

oncogenes and cellular receptors for animal viruses. Most early approaches were based on the use of pooled libraries (typically, cDNA libraries), in which a desired factor was identified by phenotype-based selection. In the 2000s, along with advancements in robotics, library screening progressively moved toward high throughput screening (HTS) analysis, based on the use of arrayed libraries. HTS has paved the way to the use not only of cDNA libraries, but also of libraries of peptides, nucleic acids (Eulalio, A., et al. Functional screening identifies miRNAs inducing cardiac regeneration. Nature 492, 376-381 (2012)) and small molecules. Today, the advances in gene transfer permit the field to take a further step forward, namely to screen libraries directly in animals, thus moving from biochemical or phenotypic selection in vitro towards true functional selection in vivo.

SUMMARY

[12] The inventors have utilized a unique procedure based on the in vivo Functional Selection (FunSel) of factors exerting a desired function to identify factors with cardioprotective effects. The inventors' procedure is based on the use of a library of AAV vectors, which are exquisite tools for highly efficient cardiac gene transfer.

[13] Of note, factor identification by FunSel does not require that the selected factors play a role in a given tissue during normal physiology, thus extending the range of potentially therapeutic proteins to all secreted factors encoded by the genome.

[14] The inventors have surprisingly found that the three factors *Chrdl1* (Chordin-like protein 1), *Fam3c* and *Fam3b*, although exerting completely different biological activities, commonly share cardioprotective effects.

[15] The three novel cardioprotective proteins *Chrdl1*, *Fam3c* and *Fam3b* are highly homologous between mouse and humans (93%, 94% and 79% respectively).

[16] These three factors protect from cardiac cell death induced by ischemia and other kind of damage, including treatment with chemotherapeutic agents.

[17] Accordingly, the present disclosure relates to the factors *Chrdl1*, *Fam3c* and *Fam3b* for use as medicaments.

[17a] In one aspect, the present disclosure refers to a use of a protein selected from the group consisting of *Chrdl1*, *Fam3c*, *Fam3b* and fragments thereof, or a polynucleotide encoding therefor, in the manufacture of a medicament for treating or reducing the risk of heart disease.

[17b] In another aspect, the present disclosure refers to a use of a protein selected from the group consisting of *Chrdl1*, *Fam3c*, *Fam3b* and fragments thereof, or a polynucleotide encoding therefor, in the manufacture of a medicament for preserving cardiac muscle cell viability.

[18] In one example, the disclosure provides a protein selected from the group consisting of *Chrdl1*, *Fam3c*, *Fam3b* and fragments thereof, or a polynucleotide encoding therefor, for use in treating or reducing the risk of heart disease.

[19] In another example, the disclosure provides Chrdl1 or a fragment thereof, or a polynucleotide encoding therefor, for use in treating or reducing the risk of heart disease. In another example, the disclosure provides Fam3c or a fragment thereof, or a polynucleotide encoding therefor, for use in treating or reducing the risk of heart disease. In another example, the disclosure provides a Fam3b or a fragment thereof, or a polynucleotide encoding therefor, for use in treating or reducing the risk of heart disease.

[20] In another example, the disclosure provides a method for treating or reducing the risk of heart disease, wherein the method comprises administering a protein selected from the group consisting of Chrdl1, Fam3c, Fam3b and fragments thereof, or a polynucleotide encoding therefor, to a subject in need thereof.

[21] In some examples, the use reduces the risk of heart failure. In some examples, the risk of heart failure is reduced in a subject suffering from a heart disease. In some examples, the risk of heart failure is reduced in a subject at risk of heart disease.

[22] In one example, the disclosure provides a protein selected from the group consisting of Chrdl1, Fam3c, Fam3b and fragments thereof, or a polynucleotide encoding therefor, for use in reducing the risk of heart failure.

[23] In another example, the disclosure provides Chrdl1 or a fragment thereof, or a polynucleotide encoding therefor, for use in reducing the risk of heart failure. In another example, the disclosure provides Fam3c or a fragment thereof, or a polynucleotide encoding therefor, for use in reducing the risk of heart failure. In another example, the disclosure provides a Fam3b or a fragment thereof, or a polynucleotide encoding therefor, for use in reducing the risk of heart failure.

[24] In another example, the disclosure provides a method for reducing the risk of heart failure, wherein the method comprises administering a protein selected from the group consisting of Chrdl1, Fam3c, Fam3b and fragments thereof, or a polynucleotide encoding therefor, to a subject in need thereof.

[25] In some examples, the risk of heart failure is reduced in a subject suffering from a heart disease. In some examples, the risk of heart failure is reduced in a subject at risk of heart disease.

[26] In another example, the disclosure provides a protein selected from the group consisting of Chrdl1, Fam3c, Fam3b and fragments thereof, or a polynucleotide encoding therefor, for use in preserving cardiac muscle cell viability.

[27] In another example, the disclosure provides Chrdl1 or a fragment thereof, or a polynucleotide encoding therefor, for use in preserving cardiac muscle cell viability. In another example, the disclosure provides Fam3c or a fragment thereof, or a polynucleotide encoding therefor, for use in preserving cardiac muscle cell viability. In another example, the disclosure provides Fam3b or a fragment thereof, or a polynucleotide encoding therefor, for use in preserving cardiac muscle cell viability.

[28] In another example, the disclosure provides a method for preserving cardiac muscle cell viability, wherein the method comprises administering a protein selected from the group consisting of Chrdl1, Fam3c, Fam3b and fragments thereof, or a polynucleotide encoding therefor, to a subject in need thereof.

[29] In some examples, cardiac muscle cell viability is preserved in a subject suffering from a heart disease. In some examples, cardiac muscle cell viability is preserved in a subject at risk of a heart disease.

[30] An example amino acid sequence of Chrdl1 is SEQ ID NO: 1. An example nucleotide sequence encoding Chrdl1 is SEQ ID NO: 4.

[31] An example amino acid sequence of Fam3c is SEQ ID NO: 2. An example nucleotide sequence encoding Fam3c is SEQ ID NO: 5.

[32] An example amino acid sequence of Fam3b is SEQ ID NO: 3. An example nucleotide sequence encoding Fam3b is SEQ ID NO: 6.

[33] In some examples, the protein comprises an amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 1. In some examples, the protein comprises an amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 2. In some examples, the protein comprises an amino acid sequence that has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 3.

[34] In some examples, the protein comprises the amino acid sequence of SEQ ID NO: 1. In some examples, the protein comprises the amino acid sequence of SEQ ID NO: 2. In some examples, the protein comprises the amino acid sequence of SEQ ID NO: 3.

[35] In some examples, the polynucleotide comprises a nucleotide sequence that has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 4. In some examples, the polynucleotide comprises a nucleotide sequence that has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 5. In some examples, the polynucleotide comprises a nucleotide sequence that has at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO: 6.

[36] In some examples, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 4. In some examples, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 5. In some examples, the polynucleotide comprises the nucleotide sequence of SEQ ID NO: 6.

[37] The heart disease may, for example, be due to loss of cardiomyocytes as a consequence of myocardial infarction, reperfusion-injury after percutaneous coronary intervention (coronary angioplasty), hypertension, cardiac toxic damage (in particular, by cancer chemotherapy), myocarditis, cardiomyopathy of genetic and non-genetic origin.

[38] In some examples, the heart disease is associated with cardiac ischemia. In some examples, the heart disease is associated with loss of cardiomyocytes.

[39] In some examples, the heart disease is selected from myocardial infarction; the consequences of myocardial infarction; reperfusion-injury after percutaneous coronary intervention (coronary angioplasty); myocarditis; hypertension; cardiac toxic damage (in particular, by cancer chemotherapy); or cardiomyopathy.

[40] In some examples, the heart disease is ventricular dysfunction.

[41] In some examples, the heart is protected from myocardial infarction. In some examples, cardiac function is preserved after myocardial infarction or percutaneous coronary intervention (coronary angioplasty). In some examples, fibrosis after infarction is reduced.

[42] In some examples, the protein or polynucleotide results in heart protection from myocardial infarction and/or other conditions leading to loss of cardiomyocytes, preferably preserving cardiac function and reducing reparative fibrosis.

[43] In some examples, heart failure is prevented.

[44] In some examples, the protein is administered by direct protein delivery. In some examples, the polynucleotide is used in gene therapy.

- [45] In some examples, the protein is a recombinant protein.
- [46] In some examples, the protein is obtained from bacterial, yeast or mammalian cell culture. In some examples, the protein is obtained from *E. coli*, *Pichia pastoris* or *Chinese Hamster Ovary cells*.
- [47] In some examples, the protein is glycosylated.
- [48] In some examples, the protein is a fusion protein. In some examples, the protein is an Fc fusion protein.
- [49] In some examples, the polynucleotide is in the form of a vector.
- [50] In some examples, the polynucleotide is in the form of a viral vector.
- [51] In some examples, the vector is an adeno-associated viral (AAV) vector, retroviral vector, lentiviral vector or adenoviral vector. In preferred examples, the vector is an adeno-associated viral (AAV) vector.
- [52] In some examples, the vector is an AAV2 vector.
- [53] In some examples, the vector is an AAV9 vector.
- [54] In some examples, the vector is an AAV8 vector.
- [54a] In another aspect, the present disclosure refers to a use of a vector in the manufacture of a medicament for treating or reducing the risk of heart disease, wherein the vector comprises a polynucleotide as defined herein.
- [55] In another example, the disclosure provides a vector for use in treating or reducing the risk of heart disease, wherein the vector comprises a polynucleotide as disclosed herein. In another example, the disclosure provides a vector for use in reducing the risk of heart failure, wherein the vector comprises a polynucleotide as disclosed herein. In another example, the disclosure provides a vector for use in preserving cardiac muscle cell viability, wherein the vector comprises a polynucleotide as disclosed herein.
- [56] In some examples, the vector is a viral vector.
- [57] In some examples, the vector is an adeno-associated viral (AAV) vector, retroviral vector, lentiviral vector or adenoviral vector. In preferred examples, the vector is an adeno-associated viral (AAV) vector.
- [58] In some examples, the vector is an AAV2 vector.
- [59] In some examples, the vector is an AAV9 vector.

[60] In some examples, the vector is an AAV8 vector.

[61] In some examples, the protein is administered parenterally. In some examples, the protein is administered intramyocardially. In some examples, the polynucleotide is administered parenterally. In some examples, the polynucleotide is administered intramyocardially.

[62] In another aspect, the disclosure provides a pharmaceutical composition comprising the protein as disclosed herein and a pharmaceutically acceptable vehicle and/or excipient.

[63] In another example, the disclosure provides a pharmaceutical composition comprising the vector as disclosed herein and a pharmaceutically acceptable vehicle and/or excipient.

[64] In some examples, the composition is formulated for injection. In preferred examples, the composition is formulated for intracardiac or intravenous administration.

[64a] In another aspect, the present disclosure refers to a use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating or reducing the risk of heart disease.

[64b] In another aspect, the present disclosure refers to a use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for preserving cardiac muscle cell viability.

[65] In another example, the disclosure provides the pharmaceutical composition disclosed herein for use in treating or reducing the risk of heart disease. In another example, the disclosure provides the pharmaceutical composition disclosed herein for use in reducing the risk of heart failure. In another example, the disclosure provides the pharmaceutical composition disclosed herein for use in preserving cardiac muscle cell viability.

[66] In another example, the disclosure provides a protein selected from the group consisting of Chrdl1, Fam3c, Fam3b and fragments thereof, or a polynucleotide encoding therefor, for use as medicament.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. FunSel, an in vivo selection procedure to identify novel cardiac therapeutics for myocardial infarction (MI)

[67] **A.** Schematic representation of the pGi vector plasmid used for the Secretome library generation. **B.** Outline of the functional selection (FunSel) procedure to identify cardioactive factors after myocardial infarction (MI). Briefly, Pools of 50 vectors are used to transduce the heart left ventricle; each vector enters a different cardiomyocyte. MI is then induced, which kills most of the cardiomyocytes, however not those that express a cardioprotective factor. After 3 weeks, barcode DNA is recovered from the heart by PCR amplification and frequency of vectors is determined by next generation sequencing (NGS). The number of sequencing reads of vectors with or without infarction are then compared; enriched factors are those that exert cardioprotective activity. **C.** Cumulative results obtained from the in vivo

CLAIMS

1. Use of a protein selected from the group consisting of *Chrdl1*, *Fam3c*, *Fam3b* and fragments thereof, or a polynucleotide encoding therefor, in the manufacture of a medicament for treating or reducing the risk of heart disease.
2. Use of a protein selected from the group consisting of *Chrdl1*, *Fam3c*, *Fam3b* and fragments thereof, or a polynucleotide encoding therefor, in the manufacture of a medicament for preserving cardiac muscle cell viability.
3. The use according to claim 1 or 2, wherein the protein comprises an amino acid sequence that has at least 70% identity to SEQ ID NO: 1, 2 or 3.
4. The use according to claim 1 or 2, wherein the polynucleotide comprises a nucleotide sequence that has at least 70% identity to SEQ ID NO: 4, 5 or 6.
5. The for use according to any one of claims 1, 3 or 4, wherein the heart disease is associated with cardiac ischemia or loss of cardiomyocytes.
6. The use according to any one of claims 1 or 3-5, wherein the heart disease is selected from myocardial infarction; the consequences of myocardial infarction; reperfusion-injury after percutaneous coronary intervention; myocarditis; hypertension; cardiac toxic damage (in particular, by chemotherapy); or cardiomyopathy.
7. The use according to any preceding claim, wherein the heart is protected from myocardial infarction; cardiac function is preserved after myocardial infarction or percutaneous coronary intervention; or fibrosis after infarction is reduced.
8. The use according to any preceding claim, wherein heart failure is prevented.
9. The use according to any preceding claim, wherein the protein is glycosylated.
10. The use according to any preceding claim, wherein the protein is a fusion protein, preferably an Fc fusion protein.
11. The use according to any one of claims 1-8, wherein the polynucleotide is in the form of a vector, optionally a viral vector, preferably an adeno-associated viral (AAV) vector.

12. Use of a vector in the manufacture of a medicament for treating or reducing the risk of heart disease, wherein the vector comprises a polynucleotide as defined in any one of claims 1-8 or 11.
13. The use according to claim 12, wherein the vector is a viral vector, optionally wherein the viral vector is an adeno-associated viral (AAV) vector.
14. A pharmaceutical composition comprising the protein as defined in any one of claims 1-10 and a pharmaceutically acceptable vehicle and/or excipient.
15. The pharmaceutical composition according to claim 14, wherein the composition is formulated for injection.
16. A pharmaceutical composition comprising the vector as defined in claim 12 or 13 and a pharmaceutically acceptable vehicle and/or excipient.
17. Use of the pharmaceutical composition according to any one of claims 14-16 in the manufacture of a medicament for treating or reducing the risk of heart disease.
18. Use of the pharmaceutical composition according to any one of claims 14-16 in the manufacture of a medicament for preserving cardiac muscle cell viability.