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2	Article Sub- Title		
3	Article Copyright - Year	**Springer Science+Business Media, LLC, part of Springer Nature 2018 (This will be the copyright line in the final PDF)**	
4	Journal Name	Heart Failure Reviews	
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38	Schedule	Received
39		Revised
40		Accepted

41	Abstract	Ischemic cardiomyopathy is the cardiovascular condition with the highest impact on the Western population. In mammals (humans included), prolonged ischemia in the ventricular walls causes the death of cardiomyocytes (myocardial infarction, MI). The loss of myocardial mass is soon compensated by the formation of a reparative, non-contractile fibrotic scar that ultimately affects heart performance. Despite the enormous clinical relevance of MI, no effective therapy is available for the long-term treatment of this condition. Moreover, since the human heart is not able to undergo spontaneous regeneration, many researchers aim at designing cell-based therapies that allow for the substitution of dead cardiomyocytes by new, functional ones. So far, the majority of such strategies rely on the injection of different progenitor/stem cells to the infarcted heart. These cardiovascular progenitors, which are expected to differentiate into cardiomyocytes de novo, seldom give rise to new cardiac muscle. In this context, the most important challenge in the field is to fully disclose the molecular and cellular mechanisms that could promote active myocardial regeneration after cardiac damage. Accordingly, we suggest that such strategy should be inspired by the unique regenerative and reparative responses displayed by non-human animal models, from the restricted postnatal myocardial regeneration abilities of the murine heart to the full ventricular regeneration of some bony fishes (e.g., zebrafish). In this review article, we will discuss on current scientific approaches to study cardiac reparative and regenerative phenomena using animal models.

42	Keywords separated by ' - '	Myocardial infarction - Cell-based therapies - Tissue regeneration - Tissue repair - Animal models

43	Foot note information	

Heart Failure Reviews
https://doi.org/10.1007/s10741-018-9750-8

Cell based therapies for the treatment of myocardial infarction: lessons from cardiac regeneration and repair mechanisms in non human vertebrates

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Abstract

Ischemic cardiomyopathy is the cardiovascular condition with the highest impact on the Western population. In mammals humans included), prolonged ischemia in the ventricular walls causes the death of cardiomyocytes myocardial infarction, MI). The loss of myocardial mass is soon compensated by the formation of a reparative, non-contractile fibrotic scar that ultimately affects heart performance. Despite the enormous clinical relevance of MI, no effective therapy is available for the long-term treatment of this condition. Moreover, since the human heart is not able to undergo spontaneous regeneration, many researchers aim at designing cell-based therapies that allow for the substitution of dead cardiomyocytes by new, functional ones. So far, the majority of such strategies rely on the injection of different progenitor/stem cells to the infarcted heart. These cardiovascular progenitors, which are expected to differentiate into cardiomyocytes de novo, seldom give rise to new cardiac muscle. In this context, the most important challenge in the field is to fully disclose the molecular and cellular mechanisms that could promote active myocardial regeneration after cardiac damage. Accordingly, we suggest that such strategy should be inspired by the unique regenerative and reparative responses displayed by non-human animal models, from the restricted postnatal myocardial regeneration abilities of the murine heart to the full ventricular regeneration of some bony fishes e.g., zebrafish). In this review article, we will discuss on current scientific approaches to study cardiac reparative and regenerative phenomena using animal models.

Keywords Myocardial infarction · Cell-based therapies · Tissue regeneration · Tissue repair · Animal models

Introduction

Cardiovascular diseases kill more people every year than cancer http://www.who.int/classifications/icd). Among cardiovascular conditions, cardiac ischemic disease is the one with the highest prevalence in Western populations [1] see also Fig. 1). Cardiac ischemia is the most frequent cause of myocardial infarction MI). From a pathophysiological perspective, MI is characterized by the loss of myocardium after sustained oxygen deprivation. Such ischemic episodes are linked to coronary flow obstructive events related to coronary artery atherosclerosis [2].

Unless other vertebrates, mammals including humans) cannot compensate for the loss of heart muscle, and therefore show very little cardiac regenerative ability. Instead of regenerating, the injured heart substitutes the dead myocardium by fibrous connective tissue secreted by activated cardiac fibroblasts myofibroblasts). This reparative, non-contractile fibrosis, which prevents cardiac wall rupture, soon transforms into a reactive fibrosis that continuously expands at the expense of the surviving myocardium. Post-MI scar ultimately alters cardiac function, leading to heart failure [3]. Despite the many advances in the field of pharmacotherapy including the design of new thrombolytic, beta-blocker, or antiarrhythmic drugs), the continuous surgical improvements and the use of ventricular assist devices catheters, pacemakers, implantable defibrillators, artificial valves and stents) [4], the mortality and morbidity rates associated to MI continue growing health costs for cardiac ischemic disease in the EU are estimated to

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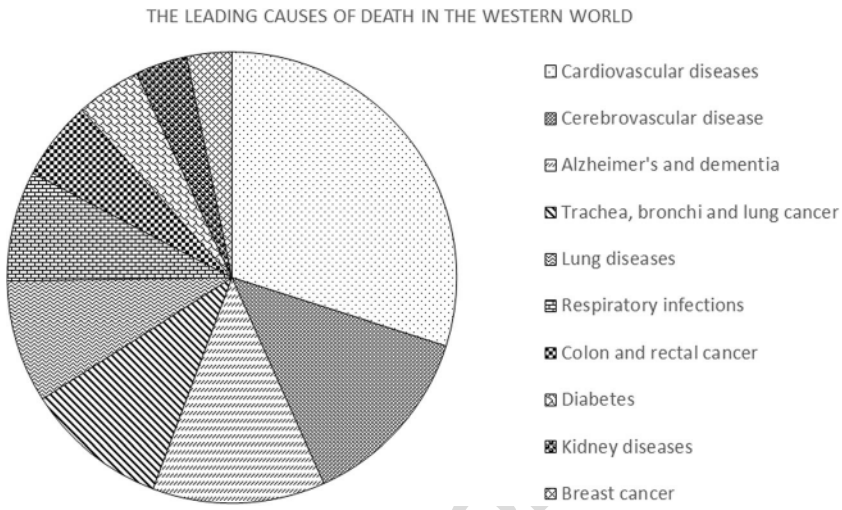

Fig. 1 Leading causes of death in the Western World from World Health Organization, 2015). Modified from http://www.who.int/classifications/icd

be 111.000 10^6€ per year, see http://www.ehnheart.org/). Both the increasing incidence of MI and its chronification in many patients urge for the development of alternative cardiac therapies to treat the infarcted heart [5].

Post MI ventricular remodeling disrupts heart performance

Heart responses to myocardial death are first triggered by cardiac macrophage-induced local inflammation. This primary inflammation is soon amplified through the recruitment of blood-circulating cells to the injured heart, first granulocytes and then monocytes [6]. These latter cells infiltrate into the ventricular walls to eliminate dead cardiomyocytes but also initiate a reparative response that depends on the recruitment of cardiac resident fibroblast progenitors to the damage site [7]. These activated fibroblasts myofibroblasts) synthetize large amounts of extracellular matrix proteins, forming a non-contractile fibrous tissue that progressively transforms into a poorly cellularized stiff scar. All these post-MI phenomena are jointly referred to as "ventricular remodeling" and are responsible for the loss of ventricular wall contractility.

Human heart repairs but does not regenerate

As discussed above, the formation of a non-contractile fibrous tissue in the infarcted human heart prevents ventricular wall rupture and cardiac tamponade. This characteristic substitution of a damaged tissue by another one displaying different functional properties is called "repair." On the contrary, the substitution of a damaged tissue by the same one is known as "regeneration." Regenerative phenomena are tightly regulated by complex molecular mechanisms. Unfortunately, the terms "repair" and "regeneration" are often used interchangeably, and although they can occur in the same organ, these two processes should be considered different biological entities.

Repair events based in the massive deposition of fibrotic extracellular matrix are a common animal tissue response to damage. In humans, these reparative responses can become clinically relevant when an organ cannot undergo regeneration. The adult mammalian heart is the perfect example of an organ lacking the ability to regenerate spontaneously. However, some experimental studies have shown that during a short postnatal temporal window around a week after birth), the murine heart can undergo regeneration [8]. We do not know whether such intrinsic regenerative ability is also present in the human postnatal heart. Relevant to this discussion, it is important to mention that other researchers have reported data on the existence of different populations of adult cardiac resident stem cells CSCs) with the ability of differentiating into cardiovascular cell types [9, 10]. Taken together, all these findings strongly suggest that the mammalian heart has an intrinsic regeneration potential that, for unknown reasons, is not effectively displayed after a severe injury. Conducting research on this specific topic may be an excellent strategy to discover new therapies to regenerate the injured heart.

Cardiac regeneration in different non human vertebrates

The ability of regenerating tissues and organs can be present or absent in organisms of the same supraspecific taxon [11]. Remarkably, while anamniote vertebrate fishes and amphibians) tissues have a relatively high regenerative capacity [12, 13], other groups of amniote vertebrates reptilians, avians, and mammals) have a limited regenerative potential [14]. We believe that a comparative analysis of the regenerative properties of cardiac tissues in different animal models will allow for the identification of relevant similarities and

differences in the cellular and molecular motifs that control cardiac regeneration.

namniote vertebrates

Recent studies have shown that different species of anamniote vertebrates such as the goldfish [15], the zebrafish [16, 17], the axolotl [18], or the newt [19] regenerate their cardiac ventricle after injury. In particular, the zebrafish, which is the most studied animal model in the field of cardiac regeneration, cardiomyocyte de-differentiation [20], and proliferation [17] are both involved in ventricular chamber regeneration and functional restoration. The formation of a blastema-like structure a rather undifferentiated, proliferative mass of mesenchyme) is instrumental to zebrafish myocardial regeneration [21]. Moreover, zebrafish cardiomyocyte proliferation is tightly related with the mononucleated, diploid status of adult cardiac myocytes [22]. This ability is also present in other cyprinid teleostei like the goldfish [15] but seems to have been lost in other species such as medaka [23], whose main response to apical ventricular resection is extensive fibrosis of the heart walls.

Despite the reported regenerative ability of adult amphibian tissues [24–27], amphibian heart responses to induced damage are not still well understood, and certain amount of controversy remains on whether all amphibians have the same regeneration abilities. It has been recently shown that different anuran species frogs and toads) display different cardiac responses to damage. While *Xenopus tropicalis* heart is able to regenerate after an endoscopy-based ventricular resection [28], *Xenopus laevis* fails to do so after suffering a similar loss of myocardial mass [29]. These differences can be explained by the specific methods applied to create the experimental insult [30], being the wound size a determinant feature for the initiation of the regeneration process, as shown to be also the case in the zebrafish [31]. In addition, it has been shown that external factors as environmental conditions and age could influence the regenerative properties of both the zebrafish [32] and the anuran [31] heart. Furthermore, it is not clear whether regeneration is a common response of urodeles salamanders and newts) to cardiac injury. Although the axolotl *mbystoma*) ventricle can fully regenerate without developing a scarring process [18, 33, 34], other species seem to be less prone to regeneration. Indeed, the adult myocardium of *Notphthalmus* increases cardiomyocyte proliferation at the wound area after an apical amputation of the ventricle [35], but myocardial regeneration is never completed in these organisms and the prevalent response to damage remains fibrotic repair [36].

Mammals

Several studies have shown that neonatal mice submitted to apical ventricular resection can also regenerate the myocardium during the first week after birth [8, 37] Fig. 2). This suggests that the cardiac regenerative ability of mammals progressively decreases from embryonic stages to adult life. This process implies an increase of cardiomyocyte proliferation rate combined with a marked reduction of cardiac fibrosis [38].

It is also obvious that the regenerative potential of the neonatal mouse heart depends on the extent of the damage, since a complete tissue regeneration does not occur after an experimental transmural cryodamage i.e., an injury affecting the whole ventricular wall thickness) [39]. On the other hand, despite the high proliferation of some adult anamniote vertebrate tissues including the myocardium), the regenerative properties of such tissues decrease in elderly animals, probably due to the aging of the cellular component of the regenerative processes [40].

In addition to the proliferative activity of cardiac tissues after myocardial infarction, it has also been argued that the presence of different populations of cardiac resident cells expressing stem cell markers is an evidence for the regenerative potential of the adult mammalian heart. Many of these cells are supposed to be real cardiac stem cells CSCs); the majority of CSCs express the c-Kit receptor, although they also express other markers like Sca-1 [9, 41]. Interestingly, c-Kit$^+$ cells play a fundamental role in the early regenerative capacity of the neonatal but not the adult heart) mouse heart, most probably through a paracrine mechanism that still needs to be characterized in detail [42].

Can we thus conclude that the mammalian heart has an endogenous regenerative potential, which is lost or actively repressed during postnatal life? This is a difficult question to answer, but all the evidences we have discussed suggest that this is the case. In this regard, a specific mouse strain MRL line) can completely regenerate different damaged adult tissues, including the heart [43, 44]. MRL CSC populations are different from those of other mice, and their control of cell cycle is also different. Moreover, MRL mice do not produce significant scar tissue after myocardial damage. We should thus conclude that 1) the murine genetic background is relevant to the study of mammalian heart regeneration; 2) theoretically, there are no evolutionary constraints limiting mammalian heart regeneration Fig. 2).

Cardiac vertebrate regeneration: biomedical implications

The analysis of regenerative phenomena in different animal species has shown that there are two main regeneration modes in the Animal Kingdom, both of which seem to have been preserved throughout evolution in different animal lineages. These two kinds of regeneration were already described by T.H. Morgan in 1901 [45]. The first type of regeneration

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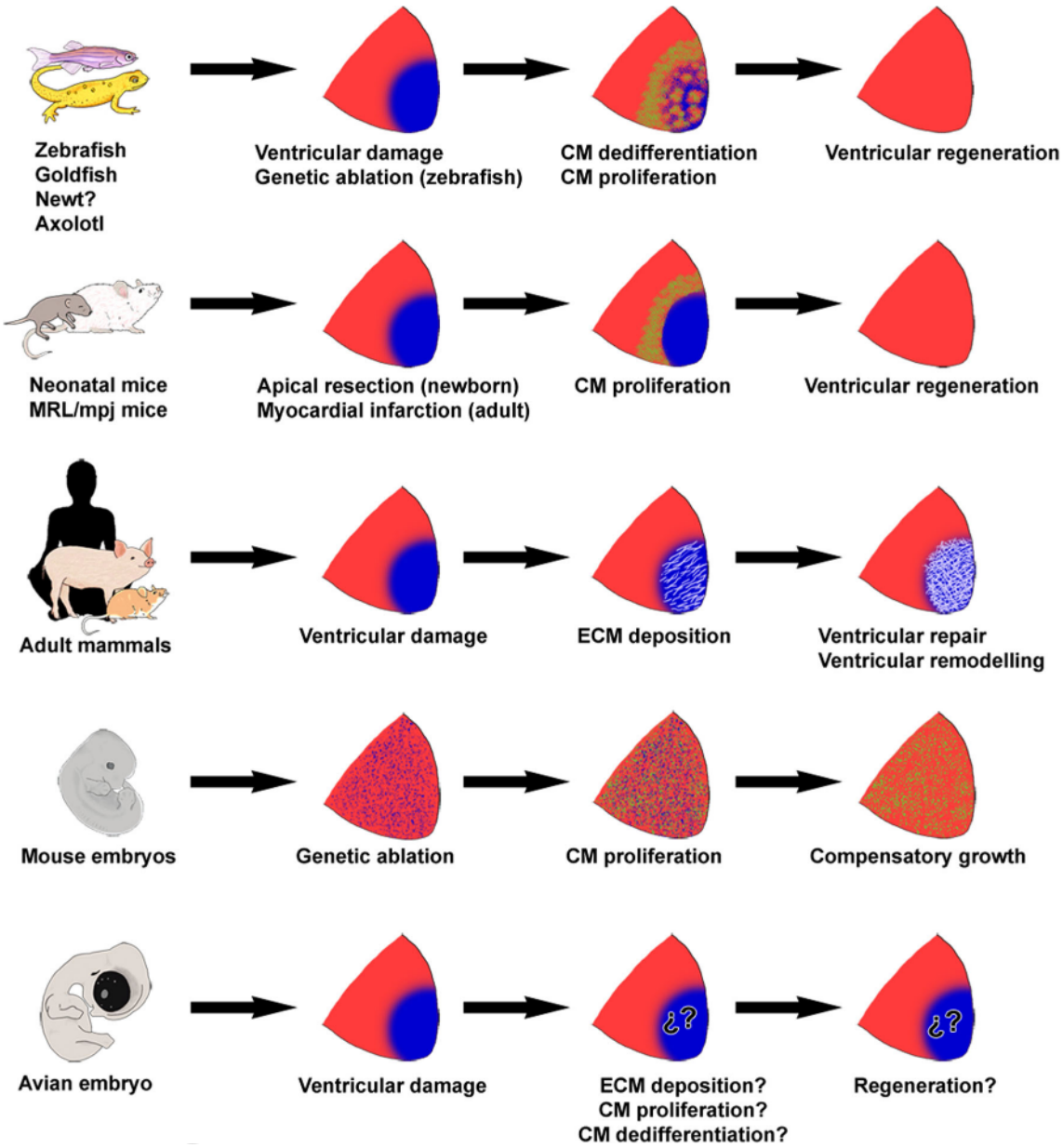

Fig. 2 Cardiac regeneration and repair in vertebrate phylogeny. Differences on the incidence of cardiac reparative or regenerative processes in different animal taxa are shown. Abbreviations: CM cardiomyocyte, ECM extracellular matrix

involves the increase of the proliferation rate of stromal cells adjacent to the damaged area, usually associated with the formation of a blastemal, i.e., a mass of highly proliferative, undifferentiated mesenchymal cells. This type of process, which is known as "epimorphic regeneration," is complex but can fully and efficiently regenerate sophisticated anatomical structures e.g., full limbs in salamander). The second type of regeneration implies a marked change in cell size, morphology, and spatial organization which is often linked to transdifferentiation phenomena direct differentiation of a given type of somatic cell into a different one). This process is known as "morphallactic regeneration" and is frequent in many invertebrate animals. We suggest that some characteristic responses of mammalian cells to pathologic stimuli e.g., cell hypertrophy) could result from the activation of mechanisms involved in morphallactic regeneration [46].

According to the criteria discussed above, cardiac regeneration in vertebrates such as the zebrafish or the newt can be ranked among epimorphic regeneration events [17, 21, 47, 48], being cell proliferation the key cellular feature of the regenerating tissue. In accordance with these findings, we can conclude that epimorphic regeneration cannot take place in the adult mammalian heart including the human one) because neither the low number of proliferating cardiomyocytes [9, 49] nor the absence of a true blastema support the formation of new cardiac tissue [50]. But, is parenchymal cell

proliferation the only factor we have to take into account when dissecting epimorphic organ regeneration? In the context of heart regeneration, the formation of a primary reparative fibrotic response following mammalian cardiac muscle death is likely to interfere with regeneration phenomena [51]. It is not known how anamniote vertebrates like the zebrafish manage to avoid the formation of such fibrotic scar, allowing for the full expansion of newly differentiated cardiomyocytes. However, it is tempting to hypothesize that differences between lower anamniote) and higher amniote) vertebrate proteolytic machineries could underlie this divergent vertebrate heart response to injury.

In addition to epimorphic and morphallactic regeneration, many animal tissues e.g., skin, blood) continuously renew some tissues as based in the presence of adult resident cells in various organs [52]. This process shares some features with classic epimorphic regeneration phenomena including the presence of highly proliferative cells in the tissue) but does not involve a blastema formation. In the case of the heart, different populations of resident cardiac stem cells CSCs) [9, 10, 53, 54] have been shown to be able to differentiate into cardiomyocytes, albeit at a very low rate [55]. The embryonic origin of these cells remains a mystery, and several cell sources including the bone marrow [56], embryonic cardiac progenitor fields [57], or the embryonic epicardium [58] have been suggested to be sources of CSC. The case of epicardial contribution to cardiac regeneration is indeed relevant, since many embryonic epicardially expressed genes are activated shortly after adult myocardial injury [48]. Recent studies have concluded that the epicardium and its derived cells do not materially contribute new cardiomyocytes to the regenerating heart, but rather secrete instructive molecules that promote cardiac regeneration and cell survival [59–61], also having an essential role in the revascularization of the wound [48]. However, further research is necessary to detail the specific roles played by this tissue in cardiac regeneration and repair. The activation of reparative fibrotic mechanisms after myocardial death seems to be the most important factor limiting cardiac regeneration, and some authors have suggested that this fibrosis interferes with CSC differentiation into new cardiomyocytes by disrupting their niche extracellular microenvironment) [62] and severely hampers CSC activation, expansion, and differentiation.

Lessons learnt from animal models regarding cardiac regeneration can be summarized as follows. First, effective cardiac regeneration exists in some animal vertebrate species, and these regeneration events depend on the ability of pre-existing myocardial tissue to generate new functional muscle. This means that, while the proliferative properties of adult higher vertebrate cardiac tissues are restricted to a low number of adult cardiomyocytes and CSC, adult cardiomyocyte proliferation in lower vertebrate animals is robust and can easily provide high numbers of new cardiomyocytes to the injured heart. Second, the fibrosis that develops after heart damage only persists in higher vertebrates, and it interferes with the replacement of dead muscle. Therefore, to minimize, post-MI fibrosis should be a major objective of novel therapeutic strategies aiming to treat the infarcted heart. Finally, the viability of any regenerative process in mammals depends on the efficacy of the neovascularization phenomena taking place in the regenerating tissues. To discover how to stabilize the primitive vascular structures that irrigate de novo formed cardiac tissues remains a scientific challenge for the field of cardiovascular regenerative medicine.

Experimental perspectives on cardiac response after damage: choosing an animal model

As it can be inferred from the previous discussion, the development of new, viable cell-based therapies to treat the infarcted heart must be grounded on extensive research using animal experimental models. Our laboratory, following a counterintuitive rationale, has been studying cardiac reparative and regenerative mechanisms in avian embryos. Our preliminary results show that the activation of cellular and molecular mechanisms related to both tissue repair e.g., fibrosis) and regeneration e.g., proliferation and differentiation of cardiomyocytes) are fully active in the embryonic heart. The choice of the mouse model to study adult cardiac responses to myocardial death is evident, but research on the embryonic developmental basis of mammalian cardiac repair and regeneration is challenging for various reasons. While mouse embryo genetic manipulation allows for the detailed study of cell lineage fate and the molecular control of cardiac morphogenesis, in vivo microsurgical manipulation and reincubation of the mammalian embryo does not seem an option. It is known that, in mammals, the loss of embryonic cardiomyocytes is quickly compensated by the proliferation of adjacent cardiomyocytes [63, 64]. This response, which might be reminiscent of standard regenerative processes, should be rather interpreted as the result of a compensatory growth of the developing tissue Fig. 2). Given the experimental limitations of performing research with mammalian embryos, we would like to suggest that research using the avian embryo that perfectly complements the work with mammalian ones, as *in ovo* microsurgical manipulation of the avian embryos, is an efficient experimental tool to produce experimental damage in avian embryo tissues that can then be combined with different methods to trace cell fate and differentiation Fig. 3) [65].

The promise of the new advanced therapies to repair the damaged heart

The development of efficient cell-based therapies is the main objective of clinical laboratories performing research

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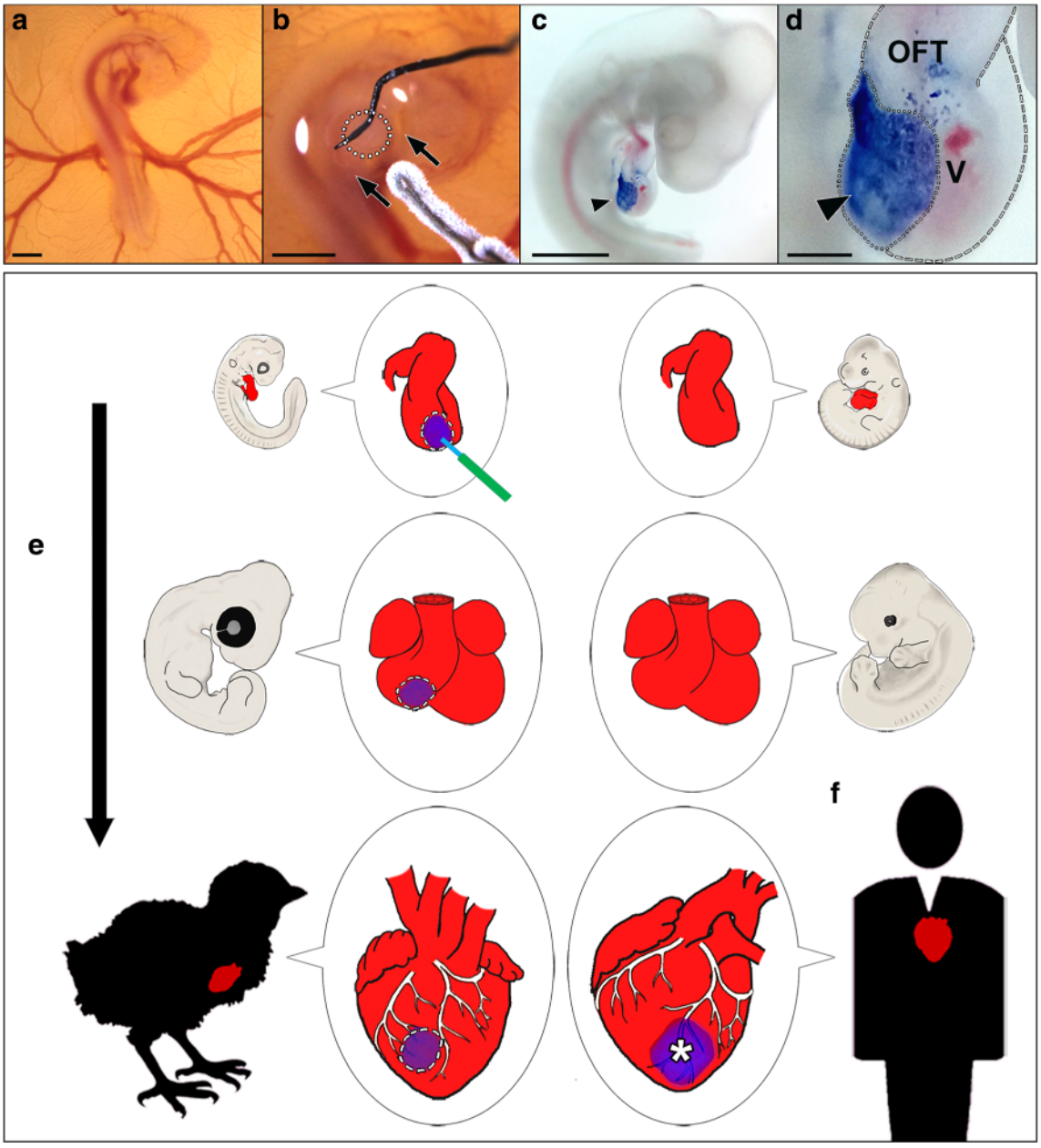

Fig. 3 The avian embryo as a model for the study of cardiac repair and regeneration. *In ovo* avian embryonic cardiac cryodamage (**a**, **b**) leads to cell death in a concrete region of the ventricle (trypan blue^{+} cells, **c**, **d**). This procedure is used in avian embryos (**e**) to study embryonic and postnatal heart responses to damage. The avian model shows parallels with cardiac human development and responses to injury (**f**). Comparing results from these two species may be useful to study cardiac responses to myocardial death

on cardiac regeneration. Cell therapy products are considered drugs by the European Union since 2003 (medicinal products were introduced in the European legislation through Directive 2003/63/EC); in 2007, cell therapy, gene therapy, and tissue engineering products were redefined as advanced therapy medicinal products by the Regulation (EC) No.1394/2007 [66]. The whole strategy of cell-based therapies is, in short, to substitute dead or damaged cells by new functional ones. In the case of the heart, the identification of an appropriate cell type to promote efficient and extensive heart muscle regeneration is still required.

In this context, the use of stem cells seemed to be the best option, as their developmental pluri/multipotency was expected to lead to significant cardiomyocyte differentiation. However, although pluripotent and multipotent stem cells have been shown to be able to differentiate into cardiomyocytes in vitro [67, 68], their ability to differentiate into cardiomyocytes in vivo (as well as their effective electrical coupling to the host tissue) has not been proven to be

successful as yet. Bone marrow mesenchymal stem cells MSCs) have also been used in experimental cell-based therapies because they show multipotency and are suitable for autologous transplantations. MSC may also be thought to promote cardiac regeneration through paracrine-like interactions [69]. Furthermore, MSCs have some immunomodulatory properties and display no significant genetic alterations during their early in vitro expansion e.g., chromosomal abnormalities) [66, 70]. Unfortunately, the results from clinical trials using MSCs have shown that efficient cardiomyocyte differentiation from these cells does not occur in vivo [71].

Pluripotent stem cells PSCs) are another alternative cell source for cardiac regeneration therapies. Embryonic and induced stem cells ESC and iPS) are prototypical PSC examples. PSC can differentiate into cell derivatives from the three blastoderm layers cells ectoderm, mesoderm and endoderm), are clonogenic, and display sustained self-renewal capacity [72], but the clinical use of these cells has many drawbacks: PSC autologous transplantation is only possible in the case of iPS, whose generation in the laboratory remains relatively expensive, and the risk of developing teratomas remains a fundamental disadvantage of PSC use as a medicinal product [73, 74].

In addition, in vivo reprogramming of resident non-cardiomyocytes cells like cardiac fibroblasts [75] could be an alternative treatment of the infarcted heart, offering several advantages over iPSC transplantation. The prevention of pluripotency reversion after cell grafting, the swiftness and simplicity of the process, and the low risk of contamination with immature cells are the main advantages of in vivo reprogramming methods compared with iPSC transplantation procedures [76]. Although in vitro reprogramming of human fibroblasts to cardiac-like myocytes is possible [77], the low reprogramming efficiency of this process is the main problem of using in vivo direct cardiac reprogramming as a clinical therapy.

In a similar way, some researchers have proposed the use of resident cardiac stem cells c-Kit^{+}) [78] and cardiosphere-derived cells [79] for cell-based cardiac therapies as a plausible alternative to PSC. Both cell types would be suitable for autologous cell transplantation, but cardiomyocyte differentiation from these cells is still suboptimal for their future possible use in the clinics.

Finally, some other authors have suggested to combine cell-based therapies with microRNA and anti-microRNA to promote in vivo cardiac regeneration [80]. As a matter of fact, some experiments demonstrate that the overexpression of some of these factors, like miR-15, induces an increase in cardiomyocyte proliferation in damaged hearts [81]. Since an increase of cardiomyocyte proliferation rate is the main response to damage in the regenerating zebrafish [17] and neonatal mouse [8] hearts, the ability to control cardiomyocyte cell cycle entry in the adult mammalian heart would represent a powerful therapy to treat myocardial infarction consequences. In this way, there are some recent studies that seek the in vivo stimulation of adult cardiomyocyte proliferation to regenerate the infarcted heart [82]. Although these treatments decrease the infarcted wound size and improve cardiac function after myocardial infarction, full ventricular regeneration is not achieved. In addition, the potential of ectopic proliferation calls for caution in any tissue with strong mitotic properties, as it could lead to the generation of tumors.

Conclusions

Lessons from animal models both during embryonic development and adult life) suggest that additional efforts must be done to study: 1) the regulation of cardiomyocyte proliferation. 2) CSC-dependent mammalian heart endogenous regenerative potential, and 3) reparative fibrotic event. All these tasks require initiating systematic research on the study of the cellular and molecular mechanisms that control cardiac regeneration in a major number of animal species, avoiding the assumption that phylogenetically close animal taxa have the same ability to regenerate tissues and organs. In order to appropriately tackle this objective, it is necessary to choose a proper preclinical animal model to then evaluate the significance of these studies in the human context Fig. 3). In summary, extensive basic research and preclinical studies are to be accomplished before cells can be successfully used as a medicinal product to regenerate the infarcted heart.

Acknowledgements We thank all present and past members of our laboratory for fruitful discussions.

Funding This work was supported by the Spanish Ministry of Economy MINECO) [grant number BFU2015-65783-R] and [grant number SAF2015-71863] to JMPP); Instituto de Salud Carlos III MINECO-ISCIII) [grant number RD16/0011/0030-TERCEL] to JMPP); Plan Propio-Universidad de Málaga to JAG); Spanish Society of Cardiology grant, "Proyectos de la SEC para Investigación Básica en Cardiología 2018" to JAG).

Compliance with ethical standards

Conflict of interests The authors declare that they have no conflict of interest.

References

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, Ferranti S, De Després JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jiménez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, MacKey RH, Magid DJ, McGuire DK, Mohler ER, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD,

Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB 2016) Heart disease and stroke statistics-2016 update a report from the American Heart Association. Circulation 133:e38–e48. https://doi.org/10.1161/CIR.0000000000000350

2. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimský P, Baumbach A, Bugiardini R, Coman IM, Delgado V, Fitzsimons D, Gaemperli O, Gershlick AH, Gielen S, Harjola VP, Katus HA, Knuuti J, Kolh P, Leclercq C, Lip GYH, Morais J, Neskovic AN, Neumann FJ, Niessner A, Piepoli MF, Richter DJ, Shlyakhto E, Simpson IA, Steg PG, Terkelsen CJ, Thygesen K, Windecker S, Zamorano JL, Zeymer U 2018) 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. Eur Heart J 39:119–177. https://doi.org/10.1093/eurheartj/ehx393

3. Jessup M, Brozena S 2003) Heart failure. N Engl J Med 348:2007–2018

4. Hall R, Simpson I 2009) The ESC textbook of cardiovascular medicine: second edition. Oxford Univ Press, Oxford. https://doi.org/10.1093/med/9780199566990.001.0001

5. Taylor DO, Edwards LB, Boucek MM, Trulock EP, Aurora P, Christie J, Dobbels F, Rahmel AO, Keck BM, Hertz MI 2007) Registry of the International Society for Heart and Lung Transplantation: Twenty-fourth Official Adult Heart Transplant report-2007. J Hear Lung Transplant 26:769–781. https://doi.org/10.1016/j.healun.2007.06.004

6. Sager HB, Kessler T, Schunkert H 2017) Monocytes and macrophages in cardiac injury and repair. J Thorac Dis 9:S30–S35. https://doi.org/10.21037/jtd.2016.11.17

7. Ruiz-Villalba A, Simón AM, Pogontke C, Castillo MI, Abizanda G, Pelacho B, Sánchez-Domínguez R, Segovia JC, Prósper F, Pérez-Pomares JM 2015) Interacting resident epicardium-derived fibroblasts and recruited bone marrow cells form myocardial infarction scar. J Am Coll Cardiol 65:2057–2066. https://doi.org/10.1016/j.jacc.2015.03.520

8. Porrello ER, Mahmoud AI, Simpson E, Hill JA, Richardson JA, Olson EN, Sadek HA 2011) Transient regenerative potential of the neonatal mouse heart. Science 331:1078–1080. https://doi.org/10.1126/science.1200708

9. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal-Ginard B, Anversa P 2003) Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 114:763–776. https://doi.org/10.1016/S0092-8674 03)00687-1

10. Messina E, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso F, Salio M, Battaglia M, Latronico MVG, Coletta M, Vivarelli E, Frati L, Cossu G, Giacomello A 2004) Isolation and expansion of adult cardiac stem cells from human and murine heart. Circ Res 95:911–921. https://doi.org/10.1161/01.RES.0000147315.71699.51

11. Lai AG, Aboobaker AA 2017) EvoRegen in animals: time to uncover deep conservation or convergence of adult stem cell evolution and regenerative processes. Dev Biol 433:118–131. https://doi.org/10.1016/j.ydbio.2017.10.010

12. Sehring IM, Jahn C, Weidinger G 2016) Zebrafish fin and heart: what s special about regeneration? Curr Opin Genet Dev 40:48–56. https://doi.org/10.1016/j.gde.2016.05.011

13. Godwin JW, Rosenthal N 2014) Scar-free wound healing and regeneration in amphibians: immunological influences on regenerative success. Differentiation 87:66–75. https://doi.org/10.1016/j.diff.2014.02.002

14. Galliot B, Crescenzi M, Jacinto A, Tajbakhsh S 2017) Trends in tissue repair and regeneration. Development 144:357–364. https://doi.org/10.1242/dev.144279

15. Grivas J, Haag M, Johnson A, Manalo T, Roell J, Das TL, Brown E, Burns AR, Lafontant PJ 2014) Cardiac repair and regenerative potential in the goldfish Carassius auratus) heart. Comp Biochem Physiol Part C Toxicol Pharmacol 163:14–23. https://doi.org/10.1016/j.cbpc.2014.02.002

16. Poss KD, Wilson LG, Keating MT 2002) Heart regeneration in zebrafish. Science 298:2188–2190. https://doi.org/10.1126/science.1077857

17. Jopling C, Sleep E, Raya M, Martí M, Raya A, Belmonte JCI 2010) Zebrafish heart regeneration occurs by cardiomyocyte dedifferentiation and proliferation. Nature 464:606–609. https://doi.org/10.1038/nature08899

18. Vargas-González A, Prado-Zayago E, León-Olea M, Guarner-Lans V, Cano-Martínez A 2005) Regeneración miocárdica en Ambystoma mexicanum después de lesión quirúrgica. Arch Cardiol Mex 75:21–29

19. Witman N, Murtuza B, Davis B, Arner A, Morrison JI 2011) Recapitulation of developmental cardiogenesis governs the morphological and functional regeneration of adult newt hearts following injury. Dev Biol 354:67–76. https://doi.org/10.1016/j.ydbio.2011.03.021

20. Sánchez-Iranzo H, Galardi-Castilla M, Minguillón C, Sanz-Morejón A, González-Rosa JM, Felker A, Ernst A, Guzmán-Martínez G, Mosimann C, Mercader N 2018) Tbx5a lineage tracing shows cardiomyocyte plasticity during zebrafish heart regeneration. Nat Commun 9:428. https://doi.org/10.1038/s41467-017-02650-6

21. Sallin P, de Preux Charles AS, Duruz V, Pfefferli C, Jaźwi ska A 2015) A dual epimorphic and compensatory mode of heart regeneration in zebrafish. Dev Biol 399:27–40. https://doi.org/10.1016/j.ydbio.2014.12.002

22. Patterson M, Barske L, Van Handel B, Rau CD, Gan P, Sharma A, Parikh S, Denholtz M, Huang Y, Yamaguchi Y, Shen H, Allayee H, Crump JG, Force TI, Lien CL, Makita T, Lusis AJ, Kumar SR, Sucov HM 2017) Frequency of mononuclear diploid cardiomyocytes underlies natural variation in heart regeneration. Nat Genet 49:1346–1353. https://doi.org/10.1038/ng.3929

23. Ito K, Morioka M, Kimura S, Tasaki M, Inohaya K, Kudo A 2014) Differential reparative phenotypes between zebrafish and medaka after cardiac injury. Dev Dyn 243:1106–1115. https://doi.org/10.1002/dvdy.24154

24. Reyer R 1954) Regeneration of the lens in amphibians. Q Rev Biol 29:1–46

25. Young HE, Bailey CF, Markwald RR, Dalley BK 1985) Histological analysis of limb regeneration in postmetamorphic adult Ambystoma. Anat Rec 212:183–194

26. Ghosh S, Thorogood P, Ferretti P 1994) Regenerative capability of upper and lower jaws in the newt. Int J Dev Biol 38:479–490

27. Mitashov VI 1996) Mechanisms of retina regeneration in urodeles. Int J Dev Biol 40:833–844

28. Liao S, Dong W, Lv L, Guo H, Yang J, Zhao H, Huang R, Yuan Z, Chen Y, Feng S, Zheng X, Huang J, Huang W, Qi X, Cai D 2017) Heart regeneration in adult Xenopus tropicalis after apical resection. Cell Biosci 7:1–16. https://doi.org/10.1186/s13578-017-0199-6

29. Marshall L, Vivien C, Girardot F, Péricard L, Demeneix BA, Coen L, Chai N 2017) Persistent fibrosis, hypertrophy and sarcomere disorganisation after endoscopyguided heart resection in adult Xenopus. PLoS One 12:1–24. https://doi.org/10.1371/journal.pone.0173418

30. Marshall L, Girardot F, Demeneix BA, Coen L 2018) Is adult cardiac regeneration absent in Xenopus laevis yet present in Xenopus tropicalis? Cell Biosci 8:1–4. https://doi.org/10.1186/s13578-018-0231-5

31. Liao S, Dong W, Zhao H, Huang R, Qi X, Cai D 2018) Cardiac regeneration in Xenopus tropicalis and Xenopus laevis:

discrepancies and problems. Cell Biosci 8:32. https://doi.org/10.1186/s13578-018-0230-6

32. Sallin P, Jaźwi ska A 2016) Acute stress is detrimental to heart regeneration in zebrafish. Open Biol 6:160012. https://doi.org/10.1098/rsob.160012

33. Flink IL 2002) Cell cycle reentry of ventricular and atrial cardiomyocytes and cells within the epicardium following amputation of the ventricular apex in the axolotl, Amblystoma mexicanum: confocal microscopic immunofluorescent image analysis of bromodeoxyuridine-label. Anat Embryol Berl) 205:235–244. https://doi.org/10.1007/s00429-002-0249-6

34. Cano-Martínez A, Vargas-González A, Guarner-Lans V, Prado-Zayago E, León-Olea M, Nieto-Lima B 2010) Functional and structural regeneration in the axolotl heart Ambystoma mexicanum) after partial ventricular amputation. Arch Cardiol Mex 80:79–86

35. Oberpriller J, Oberpriller J 1974) Response of the adult newt ventricle to injury. J Exp Zool 187:249–253. https://doi.org/10.1002/jez.1401870208

36. Neff AW, Dent AE, Armstrong JB 1996) Heart development in urodeles. Science 725:719–725

37. Porrello ER, Olson EN 2014) A neonatal blueprint for cardiac regeneration. Stem Cell Res 13:556–570. https://doi.org/10.1016/j.scr.2014.06.003

38. Bryant DM, O Meara CC, Ho NN, Gannon J, Cai L, Lee RT 2015) A systematic analysis of neonatal mouse heart regeneration after apical resection. J Mol Cell Cardiol 79:315–318. https://doi.org/10.1016/j.yjmcc.2014.12.011

39. Darehzereshki A, Rubin N, Gamba L, Kim J, Fraser J, Huang Y, Billings J, Mohammadzadeh R, Wood J, Warburton D, Kaartinen V, Lien C-L 2015) Differential regenerative capacity of neonatal mouse hearts after cryoinjury. Dev Biol 399:91–99. https://doi.org/10.1016/j.ydbio.2014.12.018

40. Sousounis K, Baddour JA, Tsonis PA 2014) Aging and regeneration in vertebrates, 1st ed. Elsevier Inc, Amsterdam

41. Matsuura K, Nagai T, Nishigaki N, Oyama T, Nishi J, Wada H, Sano M, Toko H, Akazawa H, Sato T, Nakaya H, Kasanuki H, Komuro I 2004) Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. J Biol Chem 279:11384–11391. https://doi.org/10.1074/jbc.M310822200

42. Eschenhagen T, Bolli R, Braun T, Field LJ, Fleischmann BK, Frisén J, Giacca M, Hare JM, Houser S, Lee RT, Marbán E, Martin JF, Molkentin JD, Murry CE, Riley PR, Ruiz-Lozano P, Sadek HA, Sussman MA, Hill JA 2017) Cardiomyocyte regeneration. Circulation 136:680–686. https://doi.org/10.1161/CIRCULATIONAHA.117.029343

43. McBrearty B a, Clark LD, Zhang XM, Blankenhorn EP, Heber-Katz E 1998) Genetic analysis of a mammalian wound-healing trait. Proc Natl Acad Sci U S A 95:11792–11797. https://doi.org/10.1073/pnas.95.20.11792

44. Leferovich JM, Bedelbaeva K, Samulewicz S, Zhang XM, Zwas D, Lankford EB, Heber-Katz E 2001) Heart regeneration in adult MRL mice. Proc Natl Acad Sci U S A 98:9830–9835. https://doi.org/10.1073/pnas.181329398

45. Morgan TH 1901) Regeneration. Columbia Univ Biol Ser VII:1–316

46. Haque ZK, Wang DZ 2017) How cardiomyocytes sense pathophysiological stresses for cardiac remodeling. Cell Mol Life Sci 74:983–1000. https://doi.org/10.1007/s00018-016-2373-0

47. Bettencourt-Dias M, Mittnacht S, Brockes JP 2003) Heterogeneous proliferative potential in regenerative adult newt cardiomyocytes. J Cell Sci 116:4001–4009. https://doi.org/10.1242/jcs.00698

48. Lepilina A, Coon AN, Kikuchi K, Holdway JE, Roberts RW, Burns CG, Poss KD 2006) A dynamic epicardial injury response supports progenitor cell activity during zebrafish heart regeneration. Cell 127:607–619. https://doi.org/10.1016/j.cell.2006.08.052

49. Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Walsh S, Zupicich J, Alkass K, Buchholz BA, Jovinge S, Frisén J, Bergmann O, Bhardwaj RD, Bernard S, Zdunek S, Barnabi-heider F, Walsh S, Zupicich J, Alkass K, Buchholz BA, Druid H, Jovinge S, Frisnt J 2009) Evidence for cardiomyocyte renewal in humans. Science 324:98–102. https://doi.org/10.1126/science.1164680.Evidence

50. Pasumarthi KBS, Field LJ 2002) Cardiomyocyte cell cycle regulation. Circ Res 90:1044–1054. https://doi.org/10.1161/01.RES.0000020201.44772.67

51. Ramkisoensing AA, De Vries AAF, Atsma DE, Schalij MJ, Pijnappels DA 2014) Interaction between myofibroblasts and stem cells in the fibrotic heart: balancing between deterioration and regeneration. Cardiovasc Res 102:224–231. https://doi.org/10.1093/cvr/cvu047

52. Slack JM 2000) Stem cells in epithelial tissues. Science 287:1431–1433. https://doi.org/10.1126/science.287.5457.1431

53. Martin CM, Meeson AP, Robertson SM, Hawke TJ, Richardson JA, Bates S, Goetsch SC, Gallardo TD, Garry DJ 2004) Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. Dev Biol 265:262–275. https://doi.org/10.1016/j.ydbio.2003.09.028

54. Laugwitz K-L, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, Lin L-Z, Cai C-L, Lu MM, Reth M, Platoshyn O, Yuan JX-J, Evans S, Chien KR 2005) Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. Nature 433:647–653. https://doi.org/10.1038/nature03215

55. van Berlo JH, Kanisicak O, Maillet M, Vagnozzi RJ, Karch J, Lin S-CJ, Middleton RC, Marbán E, Molkentin JD 2014) c-kit+ cells minimally contribute cardiomyocytes to the heart. Nature 509:337–341. https://doi.org/10.1038/nature13309

56. Czarna A, Sanada F, Matsuda A, Kim J, Signore S, Pereira JD, Sorrentino A, Kannappan R, Cannatà A, Hosoda T, Rota M, Crea F, Anversa P, Leri A 2017) Single-cell analysis of the fate of c-kit-positive bone marrow cells. npj Regen Med 2:27. https://doi.org/10.1038/s41536-017-0032-1

57. Laugwitz K-L, Moretti A, Caron L, Nakano A, Chien KR 2007) Islet1 cardiovascular progenitors: a single source for heart lineages? Development 135:193–205. https://doi.org/10.1242/dev.001883

58. Wessels A, Pérez-Pomares JM 2004) The epicardium and epicardially derived cells EPDCs) as cardiac stem cells. Anat Rec Part A Discov Mol Cell Evol Biol 276A:43–57. https://doi.org/10.1002/ar.a.10129

59. Bilbija D, Haugen F, Sagave J, Baysa A, Bastani N, Levy FO, Sirsjö A, Blomhoff R, Valen G 2012) Retinoic acid signalling is activated in the postischemic heart and may influence Remodelling. PLoS One 7:1–9. https://doi.org/10.1371/journal.pone.0044740

60. Wei K, Serpooshan V, Hurtado C, Diez-Cunado M, Zhao M, Maruyama S, Zhu W, Fajardo G, Noseda M, Nakamura K, Tian X, Liu Q, Wang A, Matsuura Y, Bushway P, Cai W, Savchenko A, Mahmoudi M, Schneider MD, Van Den Hoff MJB, Butte MJ, Yang PC, Walsh K, Zhou B, Bernstein D, Mercola M, Ruiz-Lozano P 2015) Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. Nature 525:479–485. https://doi.org/10.1038/nature15372

61. Cao J, Poss KD 2018) The epicardium as a hub for heart regeneration. Nat Rev Cardiol 8:53–69. https://doi.org/10.1038/s41569-018-0046-4

62. Leri A, Rota M, Hosoda T, Goichberg P, Anversa P 2014) Cardiac stem cell niches. Stem Cell Res 13:631–646. https://doi.org/10.1016/j.scr.2014.09.001

63. Drenckhahn JD, Schwarz QP, Gray S, Laskowski A, Kiriazis H, Ming Z, Harvey RP, Du XJ, Thorburn DR, Cox TC 2008)

Compensatory growth of healthy cardiac cells in the presence of diseased cells restores tissue homeostasis during heart development. Dev Cell 15:521–533. https://doi.org/10.1016/j.devcel.2008.09.005

64. Sturzu AC, Rajarajan K, Passer D, Plonowska K, Riley A, Tan TC, Sharma A, Xu AF, Engels MC, Feistritzer R, Li G, Selig MK, Geissler R, Robertson KD, Scherrer-Crosbie M, Domian IJ, Wu SM 2015) Fetal mammalian heart generates a robust compensatory response to cell loss. Circulation 132:109–121. https://doi.org/10.1161/CIRCULATIONAHA.114.011490

65. Palmquist-Gomes P, Guadix JA, Pérez-Pomares JM 2016) A chick embryo cryoinjury model for the study of embryonic organ development and repair. Differentiation 91:72–77. https://doi.org/10.1016/j.diff.2015.10.011

66. Guadix JA, Zugaza JL, Gálvez-Martín P 2017) Characteristics, applications and prospects of mesenchymal stem cells in cell therapy. Med Clin 148:408–414. https://doi.org/10.1016/j.medcle.2017.04.018

67. Brandão KO, Tabel VA, Atsma DE, Mummery CL, Davis RP 2017) Human pluripotent stem cell models of cardiac disease: from mechanisms to therapies. Dis Model Mech 10:1039–1059. https://doi.org/10.1242/dmm.030320

68. Oh H, Ito H, Sano S 2016) Challenges to success in heart failure: cardiac cell therapies in patients with heart diseases. J Cardiol 68:361–367. https://doi.org/10.1016/j.jjcc.2016.04.010

69. Loffredo FS, Steinhauser ML, Gannon J, Lee RT 2011) Bone marrow-derived cell therapy stimulates endogenous cardiomyocyte progenitors and promotes cardiac repair. Cell Stem Cell 8:389–398. https://doi.org/10.1016/j.stem.2011.02.002

70. Caplan A 2009) Why are MSCs therapeutic? New data: new insight. J Pathol 217:318–324. https://doi.org/10.1002/path.2469

71. Abdel-latif A, Bolli R, Tleyjeh IM, Montori VM, Perin EC, Hornung CA, Zuba-Surma EK, Al-Mallah M, Dawn B 2007) Adult bone marrow–derived cells for cardiac repair. Arch Intern Med 167:989–997. https://doi.org/10.1001/archinte.167.10.989

72. Posfai E, Tam OH, Rossant J 2014) Mechanisms of pluripotency in vivo and in vitro. In: Current Topics in Developmental Biology, 1st ed. Elsevier Inc., Amsterdam, pp 1–37

73. Fong C-Y, Gauthaman K, Bongso A 2010) Teratomas from pluripotent stem cells: a clinical hurdle. J Cell Biochem 111:769–781. https://doi.org/10.1002/jcb.22775

74. Romano G, Morales F, Marino IR, Giordano A 2014) A commentary on iPS cells: potential applications in autologous transplantation, study of illnesses and drug screening. J Cell Physiol 229:148–152. https://doi.org/10.1002/jcp.24437

75. Klose K, Gossen M, Stamm C 2018) Turning fibroblasts into cardiomyocytes: technological review of cardiac transdifferentiation strategies. FASEB J:fj.201800712R. https://doi.org/10.1096/fj.201800712R

76. Tani H, Sadahiro T, Ieda M 2018) Direct cardiac reprogramming: a novel approach for heart regeneration. Int J Mol Sci 19 9). https://doi.org/10.3390/ijms19092629

77. Nam YJ, Song K, Luo X, Daniel E, Lambeth K, West K, Hill JA, DiMaio JM, Baker LA, Bassel-Duby R, Olson EN 2013) Reprogramming of human fibroblasts toward a cardiac fate. Proc Natl Acad Sci 110:5588–5593. https://doi.org/10.1073/pnas.1301019110

78. Jesty S a, Steffey M a, Lee FK, Breitbach M, Hesse M, Reining S, Lee JC, Doran RM, Nikitin AY, Fleischmann BK, Kotlikoff MI 2012) c-kit+ precursors support postinfarction myogenesis in the neonatal, but not adult, heart. Proc Natl Acad Sci 109:13380–13385. https://doi.org/10.1073/pnas.1208114109

79. Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marbán L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G, Marbán E 2012) Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction CADUCEUS): a prospective, randomised phase 1 trial. Lancet 379:895–904. https://doi.org/10.1016/S0140-6736 12)60195-0

80. van Rooij E, Olson EN 2012) MicroRNA therapeutics for cardiovascular disease: opportunities and obstacles. Nat Rev Drug Discov 11:860–872. https://doi.org/10.1038/nrd3864

81. Porrello ER, Mahmoud AI, Simpson E, Johnson BA, Grinsfelder D, Canseco D, Mammen PP, Rothermel BA, Olson EN, Sadek HA 2013) Regulation of neonatal and adult mammalian heart regeneration by the miR-15 family. Proc Natl Acad Sci 110:187–192. https://doi.org/10.1073/pnas.1208863110

82. Mohamed TMA, Ang YS, Radzinsky E, Zhou P, Huang Y, Elfenbein A, Foley A, Magnitsky S, Srivastava D 2018) Regulation of cell cycle to stimulate adult cardiomyocyte proliferation and cardiac regeneration. Cell 173 1):104–116. https://doi.org/10.1016/j.cell.2018.02.014

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