organ of any one of claims 17-20.

22. Use of the engineered porcine cell of any one of claims 1-15 in the manufacture of a medicament for the treatment of a disease selected from the group consisting of fire burn, acid burn, autoimmune disease, heart disease, diabetes Type I or Type II, hepatitis, cystic fibrosis, cirrhosis, organ failure, lupus, scleroderma, IgA nephropathy, polycystic kidney disease, myocardial infarction, emphysema, chronic bronchitis, bronchiolitis obliterans, pulmonary hypertension, congenital diaphragmatic hernia, congenital surfactant protein B deficiency, congenital cystic emphysematous lung disease, primary biliary cholangitis, sclerosing cholangitis, biliary atresia, alcoholism, Wilson’s disease, hemochromatosis, and alpha-1 antitrypsin deficiency.

23. Use of the engineered porcine cell of any one of claims 1-15 in the manufacture of a medicament for the treatment of liver failure or kidney failure.

24. The use of claim 22 or 23, wherein the engineered porcine cell, or a tissue or an organ comprising the engineered porcine cell is to be transplanted into a human subject.

25. A method of generating the engineered porcine cell of any one of claims 1-15 *in vitro*, the method comprising incorporating the nucleic acid molecule into the single genomic locus of a porcine cell *in vitro*.

26. The engineered porcine cell of any one of claims 1-15 for use in therapy.