Therapeutic approaches for cardiac regeneration and repair

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Abstract

Ischaemic heart disease is a leading cause of death worldwide. Injury to the heart is followed by loss of the damaged cardiomyocytes, which are replaced with fibrotic scar tissue. Depletion of cardiomyocytes results in decreased cardiac contraction, which leads to pathological cardiac dilatation, additional cardiomyocyte loss, and mechanical dysfunction, culminating in heart failure. This sequential reaction is defined as cardiac remodelling. Many therapies have focused on preventing the progressive process of cardiac remodelling to heart failure. However, after patients have developed end-stage heart failure, intervention is limited to heart transplantation. One of the main reasons for the dramatic injurious effect of cardiomyocyte loss is that the adult human heart has minimal regenerative capacity. In the past 2 decades, several strategies to repair the injured heart and improve heart function have been pursued, including cellular and noncellular therapies. In this Review, we discuss current therapeutic approaches for cardiac repair and regeneration, describing outcomes, limitations, and future prospects of preclinical and clinical trials of heart regeneration. Substantial progress has been made towards understanding the cellular and molecular mechanisms regulating heart regeneration, offering the potential to control cardiac remodelling and redirect the adult heart to a regenerative state.

Ischaemic heart disease is the leading cause of death worldwide, accounting for 9 million deaths per year[1]. Many of these patients not only undergo the acute phase of myocardial infarction (MI) but also develop progressive heart failure derived from ventricular dysfunction caused by the ischaemic conditions, defined as ischaemic cardiomyopathy. After MI, the damaged myocardium is replaced by fibrotic scar tissue owing to the minimal regenerative capacity of cardiomyocytes in the adult human heart. The presence of scar

tissue in the heart results in loss of pump function and circulatory deficiency. Subsequently, the injured heart follows a remodelling process that results in further fibrosis, loss of myocardium, cardiac dysfunction, and dilatation, eventually leading to fatal heart failure[2].

Treatment of ischaemic heart disease has focused on protecting the heart from progression to heart failure[3]. For example, revascularization by thrombolysis, cardiac intervention, and bypass surgery serve to improve blood supply and can salvage the injured ischaemic myocardium. Pharmacological approaches that slow or reverse cardiac remodelling, such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor-neprilysin inhibitors, β-blockers, and mineralocorticoid-receptor antagonists, have decreased heart failure mortality[4–7]. In contrast to these cardioprotective therapies that target the remodelling process in the failing heart, limited therapies are available for the advanced remodelled heart at end-stage heart failure. Mechanical support therapies, such as left ventricular assist devices (LVADs) and cardiac resynchronization therapy, show beneficial outcomes in patients with end-stage heart failure, but heart transplantation remains the only current solution to renew the impaired heart[8,9]. However, pragmatically, heart transplantation is not realistic as a standard therapy because of the lack of donors worldwide and the surgical complexities[10].

To protect the failing heart, scientists have recently focused on approaches to promote heart regeneration. Initial approaches, the so-called first-generation cell- based therapies, involved transplanting noncardiac cells because researchers could not obtain adequate numbers of functional cardiomyocytes to replace the lost myocardium[11]. Initial cell candidates included skeletal myoblasts, which were expected to contribute to cardiac contraction, and bone marrow-derived cells and mesenchymal stem cells, which showed cardiogenic potential in vitro[12–14]. The next generation of cell-based therapy used resident cardiac cells with stem cell-like characteristics. These cardiac-derived cells were expandable and demonstrated multipotency, differentiating into various cell types of the heart in vitro[15]. Another approach to heart regeneration involved the generation of functional cardiomyocytes in vitro that then were transplanted into the injured heart. Preclinical studies used pluripotent stem cells, which can reliably differentiate into functional cardiomyocytes in vitro[16].

Alternative cell-free approaches for heart regeneration have targeted cardiac resident cells. For example, a reprogramming approach focused on converting cardiac fibroblasts to a cardiomyocyte fate[17,18]. Inducing proliferation of the remaining endogenous cardiomyocytes is another approach to repair the heart[19]. Additionally, the fibrotic response after myocardial injury has also been targeted to block cardiac remodelling[20]. Deciphering and harnessing the molecular mechanisms regulating the transient regenerative capacity of the neonatal mammalian heart might also provide insights into regenerating the adult mammalian heart[21,22].

Treating patients with ischaemic heart disease is the ultimate goal of therapeutic approaches for cardiac regeneration[11]. Despite the enthusiasm and effort invested in many clinical trials of heart repair and regeneration, to date, no effective approaches are available to regenerate the damaged human heart. With an eye on future clinical trials, we focus this Review on the advances in regenerative therapies that have clinical potential for the treatment of ischaemic

heart disease. The objective of this Review is to present a comprehensive overview of therapeutic approaches for cardiac regeneration and repair. However, we do not include information on therapeutic approaches targeting the inflammatory process and the epicardium for heart repair, as these topics are reviewed elsewhere in this Focus Issue[23,24].

Regenerative capacity of the heart

Cardiomyocytes are considered to be in a terminally differentiated state. Nevertheless, cardiomyocyte cell cycle activity and proliferative capacity differ in species and life stages. Cardiomyocytes from certain animals, such as axolotls, frogs, newts, and zebrafish, retain lifelong capacity to proliferate[25,26]. By contrast, cardiomyocytes from adult mammals are permanently quiescent. However, upon injury, the heart of a mouse aged 1 day can regenerate over a period of 3 weeks, whereas the injured heart of a mouse aged ≥7 days cannot repopulate lost cardiomyocytes[22] (FIG. 1). These studies define a transitional period in the first week after birth when the mouse heart loses its regenerative capacity. Unsurprisingly, this regenerative time window correlates with the period after birth when mammalian cardiomyocytes withdraw from the cell cycle[27]. Genetic lineage tracing showed that the majority of cardiomyocytes in the recovered area of hearts from mice aged 1 day were derived from pre-existing cardiomyocytes[22]. These findings offer an opportunity to study the molecular mechanisms whereby the mammalian heart transitions from a regenerative to a non-regenerative organ.

Shifting the focus to humans, a case report of a newborn baby demonstrating functional cardiac recovery after MI suggests a degree of regenerative capacity in the human neonatal heart[28]. Furthermore, accumulating evidence shows that cardiomyocyte turnover occurs in the adult mammalian heart, including in humans[29]. However, the turnover of cardiomyocytes is obviously insufficient to restore the contractile function of an injured human heart, which can lose up to 1 billion cardiomyocytes after an MI[30]. Therefore, establishing cardiac regenerative therapies is an important step towards repairing the damaged heart in patients with heart disease.

Cell-based therapies for cardiac repair

Noncardiomyocytes.

Noncardiac cells, which include skeletal myoblasts, bone marrow-derived cells, and mesenchymal stem cells (MSCs), have been the primary source for cell-based therapies in heart failure. The first cells used were skeletal myoblasts, which were expected to remuscularize the injured heart and restore the contractile function. In animal models, transplanted skeletal myoblasts survived and differentiated into a myogenic lineage, and the treatment improved the ejection fraction in both ischaemic and nonischaemic cardiomyopathies[31–34]. Initial clinical trials showed positive effects, with transplanted skeletal myoblasts leading to improved heart function in patients with ischaemic cardiomyopathy[12,35]. Unfortunately, long-term follow-up studies did not show beneficial effects. Moreover, adverse effects such as arrhythmogenesis occurred owing to the inability of skeletal myoblasts to integrate electromechanically with surrounding cardiomyocytes[36]. These poor outcomes ruled out the use of skeletal myoblasts in further clinical studies.

The next cells used for cardiac regeneration therapy were unselected bone marrow-derived mononuclear cells, which became widely tested in clinical studies for heart disease[37]. This approach was supported by studies demonstrating a beneficial contribution of bone marrow-derived mononuclear cells to cardiac repair in animal models after acute MI[38,39]. Early clinical trials, such as the BOOST trial[13] and the REPAIR-AMI trial[40], showed that transplantation of bone marrow-derived mononuclear cells conferred some beneficial effects in patients with acute MI by improving the ejection fraction. Unfortunately, multiple clinical trials that included larger patient populations and well-randomized and double-blinded settings did not reproduce these earlier results[41–43]. Much controversy remains, and no definite conclusion has been reached on the potential efficacy of cardiac regenerative therapies that are based on bone marrow-derived mononuclear cells.

MSCs are a subpopulation of stromal cells that can be isolated from various tissues, such as bone marrow or adipose tissue, and in vitro studies have shown the capacity of MSCs for self-renewal and multipotent differentiation into adipocytes, chondrocytes, hepatocytes, osteoblasts, neurons, and skeletal muscle cells[44]. MSCs can also differentiate into cardiomyocytes in the presence of the DNA methyltransferase inhibitor 5-azacytidine or by co-culture with primary cardiomyocytes[45–47]. Several preclinical studies showed that transplanting MSCs into the injured heart after MI led to improved cardiac function, although the mechanism was not understood[48,49]. However, in clinical trials such as the POSEIDON[14] and MSC-HF[50] trials, transplantation of MSCs led to only modest benefits for patients with ischaemic cardiomyopathy. Moreover, during the past decade, inconsistent reports in animal studies suggest that MSCs have minimal to no cardiomyogenic potential[51,52].

Therefore, noncardiomyocyte-based cell therapies have not shown consistent positive results in the treatment of heart disease. Nevertheless, these clinical trials have effectively established a standard of operation in evaluating the safety and efficacy of cell-based regeneration therapies in heart failure.

Cardiac-derived cells.

Studies cast doubt on the cardiomyogenic potential of bone marrow-derived mononuclear cells and MSCs; therefore, interest shifted to harvesting cardiac stem cells (CSCs) for repairing the failing heart. CSCs are defined as resident heart cells that show clonogenic, self-renewal, and multipotent capacity. CSCs can differentiate into at least three major cardiac cell types: cardiomyocytes, smooth muscle cells, and endothelial cells[53]. Therefore, CSCs were expected to be an effective and potent cell source for replacing lost tissue in the damaged heart. Different populations of CSCs have been defined by cell surface markers such as mast/stem cell growth factor receptor KIT, stem cell antigen 1 (SCA1; also known as LY6A), and insulin gene enhancer protein ISL1 (also known as islet 1)[15,54,55]. Cardiospheres, a mixed population of CSCs (mainly Bassat, E. et al. The extracellular matrix protein agrin promotes heart regeneration in mice. *Nature* 547, 179–184 (2017). CSCs) that are isolated from cell cultures of mouse and human heart explants, are another cardiac-derived cell source for heart repair[53]. Indeed, preclinical trials showed some beneficial effects of cardiac-derived cell transplantation in animal models of MI[56,57]

(TABLE 1). However, clinical trials were initiated with limited understanding of how these cells contribute to heart regeneration[58].

Two clinical trials have been carried out using KIT[+] CSCs and cardiosphere-derived cells. The first was the SCIPIO trial[59,60], in which KIT[+] CSCs were used to treat patients with ischaemic cardiomyopathy. This study showed a slight positive effect in patients treated with CSC therapies: left ventricular ejection fraction (LVEF) increased and infarct size decreased. However, because CSCs had shown low engraftment rates in preclinical studies, other researchers questioned the capacity of CSCs to form functional cardiomyocytes. Additionally, conflicting findings on the cardiomyogenic potential of endogenous KIT[+] CSCs were reported[61,62]. A study based on lentiviral expression of Cre recombinase under the control of the *Kit* promoter, which has a pattern of expression restricted to KIT^{+} cells, showed that KIT[+] CSCs were necessary and sufficient for heart regeneration after injury[61]. By contrast, another study using a mouse model in which the tamoxifen-inducible MerCreMer protein was targeted to the Kit locus, followed by cross-breeding with the R26-GFP reporter mouse line, showed a minimal contribution of KIT^{+} cells to heart regeneration after injury[62]. In addition, a concern was expressed about the integrity of certain data in the SCIPIO trial[63]. Despite the uncertainties of the benefit of CSC-based regeneration therapy, a second clinical trial, the CADUCEUS trial[64], was initiated, in which a cardiosphere-derived cell population was transplanted into patients after MI. The results from this clinical trial showed a reduction in infarct size and an increase in viable myocardium, as shown by cardiac MRI at 6 months and 12 months after therapy. However, in the CADUCEUS trial, patients receiving the cell therapy did not show any improvement in left ventricular global function, indicating that, under current conditions, transplanting cardiosphere-derived cells is not an effective therapy to repair the heart[64]. Given the limited clinical data reported to date, additional studies are needed to determine the efficacy of CSCs in clinical regenerative therapy.

Pluripotent stem cells.

A major deficiency of the early clinical trials was that the transplanted stem cells had a limited capacity to differentiate into cardiomyocytes. Therefore, scientists were challenged to generate functional cardiomyocytes efficiently in vitro to enable transplantation of de novo cardiomyocytes to the injured heart. The first cell source studied was embryonic stem cells (ESCs) derived from the inner cell mass at the blastocyst stage of early embryos. ESCs are clonogenic, self-renewing, and pluripotent, thereby having the capacity to differentiate into all cell types of the three germ layers (endoderm, ectoderm, and mesoderm)[65]. Because ESCs are easily expandable and can differentiate into cardiomyocytes in vitro, these cells offer the opportunity to obtain sufficient amounts of cardiomyocytes for transplantation. Transplantation of ESC-derived cardiomyocytes into the injured heart in animal models of MI improved cardiac function despite low engraftment rates[66,67]. Remarkably, transplanted ESC-derived cardiomyocytes electromechanically coupled with resident cardiomyo- cytes in animal models, which was not the case with skeletal myoblasts. Nevertheless, complications associated with the ESC-derived cardiomyocyte therapy, such as arrhythmias, were detected in some species[68,69] (TABLE 1). Moreover, using ESCs poses major issues for clinical application, such as risk of tumorigenesis and immune rejection. In addition, there is also

broad ethical opposition to using human embryonic cells even for therapeutic use. Therefore, only one clinical trial using ESC-derived cardiomyocytes is ongoing[70].

Fortuitously, Yamanaka and colleagues solved the ethical issues of using ESCs when they reported that mouse and human fibroblasts could be reprogrammed to an ESC-like pluripotent state, called induced pluripotent stem cells (iPSCs), by forced expression of four genes encoding transcription factors: *0CT3/0CT4* (also known as *POU5F1), SOX2, KLF4,* and *MYC* (referred to as OSKM factors)[71,72]. Not only did Yamanaka win the 2012 Nobel Prize in Physiology or Medicine for this discovery, but iPSCs also offered a new cell-based approach for heart repair, enabling autologous or allogeneic transplantation and avoiding the ethical concerns associated with ESCs. However, because autologous transplantation is considered unrealistic owing to the vast amount of work and high cost associated with this approach, preclinical studies are predominantly performed by transplantation of allogeneic or xenogeneic cells with administration of immunosuppressants to avoid rejection of the transplanted cells. Although an early study showed that transplantation of human iPSC-derived cardiomyocytes attenuated cardiac remodelling and improved LVEF in an immunosuppressed porcine MI model, most of the transplanted human iPSC- derived cardiomyocytes did not show long-term survival in the injured heart[73]. By contrast, in a 2016 study, transplanted allogeneic iPSC-derived cardiomyocytes survived up to 12 weeks after treatment in the infarcted heart of a macaque, with evidence of improved cardiac function[74] (TABLE 1). A notable finding of this study is that the iPSC-derived cardiomyocytes showed long-term survival in the immunosuppressed macaque heart without any tumour formation. However, as predicted, the animals with cell transplants had a substantial incidence of ventricular tachycardia, which could be due to the immature state of the transplanted iPSC-derived cardiomyocytes.

Pluripotent stem cell-based approaches probably share similar problems with other cell-based approaches: inconsistencies in the reported engraftment rate and the risk of arrhythmia. Additionally, risk of tumorigenesis remains an underlying concern of pluripotent stem cell-based therapies and has been detected in immunodeficient mouse models[75,76]. However, improvements have been made in generating mature iPSC-derived cardiomyocytes with high purity and sufficient numbers, and these improvements are expected to enhance the cell retention rate by increasing the number of transplanted cells and to reduce the risk of tumorigenesis and arrhythmia associated with undifferentiated immature cells[77,78]. In addition, providing scaffolds for iPSC-derived cardiomyocytes, such as hydrogels or cell sheets, has been shown to improve therapeutic outcomes[79,80]. Regardless of the advancement in transplantation methods, before translating these therapies to clinical trials, studies in large-animal models are warranted to assess the efficacy and safety of iPSC-derived cardiomyocyte-based therapy.

Secretory factors for cardiac repair

In cell-based therapy, transplanted cells were expected to restore cardiac function by engrafting and differentiating into functional cardiomyocytes in vivo. Although this cell-based approach did provide modest cardioprotective benefits, paradoxically, the transplanted stem cells rarely differentiated into cardiomyocytes. To rationalize the beneficial effects

observed, researchers reasoned that paracrine effects from growth factors, microRNAs (miRNAs), and exosomes secreted by the stem cells were responsible and potentially crucial for cardiac repair and regeneration[81].

Growth factors to promote cardiac repair.

Growth factors are signalling molecules that contribute to multiple cellular processes. The growth factor neuregulin 1 (NRG1) and its receptors, receptor tyrosine- protein kinase ERBB2 and ERBB4, have a critical role in trabeculation and endocardial cushion formation during heart development[82]. Activation of the NRG1-ERBB2/ERBB4 signalling pathway in the injured adult mouse heart induces cardiomyocyte proliferation and improves cardiac function[83,84] (FIG. 2). In a clinical trial in patients with heart failure, systemic delivery of human recombinant NRG1 prevented cardiac remodelling, as shown by decreases in end-diastolic volume and end-systolic volume compared with placebo[85]. However, the proliferative effect of NRG1 in cardio- myocytes in vivo has been challenged on the basis of findings in a mouse model of MI where NRG1 treatment did not increase cardiomyocyte DNA synthesis[86]. Therefore, additional studies are required to explain and confirm this beneficial outcome.

Other growth factors that showed beneficial effects in preclinical studies did not realize this promise in clinical trials. For instance, administration of vascular endothelial growth factor A (VEGFA) — a well- known pro-angiogenic factor — improved regional coronary flow and restored cardiac function in an animal model of MI[87], but this benefit was not observed in clinical trials[88]. Cardiac-specific overexpression in mice of the gene encoding another pro-angiogenic factor, fibroblast growth factor 2 (FGF2), decreased infarct size and improved cardiac function after MI compared with wild-type mice[89]. Furthermore, FGF2 depletion exacerbated cardiac dysfunction after ischaemia-reperfusion injury[89]. Despite these encouraging early results, treatment with FGF2 provided only minimal cardioprotective effects in patients with ischaemic heart disease[90].

One possible explanation for these inconsistent findings is the inadequate amount of therapeutic factor available at the target sites. This low availability could be due to inefficient delivery of the therapeutic growth factors and/or to a short half-life of the growth factors. To enhance exposure of the injured heart to the therapeutic growth factors, scaffolds of biomaterials that enable sustained release of the factors to the target sites have been used and have shown improved cardioprotective effects. For instance, in a pig MI model, loading NRG1 and fibroblast growth factor 1 (FGF1) into microparticles provided sustained local release of the therapeutic factors, which improved left ventricular function associated with increased angiogenesis and reduced ventricular remodelling[91] (TABLE 1). Additionally, improved gene- based therapeutics have also led to increased efficiency of delivery and expression of therapeutic factors, such as synthetic modified RNA, a method that has been used to express human *VEGFA* in the mouse heart after MI[92]. Treatment with this modified RNA led to an increase in *VEGFA* expression, induced vascular regeneration, and improved cardiac function and long-term survival compared with the use of DNA vectors[92].

microRNAs in cardiac repair.

miRNAs have been implicated in paracrine signalling. miRNAs are highly conserved, single-strand, small non-coding RNAs that regulate gene expression post-transcriptionally by annealing with complementary sequences of mRNAs[93]. Individual miRNAs interfere with multiple target mRNAs, thereby controlling a variety of biological processes, including heart development and disease (reviewed elsewhere[94,95]). For example, miR-15 family members have been reported to control postnatal mitotic arrest in the mouse heart[96]. Cardiac overexpression of miR-195, a miR-15 family member, causes premature cardiomyocyte cell cycle arrest[96]. Moreover, inhibition of miR-15 family members with synthetic modified RNAs induced cardiomyocyte proliferation in vivo and improved cardiac function in the ischaemic adult mouse heart[96] (FIG. 2).

Other studies have identified miRNAs that activate DNA replication in cardiomyocytes. An unbiased screen using a library of 875 human miRNAs detected multiple miRNAs that promote neonatal rat cardiomyocyte proliferation[97]. Among these miRNAs, cardiac overexpression of miR-199a or miR-590 in vivo induced proliferation of postnatal and adult cardiomyocytes and improved cardiac function and decreased fibrosis in the infarcted heart of adult mice (TABLE 1). Other miRNAs such as the miR-17–92 cluster, miR-214, miR-302–367, and the miR-222 cluster have been reported to contribute to cardiac repair in vivo in animal studies[98–101]. Therefore, the positive outcomes in preclinical studies demonstrate the therapeutic potential of miRNAs.

However, lessons from clinical trials of regenerative therapies based on growth factors caution us to consider a major challenge for the clinical translation of miRNA-based approaches: how can miRNAs be efficiently delivered to the target site? Similar to the studies on growth factors, providing a scaffold seems to be beneficial for continuous local delivery of miRNAs. As an example, miR-302 is reported to promote cardio- myocyte proliferation by inhibiting Hippo signalling components[99]. Use of a hydrogel scaffold to deliver miR-302 allowed the sustained, gradual release of the miRNA in mouse hearts, and a single injection of the hydrogel-miR-302 complex into the mouse heart after MI led to continuous cardiomyocyte proliferation and improved cardiac function[102].

Exosomes in cardiac repair.

Exosomes provide a plausible therapeutic approach to cardiac repair. Exosomes are small extracellular vesicles (30–100 nm diameter) that are produced by cells and are characterized by the presence of specific surface markers such as CD9, CD63, and CD81 (REF[103]). Exosomes are released from cells by fusion of intracellular multivesicular bodies with the plasma membrane[103]. Exosomes are secreted by many cell types, including stem cells, and contain various cargos such as RNAs, lipids, or proteins. Exosomes can function as a vehicle for intercellular communication by carrying cell-specific mRNAs and miRNAs, and accumulating evidence supports a role for exosomes in cell-cell communication among cardiac cells[104,105]. For instance, after MI in mice, administration of murine cardiosphere-derived exosomes enriched in miR-451 inhibited cardiomyo- cyte apoptosis[106] (FIG. 2). Administration of extracellular vesicles, predominantly comprising exosomes, derived from human CSCs also attenuated cardiac remodelling and improved cardiac function in

preclinical studies of MI[107–109] (TABLE 1). Delivery of MSC-derived exosomes into the mouse heart reduced oxidative stress and promoted survival of cardiomyocytes after ischaemia-reperfusion injury, thereby reducing infarct size and improving cardiac function[110].

In addition to these cardioprotective properties, the capacity of exosomes to transport therapeutic factors supports the prospect of exosomes as a biological vehicle for clinical application. Interestingly, fusing the neuron-specific rabies viral glycoprotein peptide (RVG) with an exosomal membrane protein enabled targeted exosomal delivery of small interfering RNAs to knock down the expression of specific genes in the brain[111]. A cardiac-targeted exosome would be an attractive delivery vehicle for cardioprotective factors such as miRNAs or ligands because the use of exosomes could diminish the risk of activating the immune response associated with viral delivery systems[112]. Understanding the molecular mechanisms of exoso- mal biology will advance the therapeutic potential of exosomes in cardiac regenerative therapy.

Direct reprogramming for heart repair

Direct reprogramming of mouse fibroblasts into cardiomyocytes in vitro.

Two major issues arising from the use of iPSCs for heart repair and regeneration are the oncogenic potential of the remaining undifferentiated cells and the low engraftment rates of transplanted cells. An approach that could bypass these challenges is to convert resident cardiac cells directly into de novo cardiomyocytes. Fibroblasts constitute a large cell population of the heart, and considering that cardiac injury such as MI is followed by a fibrotic response, transdif- ferentiating cardiac fibroblasts to cardiomyocytes is an attractive possibility for repairing the heart and diminishing cardiac fibrosis[113]. To achieve this goal, a similar approach to that for establishing iPSCs has been taken, where multiple transcription factors related to cardiac development are combined and overexpressed in fibroblasts. Forced expression of a combination of three genes encoding cardiac transcription factors — *Gata4, Mef2c,* and *Tbx5* (a cocktail referred to as GMT) — or of GMT factors plus *Hand2* (referred to as GHMT) successfully reprogrammed murine fibroblasts into induced cardiomyocyte-like cells in vitro, and these cells expressed major cardiac genes and had cardiomyocyte characteristics (such as sarcomere structure, spontaneous intracellular calcium oscillations, and beating contractions) without going through a cardiac precursor state[17,18] (FIG. 3). Remarkably, these reprogramming cocktails are sufficient to suppress fibrotic signalling and convert fibroblasts towards a cardiac fate. However, several hurdles need to be addressed before the clinical application of this approach, including inadequate reprogramming efficiency, lack of understanding of the molecular mechanisms, and the heterogeneous population of induced cardiomyocyte-like cells[114,115].

Many approaches have been implemented to improve cardiac reprogramming efficiency, including the addition of miRNAs and growth factors to the reprogramming cocktails and the modification of endogenous signalling pathways such as RAC-α serine/ threonine-protein kinase (AKT1), transforming growth factor-β (TGFβ), WNT, and Notch signalling[116–122]. Using an unbiased screen of human transcription factors, the zinc-finger protein ZNF281 was identified as an inducer of cardiac reprogramming[123]. Addition of

ZNF281 to the reprogramming cocktail suppressed the expression of genes associated with the inflammatory response and modulated cardiac gene expression by interacting with the transcription factor GATA4 (REF[123]). In other studies, suppressing the expression of genes encoding certain factors such as the Polycomb complex protein BMI1 and the splicing factor polypyrimidine tract-binding protein 1 (PTB) enhanced cardiac reprogramming, suggesting that these factors act as barriers to cardiac repro- gramming[124,125]. However, reaching a consensus protocol for in vitro direct reprogramming of fibroblasts into cardiomyocytes is difficult because in these studies the cardiomyocyte markers, source of fibroblasts, and the time of analysis differ. Therefore, a precise comparison of the reprogramming efficiency between the different protocols should be performed.

Another strategy to reprogramme fibroblasts into cardiomyocytes has been the partial reprogramming of the cells into cardiac progenitor cells, bypassing a pluripotent state. Forced expression of a combination of five genes encoding early cardiac factors — *Mespl, Gata4, Tbx5, Nkx2–5,* and *Baf60c* (also known as *SmarcdS)* — reprogrammed murine fibroblasts into an expandable multipotent cardiac progenitor cell population[126]. These induced cardiac progenitor-like cells were transplanted into murine hearts after MI, where the cells differentiated into cardiomyocytes, endothelial cells, and smooth muscle cells, and improved survival. However, the drawbacks of transplanting cells diminish the advantages of direct reprogramming.

Direct reprogramming of human cells in vitro.

To translate reprogramming approaches to the clinic, efforts have focused on performing direct cardiac reprogramming in human cells. Previous studies reported that expression of a combination of the transcription factors protein c-ETS2 (ETS2) and mesoderm posterior protein 1 (MESP1) converted human dermal fibroblasts into cardiac progenitors that expressed early cardiac markers such as ISL1 and homeobox protein NKX2–5, which are not detected with direct reprogramming of mouse fibroblasts[127]. Interestingly, for direct reprogramming of human cells, GMT or GHMT reprogramming cocktails require additional factors such as myocar- din, MESP1, oestrogen-related receptor-γ (ESRRγ), and zinc-finger protein ZFPM2, or even miR-1 and/or miR-133, to successfully induce the conversion of human fibroblasts towards a cardiac fate[128–130] (FIG. 3). Human reprogrammed cardiomyocyte-like cells showed a similar gene expression profile to cardiomyocytes and exhibited sarcomere structure and spontaneous calcium transients[128–130]. Moreover, transdifferentiation of human fibroblasts to cardiomyocyte-like cells has also been achieved with a virus-free method consisting of a pluripotent cocktail with a combination of nine chemicals, with up to 97% of the reprogrammed cells spontaneously beating[131]. These chemically treated human fibroblasts were transplanted into an immunodeficient mouse heart after MI, where the transplanted cells were eventually reprogrammed to cardiomyocyte-like cells that expressed cardiac genes and had a well-organized sarcomere structure. However, there was no evidence that the chemically reprogrammed cardiomyocyte-like cells were directly converted from fibroblasts without passing through a progenitor state.

Direct cardiac reprogramming in human cells is more challenging than in mouse cells, given the low reprogramming efficiency and the longer time needed for human cells to exhibit cardiomyocyte characteristics. This increased difficulty is consistent with other cellular reprogramming strategies[132]. For example, human iPSC reprogramming efficiency is less than that observed for mouse iPSCs and requires a longer time to reprogramme, even though the reprogramming cocktail contains the same factors for both cell types. Similarly, neuronal cell reprogramming with human cells requires additional factors and longer duration than neuronal cell reprogramming with mouse cells[133]. The difficulty in reprogramming human cells could be attributed to the difference in the epigenetic landscape between mouse and human fibroblasts, suggesting additional epigenetic barriers for reprogramming human cells[134]. Another feature to consider is that humans have a longer developmental time than mice, potentially contributing to the species differences in cell reprogramming. Interestingly, despite the requirement of different factors for direct cardiac reprogramming between species, crucial endogenous signalling pathways such as the TGFβ1 and WNT signalling pathways contribute similarly to cardiac reprogramming in both mouse and human cells[120].

Direct reprogramming of fibroblasts into cardiomy- ocytes in vivo.

Direct reprogramming in vivo has been reported in mice with the use of GMT and GHMT reprogramming cocktails with retroviral delivery, which exclusively infects proliferating cells such as activated cardiac fibroblasts after myocardial injury[18,135,136] (FIG. 3; TABLE 1). Lineage tracing of fibroblasts with the use of *Fsp1* (also known as *Sl00a4)-Cre* and *Myh6-MerCreMer* mice demonstrated that these newly generated cardiomyocyte-like cells were derived from fibroblasts and not from cell fusion with endogenous cardiomyocytes. Interestingly, these in vivo- generated cardiomyocyte-like cells demonstrated similar characteristics to endogenous ventricular cardiomyo- cytes, such as their cardiac gene expression profile, sarcomere structure, contractility, calcium transients, and action potential; therefore, in vivo-generated cardiomyocyte-like cells seem to mimic endogenous cardiomyocytes better than in vitro-generated cardiomyocyte-like cells. Actually, this finding is not surprising considering that cardiomyocyte-like cells generated in vivo are exposed to a physiological microenvironment, including electromechanical stimulation and interactions with adjacent cells. In vivo-generated cardiomyocyte-like cells also form gap junctions with endogenous cardiomyocytes, increasing the potential for electromechanical coupling[18]. Similar results were reported with the use of a combination of muscle-specific miRNAs delivered with lentivirus to the murine heart[122].

Although direct reprogramming in vivo improves cardiac function after MI in mice, the relatively low reprogramming efficiency compared with direct reprogramming in vitro suggests that other factors besides contractility of induced cardiomyocyte-like cells contribute to the therapeutic efficacy of in vivo reprogramming[137]. For example, a decrease in the size of the fibrotic tissue suggests that paracrine effects from induced cardiomyocyte-like cells or a direct contribution of the reprogramming factors suppress the fibrotic response. In addition, we cannot exclude the possibility that the reprogramming cocktail affects other cells in the heart, such as endothelial cells, immune cells, CSCs, and even pre-existing cardiomyocytes. This possibility raises concerns because direct cardiac reprogramming has been honed using fibroblasts; therefore, in other cell lineages, the

reprogramming cocktail might induce incomplete reprogramming that could elicit unfavourable effects such as arrhythmias.

The positive outcomes observed in murine direct cardiac reprogramming in vivo give optimism to eventual clinical translation. However, several challenges remain to be addressed before clinical application, such as the low reprogramming efficiency, the immaturity and heterogeneity of induced cardiomyocyte-like cells, and the lack of a feasible delivery method for the reprogramming cocktail. In addition, in current studies, the induction of reprogramming is limited to a short period of time after coronary ligation. This short window of administration would restrict the therapeutic time window in a clinical setting. A 2018 study reported that direct cardiac reprogramming could be achieved in both mouse and human cells by delivering reprogramming factors with a Sendai virus vector, which is a non-segmented, negative-stranded RNA virus[138]. Sendai virus replicates only in the cytoplasm and does not integrate into the genome, suggesting a more feasible strategy for clinical application compared with delivery using retrovirus or lentivirus[139].

Stimulating endogenous cardiac repair

Proliferation of endogenous cardiomyocytes.

To promote endogenous cardiomyocyte proliferation, initial approaches targeted universal cell cycle regulators such as cyclins, cyclin-dependent kinases (CDKs), and tumour suppressor genes. Although modulating these factors individually in adult cardiomyocytes did enable some proliferative activity[140,141], a study published in 2018 reported that overexpression of a combination of cell cycle regulators increases the effect on cardiomyocyte proliferation and improves cardiac function after MI[142] (FIG. 4). These findings suggest that specific factors involved in postnatal cardiomyocyte cell cycle regulation are also candidates to stimulate the proliferative capacity in adult cardiomyocytes. For example, the transcription factor homeobox protein MEIS1 is reported as a regulator of postnatal cardiomyocyte cell cycle arrest[143]. Cardiac-specific overexpression of *Meis1* in neonatal mice reduced cardiomyocyte proliferation and abolished heart regeneration. Conversely, genetic ablation of *Meis1* in neonatal mice extended the time window of postnatal cardiomyocyte proliferation and induced cardio- myocytes to re-enter the cell cycle in adult mice[143]. Gene expression analysis revealed that MEIS1 is required for transcriptional activation of the synergistic CDK inhibitors p15 (also known as CDK inhibitor 2B; encoded by *Cdkn2b),* p16 (also known as CDK inhibitor 2A; encoded by *Cdkn2a),* and p21(also known as CDK inhibitor 1A; encoded by *Cdkn1a),* which probably contribute to postnatal cardiomyocyte cell cycle withdrawal.

The Hippo signalling pathway is an evolutionarily conserved pathway shown to control cell proliferation and organ size[144]. This pathway is activated through phosphorylation of a series of factors, such as mammalian STE20-like protein kinase 1 (MST1; also known as STK4) and MST2 (also known as STK3), protein salvador homologue 1 (SAV1), and large tumour suppressor homologue 1 (LATS1) and LATS2, which together phosphorylate and inactivate the transcriptional co-activator Yes-associated protein 1 (YAP1). Phosphorylation of YAP1 blocks nuclear translocation and results in YAP1 being retained in the cytoplasm. In mice, similar phenotypes of heart enlargement owing to cardiomyocyte proliferation can

be evoked by genetic ablation of *Mstl, Mst2, Lats2,* or *Savl,* or by activating YAP1 in cardio-myocytes[19,145]. Encouragingly, inhibiting the Hippo pathway in the heart after MI promotes cardiomyocyte proliferation and improves cardiac function in mice[19,146] (FIG. 4). Accordingly, cell cycle-related genes were upregulated in the heart after Hippo signalling inhibition, but stress response genes, such as those associated with an antioxidant response, and mitochondrial quality control genes were also upregulated[147,148]. Because inhibiting the Hippo pathway is associated with the risk of off-target cell proliferation, antioxidant reagents have been tested and administered into the injured adult mouse heart as an alternative approach. Interestingly, administration of antioxidants induced cardiomyocyte proliferation and promoted heart repair, suggesting that targeting oxidative stress offers a therapeutic approach for heart regeneration[147].

Targeting the fibrotic response for heart repair.

Cardiac fibroblasts are activated after myocardial injury, expressing genes associated with contractile responses and becoming myofibroblasts[149]. In the necrotic tissue after MI, myofibroblasts and inflammatory cells produce a secretome that leads to the formation of a reparative fibrotic scar (reviewed elsewhere[150]). The continuing activity of myofibroblasts produces fibrotic tissue, leading to progressive pathological cardiac remodelling. Multiple signalling pathways, such as the renin-angiotensin-aldosterone system and the TGFβ and WNT signalling pathways, contribute to this fibrotic process after injury[20]. Targeting these pathways and inhibiting the progressive phase of fibrosis has been shown to improve cardiac function by attenuating cardiac remodelling[20,151–153] (FIG. 4). Inhibiting additional targets such as chymase, which is a serine protease that activates the renin-angiotensin-aldosterone system and TGFβ signalling, also prevents cardiac fibrosis and improves cardiac function after MI in rats[154]. The hormone relaxin inhibits TGFβ signalling-mediated fibroblast activation into myofibroblasts and thereby reduces cardiac fibrosis after MI[155,156]. However, the approach of targeting the fibrotic response for heart repair is not as straightforward as anticipated. One of the reasons is that the initial phase of the fibrotic response is considered a healing process because transient scar formation is also detected during heart regeneration after injury in both zebrafish and neonatal mice[157].

At the healing site of the myocardial injury, angiotensin and TGFβ1 have a pivotal role in the secretome of myofibroblasts and inflammatory cells[150]. The macrophages at the injury site produce angiotensin II, which leads to the upregulation and secretion of TGFβ1. Macrophage-derived TGFβ1 induces fibroblast activation and differentiation into myofibroblasts, which also generate TGFβ1 (FIG. 4). TGFβ1 triggers myofibroblasts to produce matrix proteins, eventually forming fibrous tissue. Accumulating evidence from clinical trials shows that pharmacologically inhibiting the activity of angiotensin II attenuates cardiac remodelling[158]. However, blocking fibrosis by inhibiting TGFβ is not straightforward, as preclinical studies have shown that TGFβ treatment outcomes depend on the timing of intervention after the MI[159]. In mice, systemic inhibition of TGFβ in the first 24 h after MI increased mortality and impaired cardiac function, whereas TGFβ inhibition at a later phase attenuated cardiac hypertrophy and remodelling. These results support the concept that the initial fibrotic response after myocardial injury is a necessary phase for healing.

Additionally, nonfibroblast cells such as endothelial cells, epicardial cells, bone marrow-derived cells, and perivascular cells have been proposed to generate newly activated fibroblasts after MI[160–163]. For example, endothelial cells acquire a fibroblastic phenotype through endothelial-to-mesenchymal transition after myocardial injury[160]. Delivery of recombinant human bone morphogenetic protein 7 or pigment epithelium- derived factor attenuates myocardial fibrosis by inhibiting endothelial-to-mesenchymal transition after myocardial injury in animal studies[160,164]. These findings suggest endothelial cells as potent targets for heart repair. Nevertheless, additional lineage-tracing studies show that the contribution of nonfibroblasts to fibroblast activation is more limited than previously surmised[149,165].

Further understanding of the fibrotic response after myocardial injury is necessary to fulfil the therapeutic potential of targeting this process for heart regeneration. Interestingly, a study reported that injecting agrin, an extracellular matrix protein that is enriched in the neonatal mouse heart, promoted heart regeneration after MI in adult mice, partly through cardiomyocyte dedifferentiation[166] (TABLE 1). Therefore, a potential approach for heart regeneration lies in modulating, not inhibiting, the fibrotic response.

Future perspectives

New advances in basic research for heart regeneration.

The identification of a transient regenerative period in the heart of neonatal mice provided scientists with a new resource to study the mechanisms governing mammalian cardiac regeneration. In particular, a postnatal switch from glycolytic to oxidative metabolism and an increase in the oxygenation state of cardiomyocytes induce the production of reactive oxygen species (ROS) in mitochondria[167]. This increase in ROS levels leads to the activation of the DNA damage response (DDR) pathway, which induces postnatal cardiomyocyte cell cycle arrest[167]. Encouragingly, in this study, the postnatal cardiomyocyte proliferation window was extended by pharmacologically scavenging ROS or by inhibiting the DDR pathway, consistent with findings from another study[147]. Moreover, exposing adult mice to gradual, severe, systemic hypoxia led to decreases in ROS production and oxidative DNA damage, which reactivated cardiomyocyte mitosis and led to heart repair after MI[168] (TABLE 1). In addition, an increase in cardiac mechanical load after birth has been proposed to activate cardiac mitochondrial biogenesis, thereby adapting the heart to the increase in energetic demand[169]. Remarkably, mechanical unloading after implantation of LVADs in the failing human heart caused a decrease in mitochondrial content and a reduction of the DDR, with signs of cardiomyocyte proliferation[170]. These findings suggest a novel therapeutic approach for heart regeneration whereby the proliferative capacity of cardiomyocytes that is silenced in the adult mammalian heart can be reawakened by environmental adjustments rather than by directly manipulating specific signalling pathways in cardiomyocytes.

A study analysing the transcriptome and the chromatin landscape in neonatal and adult mouse hearts also revealed that noncardiomyocytes activate a distinct injury-induced transcriptional programme in response to MI[171]. Another report demonstrated an apparent difference in the immune response after MI between newborn mice at day 1 and at week 2 (REF[172]). Additionally, depletion of macrophages abolished the early neonatal cardiac

regenerative capacity, and this regenerative deficiency was associated with a defect in angiogenesis[172]. Future detailed analyses of the epigenetic landscape and the transcriptome in neonatal and adult mouse hearts will define the regulatory networks that contribute to heart regeneration.

Another novel approach to consider for the treatment of heart diseases that are caused by specific monogenic mutations is genome editing. Discovery and engineering of CRISPR technology has advanced the field by offering the possibility of genome editing targeted by only one guide RNA[173,174]. For example, Duchenne muscular dystrophy, an X-linked recessive monogenic disease caused by mutations in the dystrophin gene *(DMD),* is a severe progressive muscle disease that causes premature death usually in the mid-twenties owing to cardiac and respiratory failure[175]. In vivo genome editing with the CRISPR- Cas9 system restored muscle function by correcting the *Dmd* mutation in a mouse model of Duchenne muscular dystrophy in both the germline and the postnatal stage[176–180]. In a proof-of-principle study, genome editing technology also corrected mutations in human iPSC- derived cardiomyocytes from patients with Duchenne muscular dystrophy[181–185]. Corrected iPSC-derived cardiomyocytes had restored dystrophin expression and improved cardiomyocyte functional performance in vitro, such as improved contraction and suppression of arrhythmia (FIG. 5). Given the myriad mutations in cardiac structural and contractile protein genes that cause cardiomyopathy in humans, gene editing is an attractive means of eliminating such disease-causing mutations. Therefore, CRISPR technology has great potential for innovative therapies to treat heart disease[186].

Genome editing could potentially be adapted for heart regenerative therapies. Genetically engineered stem cells have been reported to have improved cardioprotective effects[187,188]. Priming stem cells with site-targeted modification with CRISPR technology before transplantation will reduce the risk of undesired insertional mutagenesis. This technology also opens new avenues to xenotransplantation. Xenografts could be genome-edited to silence unfavourable xenogeneic genes, which potentially could reduce the risk of immune rejection and infection after cell transplantation[189,190]. Additionally, CRISPR technology can be modified to regulate endogenous gene expression by using a cata- lytically inactive form of Cas9 (dCas9)[191,192]. It will be interesting to see whether direct cardiac reprogramming can be achieved by activating endogenous gene expression with the use of the CRISPR-dCas9 system, which has been successfully reported in the reprogramming of other cell lineages[193,194]. However, much work remains in addressing major issues before clinical application, such as off-target mutagenesis and unstable editing efficiency, as well as establishing feasible delivery methods and determining the therapeutic target population.

Challenges for clinical application.

How can we account for the disparity between the outcomes in preclinical studies of cardiac regeneration and the clinical trials of cardiac regeneration in humans? One explanation could be an insufficient delivery of therapeutic factors to the target sites, which prevents the therapeutic thresholds needed for cardiac regeneration from being reached when translated to clinical settings. To address this issue, biomaterials have attracted attention for their capacity to serve as a matrix to improve graft survival and behaviour, as well as to protect

the therapeutic factors from degradation and to act as a reservoir for sustained local delivery of therapeutic factors. Biomaterial-based delivery systems thus provide a scaffold to improve the therapeutic effects of both cellular and noncellular approaches[79,102,195–197]. Furthermore, tissue-engineering technologies have allowed the building of myocardial patches and sheets and 3D heart tissues recapitulating in vitro the complex structure of heart tissue. Engineered heart tissues can be directly transplanted to the damaged heart and have been shown to improve therapeutic outcomes[198–201]. Meanwhile, the number of deliveries of the therapeutic factor might also be important, because repetitive delivery of stem cells was shown to improve therapeutic outcomes compared with a single delivery[202]. Therefore, optimizing the delivery protocol in preclinical settings is necessary to advance the potential of therapeutic factors to clinical trials.

Another challenge in the process of clinical application of regenerative therapies is the limitations of current animal models (BOX 1). Typically, proof-of-principle studies are first performed in fish or rodent models and then repeated in larger animal models to test the feasibility, safety, and efficacy of the approach[203]. Although animal models undoubtedly have a pivotal role in translational medicine, differences between species, including the molecular basis of disease, anatomy, and physiology, might account for translational failures. In other words, no universally ideal animal model for preclinical studies is available, and for each purpose and use, an appropriate model should be selected. Additionally, preclinical studies tend to have weak statistical power because rigorously designed, large-scale studies require major financial investments. The lack of sufficient scientific rigour of clinical trials of cardiac regeneration stands as a major challenge for clinical application of cardiac regenerative therapies, because positive outcomes in initial clinical trials, which tended to be performed on a small scale, were often not reproduced in later, well-randomized, large-scale clinical trials. Altogether, study designs in preclinical settings require further refinements, and establishing a more feasible translational path could reduce the risk of clinical translational failure[204].

Conclusions

In conclusion, new discoveries and promising preclinical outcomes in the field of cardiac regeneration give us optimism regarding the establishment of novel effective regenerative therapies for the failing human heart. However, considering the disappointing results of previous clinical trials of cardiac regenerative therapies, we must acknowledge and address the limitations of preclinical studies of cardiac regeneration and the difficulties of clinical translation. In addition, mammalian heart development is a complex and precisely spatiotemporally orchestrated process[205]. Therefore, repairing the human heart will probably require a combination of multiple therapeutic approaches.

Acknowledgements

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Box 1 | Research models for translational medicine in heart regeneration

Representative research models in the flow of translational research in heart regeneration are shown in the figure.

Fish

Fish such as zebrafish are widely used model organisms in the early phase of translational research. High fertility rates, short gestation period, short lifespan, and low cost of maintenance make fish a suitable model organism for high-throughput studies using genetic models of heart disease[206]. However, fish have substantial differences compared with mammals in heart structure, having only two chambers and maintaining regenerative capacity throughout their lifetime. These properties make fish an invalid model to confirm efficacy of therapeutic approaches for human heart regeneration.

Small animals

Mammalian model organisms are phylogenetically, anatomically, and physiologically closer to humans, including the heart structure and the minimal regenerative capacity in the adult heart. Among mammals, rodents are one of the most popular model organisms to study heart regeneration. The short gestation period and lifespan of rodents, associated with well-understood molecular mechanisms of the cardiac regulatory networks, make these animal models suitable for proof-of-principle studies using genetically modified animals[207]. A fairly low cost of maintenance and the convenience of easy handling enable testing the reproducibility of regenerative approaches before proceeding to further studies. Nonetheless, rodents are substantially different from humans in many ways, including body size, cardiac output, and contractile proteins, and have a relatively homogenous genetic background; therefore, therapeutic approaches tested in rodent models require an additional confirmatory step before clinical application[208].

Large animals

Large animals such as pigs, dogs, and sheep are considered to be the bridge between small-animal studies and clinical trials. Relatively similar to humans in body size and cardiac physiology, large-animal models enable testing the feasibility, efficacy, and long-term safety of therapeutic approaches for heart regeneration[203]. However, major disadvantages of large animals include the high cost of maintenance and the long life cycle compared with small animals. In addition, obtaining approval to use some species for research purposes might be difficult. These factors hinder the design of large-animal studies at a large scale with adequate numbers to confirm reproducibility.

Nonhuman primates

Nonhuman primates are distinct from other large-animal models because these animals are phylogenetically closer to humans and their immune system has been well studied in translational medicine[209]. These features give nonhuman primate models an important role in studies of allogeneic stem cell transplantation, which is considered to be one of the main therapeutic approaches for heart regeneration[210].

Human samples

Alternative preclinical models involve the use of human ex vivo cultured cardiac tissues and human pluripotent stem cell (PSC)-derived cardiomyocytes, which theoretically should be suitable for performing in vitro proof-of-principle studies[211].
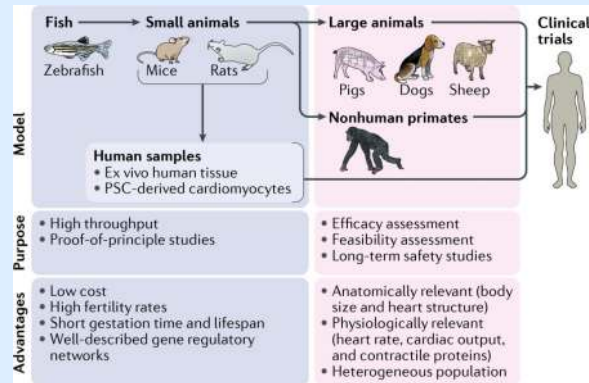
Key points

- Preclinical outcomes of cardiac regenerative therapy approaches have not translated effectively to clinical trials.

- Transplantation of induced pluripotent stem cell-derived cardiomyocytes for cardiac repair has encountered problems related to safety and low engraftment rates.

- Cell-free-based approaches for heart repair and regeneration involve cardioprotective secretory factors or direct reprogramming of resident cardiac fibroblasts to cardiomyocyte-like cells.

- Endogenous cardiomyocyte proliferation can be evoked by modulating cell cycle regulators, the Hippo signalling pathway, and the cardiac microenvironment.

- Genome editing can correct underlying mutations causing heart disease in animals and offers a state-of-the-art therapeutic approach for cardiac repair.

- The therapeutic potential of cardiac regeneration approaches can be improved by optimizing the delivery method of the therapeutic factors.

Left ventricular assist devices

(LVADs). Electromechanical devices to support circulation of a failing heart.

Cardiac resynchronization therapy

Therapy that uses an electromechanical device to resynchronize ventricular contraction in patients with heart failure.

Lineage tracing

A method to identify all progeny originating from a single cell.

MerCreMer

A fusion protein containing Cre recombinase flanked at both ends with a mutated murine oestrogen receptor (Mer) ligand binding domain. MerCreMer generates an inducible Cre recombinase activation system that can gain access to the nuclear compartment only with exposure to tamoxifen.

Hydrogels

Colloid gels composed of a network of hydrophilic polymer chains.

Paracrine effects

The effects on a cell that are induced by secreted factors from another cell

Synthetic modified RNA

Chemically synthesized RNA with changes to the chemical composition that alter function or stability of the RNA.

Xenotransplantation

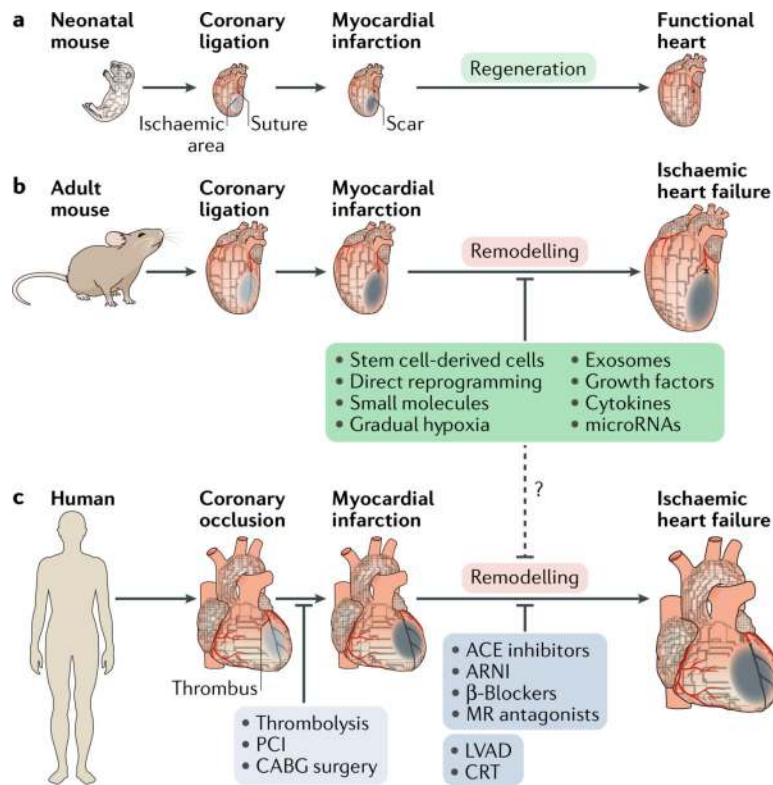Cell, tissue, or organ transplantation across different species.

Fig. 1 |. Response and therapeutic approaches to myocardial injury.
a | Response tomyocardial injury differs between developmental stages in mice. Neonatal mice (aged <1 week) are capable of regenerating the heart, with functional recovery after injury.This regenerative capacity is lost postnatally after the first week. **b,c** | In adult mice and humans, the default response to myocardial injury is fibrosis, where the infarct necrotic tissue is replaced with a fibrotic scar, causing loss of cardiac contractility. The damaged adult heart enters a negative loop of cardiac remodelling that progresses to heart failure. In humans, the current goal of clinical therapies is either to salvage the ischaemic myocardium by early revascularization (light grey box) or to prevent cardiac remodelling with drug therapy and electromechanical support (dark grey box). Accumulating evidence in preclinical studies demonstrates promising outcomes with therapeutic approaches aimed at heart regeneration (green box), although these new approaches have clinical translational problems. The dashed line indicates potential clinical therapeutic approaches. ACE, angiotensin- converting enzyme; ARNI, angiotensin receptor–neprilysin inhibitor ; CRT, cardiac resynchronization therapy; LVAD, left ventricular assist device; MR, mineralocorticoid receptor ; PCI, percutaneous coronary intervention.
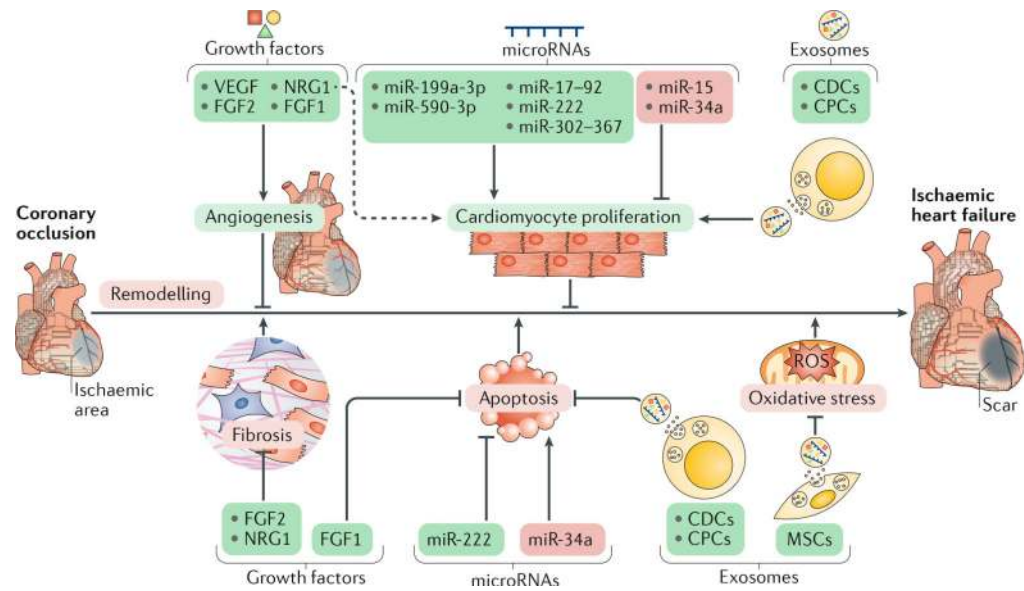
Fig. 2 |. Contributions of secretory factors to cardiac repair and regeneration.
A scheme of cardioprotective effects (green boxes) or cardiac remodelling effects (red boxes) by representative secretory factors (growth factors, microRNAs, and exosomes) is shown. Various secretory factors promote angiogenesis or cardiomyocyte proliferation, thereby promoting cardiac repair. Other secretory factors elicit cardioprotective effects by attenuating cardiac remodelling through inhibition of fibrosis, cardiomyocyte apoptosis, and oxidative stress. The dashed arrow indicates incompletely understood mechanisms. CDC, cardiosphere-derived cell; CPC, cardiac progenitor cell; FGF, fibroblast growth factor; MSC, mesenchymal stem cell; NRG1, neuregulin 1; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor.
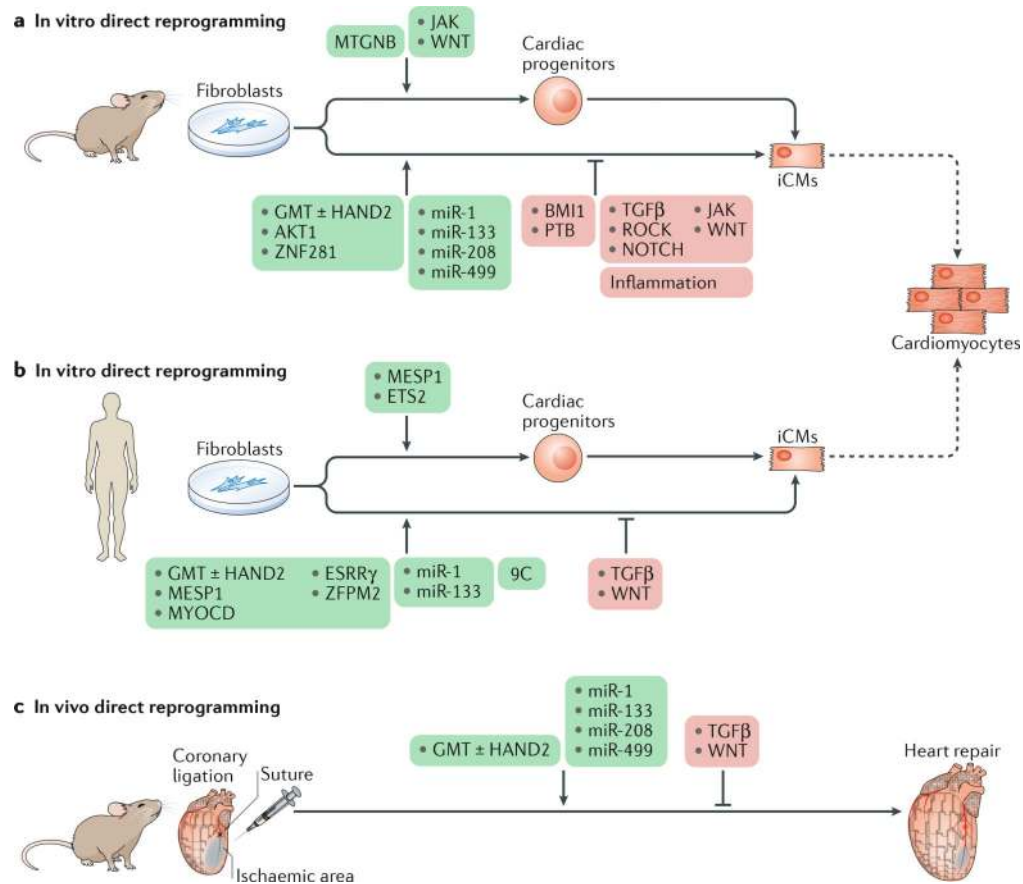
Fig. 3 |. Direct reprogramming of fibroblasts into cardiomyocytes.
a | Forced expression of cardiac transcription factors or myogenic microRNAs directly reprogrammes mouse fibroblasts to induced cardiomyocyte-like cells (iCMs) or cardiac progenitors. As iCMs are functionally and structurally different from endogenous cardiomyocytes, studies have aimed to improve the efficiency and quality of reprogrammed iCMs by adding factors (green box) or blocking the transdifferentiation barriers (red box) in vitro. **b** | In humans, the cardiac reprogramming factors are different from the factors used in mouse ceüs in vitro. However, inhibition of transforming growth factor-β (TGFβ) and WNT signalling pathways enhances reprogramming in both mouse and human cells. **c** | The reprogramming cocktails determined in vitro can reprogramme resident cardiac fibroblasts in mice in vivo. The dashed arrows indicate differentiation potential. 9C, CHIR99021, A83–01, BIX01294, AS8351, SC1, Y27632, OAC2, SU16F, and JNJ10198409; AKT1, RAC-α serine/threonine-protein kinase; BMI1, Polycomb complex protein BMI1; ESRRγ, oestrogen-related receptor-γ; GMT, transcription factor GATA4, myocyte-specific enhancer factor 2C (MEF2C), and T-box transcription factor TBX5; HAND2, heart and neural crest derivatives-expressed protein 2; JAK, Janus kinase; MESP1, mesoderm posterior protein 1; MTGNB, MESP1, TBX5, GATA4, homeobox protein NKX2–5, and BRG1-associated factor 60C (BAF60C; also known as SMARCD3); MYOCD, myocardin; PTB, polypyrimidine tract-binding protein 1; ROCK, RHO-associated protein kinase 1.
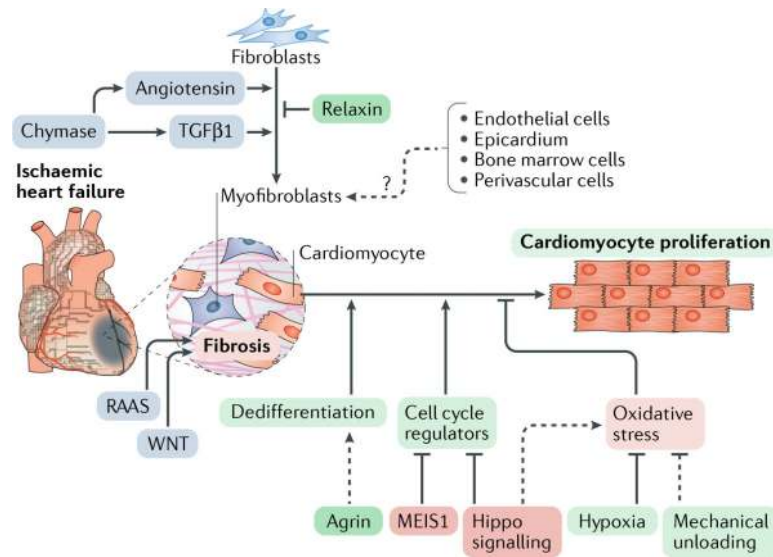
Fig. 4 |. Approaches to stimulate endogenous regenerative capacity for heart repair.
Approaches targeting endogenous cardiac regeneration (green boxes) involve activating the proliferation of endogenous cardiomyocytes and targeting the cardiac fibrotic response. Cardiomyocyte proliferation can be induced by overexpressing cell cycle-related genes or by inhibiting cell cycle suppressors such as the transcription factor homeobox protein MEIS1 or the Hippo signalling pathway (red boxes). Alternatively, approaches to mimic the neonatal cardiac environment by exposure to hypoxia or mechanical unloading or by providing the extracellular matrix protein agrin also evoke cardiomyocyte proliferation. Transforming growth factor-β1 (TGFβ1) and angiotensin have a pivotal role in inducing cardiac fibroblast differentiation into myofibroblasts during cardiac injury, thereby inducing cardiac fibrosis. Additionally, other cell lineages are proposed to transdifferentiate into myofibroblasts. The renin-angiotensin-aldosterone system (RAAS) and the WNT signalling pathway also contribute to cardiac fibrosis. These fibrotic processes (blue boxes) can be targeted to attenuate cardiac remodelling. The dashed arrows indicate incompletely understood mechanisms.
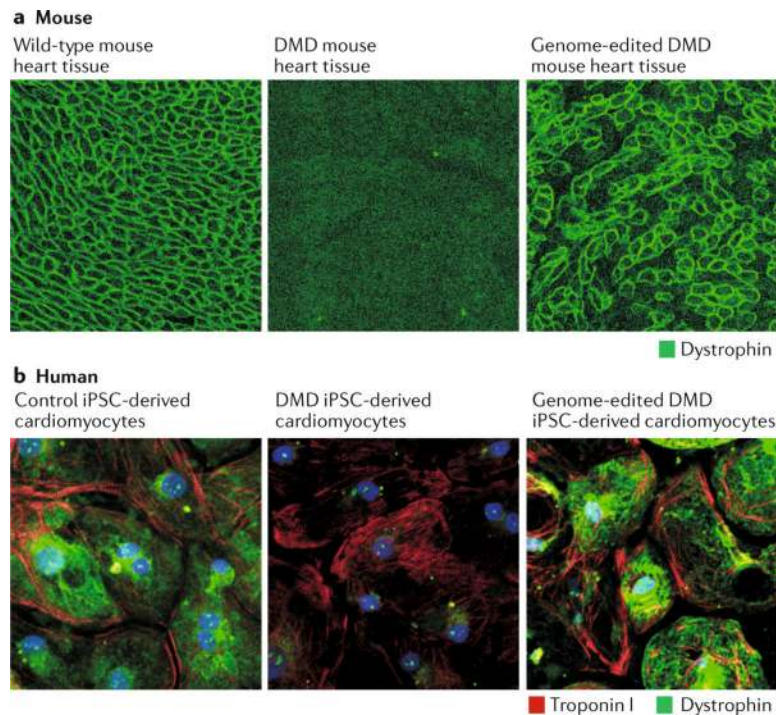
Fig. 5 |. Genome editing as a therapeutic approach to heart disease.
Genome editing offers the possibility of correcting mutations postnatally in congenital muscle diseases, such as Duchenne muscular dystrophy (DMD), to restore muscle function. DMD is caused by mutations in the dystrophin gene *(DMD)* that lead to abnormalities in the production of dystrophin protein and are associated with premature death owing to cardiac and respiratory failure. The CRISPR-Cas9 system has been successfully used to correct *Dmd* mutations and restore the expression of dystrophin in a mouse model of DMD. **a** | The panels show heart sections from a wild-type mouse, a DMD mouse model, and a CRISPR-Cas9-edited DMD mouse model. Tissues are immunostained with an antibody for dystrophin (green). **b** | The panels show induced pluripotent stem cell (iPSC)-derived cardiomyocytes from a healthy person (control) and from a patient with DMD, and iPSCs from a patient with DMD that were edited with CRISPR-Cas9 to correct the *DMD* mutation. Cells were immunostained with antibodies for dystrophin (green) and troponin I (red). Adapted with permission from REF[180], AAAS.

Table 1 |

Cardiac regenerative therapies in preclinical studies

Therapeutic source	Animal model and age	Cohort (n)	Disease model	Delivery method	Cardiac function recovery versus control; assessment method	Comments	Ref.
Cardiac stem cells							
Autologous CDCs (1 X 10^7)	• Pig • Adult	• C = 11 • T = 10	• IHF • MI	• Intramyocardial • 4 weeks after MI	• LVEF improvement (~7%) at 8 weeks • Ventriculography	*LacZ*$^+$ CDCs detected in the peri-infarct zone with the use of cardiac marker expression	56
Autologous KIT$^+$ CSCs (5 X 10^5)	• Pig • 8–10 weeks	• C = 10 • T = 11	• IHF • IR 90 min	• Intracoronary • 3–4 months after IR	• LVEF improvement (8–10%) at 8 weeks • Echocardiography	EGFP-labelled CSC- derived cells detected in the infarct zone with the use of cardiac marker expression	57
Pluripotent stem cells							
Human ESC- CMs (~1 X 10^9)	• Macaque • 5–14 years	• C = 2 • T=4	• IHF • IR 90 min	• Intramyocardial • 14 days after MI	• No significant change in LVEF • Echocardiography	• Proof-of-principle study • GFP-labelled human ESC-CMs detected in the peri-infarct zone • Electromechanical integration with host myocardium • All animals receiving cell transplants had ventricular arrhythmias	69
Allogeneic iPSC- CMs (4 X 10^8)	• Macaque • 4–5 years	• C = 5 • T = 5	• IHF • MI	• Intramyocardial • 14 days after MI	• LVEF improvement (~10%) at 12 weeks • CT imaging	• GFP-labelled iPSC-CMs detected in the peri- infarct zone • Electromechanical integration with host myocardium • All animals receiving cell transplants had ventricular arrhythmias	74
Secretory factors							
• NRG1-MPs • FGF1-MPs	• Pig • 12–24 months	• C = 6 • T (NRG1-MPs) = 5 • T (FGF1-MPs) = 6	• IHF • IR 120 min	• Intramyocardial • 1 week after MI	• LVFS improvement (~9%) at 3 months • Echocardiography	• Increase in vascularization with NRG1-MP and FGF1-MP treatment • Reduced fibrosis with NRG1-MP treatment	91
• miR-199a-3p • miR-590–3p	• Mouse • 2 months	• C = 13 • T (miR-199a-3p) = 20 • T (miR-590–3p) = 13	• IHF • MI	• Intramyocardial • After coronary ligation (with cationic lipid formulations)	• LVEF improvement (10–20%) at 8 weeks • Echocardiography	Increased numbers in the peri-infarct zone of CMs positive for the DNA synthesis marker EdU	97

Therapeutic source	Animal model and age	Cohort (n)	Disease model	Delivery method	Cardiac function recovery versus control; assessment method	Comments	Ref.
Human CDC-derived exosomes ($\sim16.5 \times 10^{11}$)	• Pig • Adult	• C = 6 • T = 6	• IHF • MI	• Intramyocardial • 4 weeks after MI	• LVEF improvement ($\sim5\%$) at 1 month • MRI	• Reduced scar size • Increased numbers in the peri-infarct zone of CMs positive for the cell cycle active phase marker Ki67	[109]
Direct cardiacreprogramming							
Retroviral GMT	• Mouse • 2 months	• C = 9 • T = 9	• IHF • MI	• Intramyocardial • After coronary ligation	• LVEF improvement ($\sim10\%$) at 12 weeks • MRI	• Reduced scar size • Fibroblast lineage- traced cells (*Fsp1*-Cre or *Postn*-Cre) expressing cardiac markers in the peri-infarct zone	[135]
Retroviral GHMT	• Mouse • 8–10 weeks	• C = 9 • T = 10	• IHF • MI	• Intramyocardial • After coronary ligation	• LVEF improvement ($\sim25\%$) at 12 weeks • MRI	• Reduced scar size • Fibroblast lineage- traced cells (*Tcf21*-Cre) expressing cardiac markers in the peri-infarct zone	[18]
Stimulation of endogenous cardiac repair							
Agrin	• Mouse • 12 weeks	• C = 7 • T = 8	• IHF • MI	• Intramyocardial • After coronary ligation	• LVEF improvement ($\sim10\%$) at 5 weeks • Echocardiography	• Reduced scar size • Increased numbers in the peri-infarct zone of CMs positive for the proliferation markers Ki67, BrdU, and AURKB	[166]
Systemic exposure to hypoxia	• Mouse • 2 months	• C = 9 • T = 9	• IHF • MI	• Gradual hypoxia induction reaching 7% O_2 level for 2 weeks • 1 week after MI	• LVEF improvement ($\sim20\%$) at ~6 weeks • Echocardiography	• Reduced scar size • Increased numbers of BrdU$^+$ CMs, PHH3$^+$ CMs, and AURKB$^+$ CMs, mainly in the MI remote zone	[168]

AURKB, aurora kinase B; BrdU, 5-bromodeoxyuridine; C, control; CDC, cardiosphere-derived cell; CM, cardiomyocyte; CSC, cardiac stem cell; EdU, 5-ethynyl-2'-deoxyuridine; EGFP, enhanced green fluorescent protein; ESC, embryonic stem cell; FGF1, fibroblast growth factor 1; GFP, green fluorescent protein; GMT, *Gata4, Mef2c,* and *Tbx5*; GHMT, *Gata4, Hand2, Mef2c,* and *Tbx5*; IHF, ischaemic heart failure; iPSC, induced pluripotent stem cell; IR, ischaemia-reperfusion; KIT, mast/stem cell growth factor receptor KIT; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; MI, myocardial infarction; MP, microparticle; NRG1, neuregulin 1; PHH3, phosphohistone H3; T, treatment.