

CLAIMS:

1. A therapeutic polynucleotide comprising sequences encoding a S100 calcium binding protein A(S100A) polypeptide, a stromal cell derived factor 1 (SDF1) polypeptide, and a vascular endothelial growth factor (VEGF) polypeptide, wherein at least two of the S100A polypeptide, the SDF1 polypeptide, and the VEGF polypeptide are connected by one or more in vivo cleavable linkers, and wherein upon administration and expression of said therapeutic polynucleotide in a subject, said one or more in vivo cleavable linkers can be cleaved to release a free functional form of the at least two polypeptides connected by said one or more in vivo cleavable linkers.
2. The polynucleotide of claim 1, wherein said SDF1 polypeptide is selected from the group consisting of SDF-1 α and SDF-1 β .
3. The polynucleotide of claim 1, wherein said VEGF polypeptide is selected from the group consisting of VEGF121, VEGF121b, VEGF145, VEGF165, VEGF165b, VEGF189, VEGF191, and VEGF206.
4. The polynucleotide of any one of claims 1 to 3, wherein said S100A polypeptide comprises a sequence as shown in SEQ ID NO: 104; said SDF1 polypeptide comprises a sequence as shown in SEQ ID NO: 102; and said VEGF polypeptide comprises a sequence as shown in SEQ ID NO: 106.
5. The polynucleotide of claim 1, wherein said one or more linkers encode a sequence that mediates a ribosome skipping translational effect .
6. The polynucleotide of claim 1, wherein said one or more linkers are selected from the group consisting of a RAKR linker, a fp2a linker, a p2a linker, a GSG-p2a linker, and a fmdv linker, or any combination thereof.
7. The polynucleotide of claim 1, wherein said one or more linkers comprise a sequence as shown in SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, or SEQ ID NO: 40.
8. The polypeptide of claim 6, wherein said one or more linkers further comprise a sequence selected from the group consisting of APVKQ (SEQ ID NO: 42), GPVKQ (SEQ ID NO: 43), VPVKQ (SEQ ID NO: 44), IPVKQ (SEQ ID NO: 45), MPVKQ (SEQ ID NO: 46), APIKQ (SEQ ID NO: 47), GPIKQ (SEQ ID NO: 48), VPIKQ (SEQ ID NO: 49), IPIKQ (SEQ ID NO: 50), MPIKQ (SEQ ID NO: 51), APAKQ (SEQ ID NO: 52), GPAKQ (SEQ ID NO: 53), VPAKQ (SEQ ID NO: 54), IPAKQ (SEQ ID NO: 55), MPAKQ (SEQ ID NO: 56), APVRQ (SEQ ID NO: 57), GPVRQ (SEQ ID NO: 58), VPVRQ (SEQ ID NO: 59), IPVRQ (SEQ ID NO: 60), MPVRQ (SEQ ID NO: 61), APIRQ (SEQ ID NO: 62), GPIRQ (SEQ ID NO: 63), VPIRQ (SEQ ID NO: 64), IPIRQ (SEQ ID NO: 65), MPIRQ (SEQ ID NO: 66), APARQ (SEQ ID NO: 67), GPARQ (SEQ ID NO: 68), VPARQ

(SEQ ID NO: 69), IPARQ (SEQ ID NO: 70), MPARQ (SEQ ID NO: 71), APVKN (SEQ ID NO: 72), GPVKN (SEQ ID NO: 73), VPVKN (SEQ ID NO: 74), IPVKN (SEQ ID NO: 75), MPVKN (SEQ ID NO: 76), APIKN (SEQ ID NO: 77), GPIKN (SEQ ID NO: 78), VPIKN (SEQ ID NO: 79), IPIKN (SEQ ID NO: 80), MPIKN (SEQ ID NO: 81), APAKN (SEQ ID NO: 82), GPAKN (SEQ ID NO: 83), VPAKN (SEQ ID NO: 84), IPAKN (SEQ ID NO: 85), IVIPAKN (SEQ ID NO: 86), APVRN (SEQ ID NO: 87), GPVRN (SEQ ID NO: 88), VPVRN (SEQ ID NO: 89), IPVRN (SEQ ID NO: 90), IVIPVRN (SEQ ID NO: 91), APIRN (SEQ ID NO: 92), GPIRN (SEQ ID NO: 93), VPIRN (SEQ ID NO: 94), IPIRN (SEQ ID NO: 95), MPIRN (SEQ ID NO: 96), APARN (SEQ ID NO: 97), GPARN (SEQ ID NO: 98), VPARN (SEQ ID NO: 99), IPARN (SEQ ID NO: 100) or MPARN (SEQ ID NO: 101).

9. A therapeutic polynucleotide encoding a first polypeptide comprising a S100 calcium-binding protein A (S100A) polypeptide, and a second polypeptide, wherein said second polypeptide is a SDF1 polypeptide or a VEGF polypeptide, wherein said first polypeptide and said second polypeptide are connected by an in vivo cleavable linker, wherein the in vivo cleavable linker can be cleaved in vivo upon administration to a subject to release a free functional form of said first polypeptide and said second polypeptide.

10. The polynucleotide of claim 9, further comprising a third polypeptide, wherein said third polypeptide is a SDF1 polypeptide or a VEGF polypeptide, wherein said third polypeptide is different from said second polypeptide and is connected to said second polypeptide by a second in vivo cleavable linker, wherein the second in vivo cleavable linker can be cleaved in vivo upon administration to the subject to release a free functional form of the third polypeptide.

11. The polynucleotide of any one of claims 9 to 10, wherein at least one of said in vivo cleavable linkers comprises furin and a 2A polypeptide.

12. The polynucleotide of claim 11, wherein at least one of said in vivo cleavable linkers comprises furin and P2A (fp2a), wherein said 2A polypeptide comprises a sequence that mediates a "ribosome skipping" translational effect.

13. The polynucleotide of any one of claims 9 to 12, wherein said in vivo cleavable linker comprises a sequence as shown in SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, or SEQ ID NO: 40, or SEQ ID NO: 42.

14. A polypeptide encoded by the polynucleotide of any one of claims 1 to 13.

15. A cell comprising the polynucleotide of any one of claims 1 to 13, or a polypeptide encoded by said polynucleotide.

16. An expression vector comprising the polynucleotide of any one of claims 1 to 13, wherein said polynucleotide is operably linked to a promoter.
17. The expression vector of claim 16, wherein said promoter is a constitutive promoter, a tissue specific promoter, or an inducible promoter.
18. The expression vector of claim 17, wherein said inducible promoter is a small molecule ligand-inducible two polypeptide ecdysone receptor-based gene switch.
19. The expression vector of any one of claims 16 to 18, wherein said expression vector is a cardiac expression vector.
20. A pharmaceutical composition comprising:
the polynucleotide of any one of claims 1 to 13, a polypeptide encoded by said polynucleotide of any one of claims 1 to 13, or the expression vector of any one of claims 16 to 19; and,
a pharmaceutically acceptable excipient.
21. A method comprising: contacting at least one cardiac cell with the polynucleotide of any one of claims 1 to 13, a polypeptide encoded by said polynucleotide of any one of claims 1 to 13, or the expression vector of any one of claims 16 to 19, wherein the contacting is ex vivo.
22. The method of claim 21, said cardiac cell is a myocardial cell.
23. Use of a therapeutically effective amount of the pharmaceutical composition of claim 20 in the manufacture of a medicament for the treatment of a cardiac condition in a subject in need thereof.
24. The use of claim 23, wherein said cardiac condition is cardiomyopathy, vasculogenesis, congestive heart failure, arrhythmia, pericardial disease, aorta disease, Marfan syndrome, or coronary artery disease.
25. The use of claim 23 or 24, wherein said subject is a human subject.
26. Use of a therapeutically effective amount of a composition in the manufacture of a medicament for the treatment of a cardiac condition in a subject in need thereof, wherein the composition comprises a microbubble complex, wherein said microbubble complex comprises a lipid, a gas, and a plasmid comprising the polynucleotide of any one of claims 1 to 13; wherein the medicament is to be administered by contacting said microbubble complex with ultrasonic energy sufficient to result in targeted microbubble destruction (UTMD).
27. The use of claim 26, wherein said lipid forms a shell enclosing said gas and said plasmid.

28. The use of any one of claims 21 to 27, wherein said contacting, treatment, or administering is to be via retrograde coronary sinus infusion (RCSI).

29. The polynucleotide of any one of claims 1 to 3, wherein said S100A polypeptide is a S100A1 polypeptide.