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SPOTLIGHT REVIEW

Novel therapeutic approaches to post-infarction remodelling

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Abstract Adverse cardiac remodelling is a major cause of morbidity and mortality following acute myocardial infarction (MI). Mechanical and neurohumoral factors involved in structural and molecular post-infarction remodelling were important targets in research and treatment for years. More recently, therapeutic strategies that address myocardial regeneration and pathophysiological mechanisms of infarct wound healing appear to be useful novel tools to prevent progressive ventricular dilation, functional deterioration, life-threatening arrhythmia, and heart failure. This review provides an overview of future and emerging therapies for cardiac wound healing and remodelling after MI.

Keywords Myocardial infarction • Ischaemia • Remodelling • MicroRNAs • Progenitor cells • Fibroblasts • Macrophages

This article is part of the Spotlight Issue on: Reducing the Impact of Myocardial Ischaemia/Reperfusion Injury

1. Introduction

Adverse cardiac remodelling after myocardial infarction (MI) precipitates impaired ventricular function and heart failure leading to increased morbidity and mortality. Advances in interventional therapies, especially early reperfusion therapy, markedly reduced short-term mortality in patients with large MI; however, the parallel increase in heart failure morbidity and mortality is of concern.[1–3]

The incidence of heart failure after MI is determined by the size of the infarcted area, infarct wound healing, and chronic left ventricular (LV) remodelling.[1–3] Timely reperfusion therapy and conventional pharmacotherapy [β-blockers, angiotensin-converting enzyme (ACE) inhibitors, mineralocorticoid receptor (MR) blockers, statins] improve prognosis in patients with acute MI by limiting infarct size, reducing arrhythmia, and attenuating progressive LV remodelling.[2–4] However, to prevent the development of heart failure especially after large or recurrent MI, additional therapeutic strategies are needed which include (i) pharmacological and non-pharmacological approaches to limit early reperfusion injury, (ii) progenitor/stem cell therapy to enhance repair of damaged myocardium, and (iii) targeting infarct wound healing to improve cardiac remodelling in the early phase of healing.

Several recent review articles provide excellent overviews of experimental and clinical studies on therapeutic management of post-infarction cardiac remodelling.[2–6] This review highlights future and emerging therapeutic approaches to improve post-infarct wound healing and remodelling.

2. Pathophysiology of healing and remodelling after myocardial ischaemia

Cardiomyocyte apoptosis and necrosis with the consequent loss of contractile myocardium trigger a cascade of immuno-inflammatory pathways and cellular activities that promote infarct wound healing and a unique pattern of LV structural remodelling, involving the infarcted region and the residual viable myocardium.[7–9] LV remodelling in the early phase of healing is a consequence of thinning and dilation of the infarcted myocardial wall (infarct expansion); in late phases, LV remodelling is secondary to architectural rearrangements of the surviving myocardium involving myocyte hypertrophy, interstitial fibrosis, and LV dilation.[1,2]

Cardiac healing and repair following myocardial ischaemia, a complex process of well-defined continuous and overlapping events,[7–10] begins with an inflammatory phase characterized by replacement of the necrotic myocardium with granulation tissue and is followed by a fibrogenic phase resulting in scar tissue formation. Optimal infarct healing depends on rapid but tightly controlled recruitment as well as coordinated activation/polarization of myeloid cells particularly monocytes, which promote resorption of cellular debris and apoptotic cells, degradation of extracellular matrix components, regulation of granulation tissue formation, and neoangiogenesis, through secretion of cytokines, proteases, and growth factors[7,8,10–13] (*Figure 1*). Despite detailed histological analysis of the cellular composition of the healing infarct over time in

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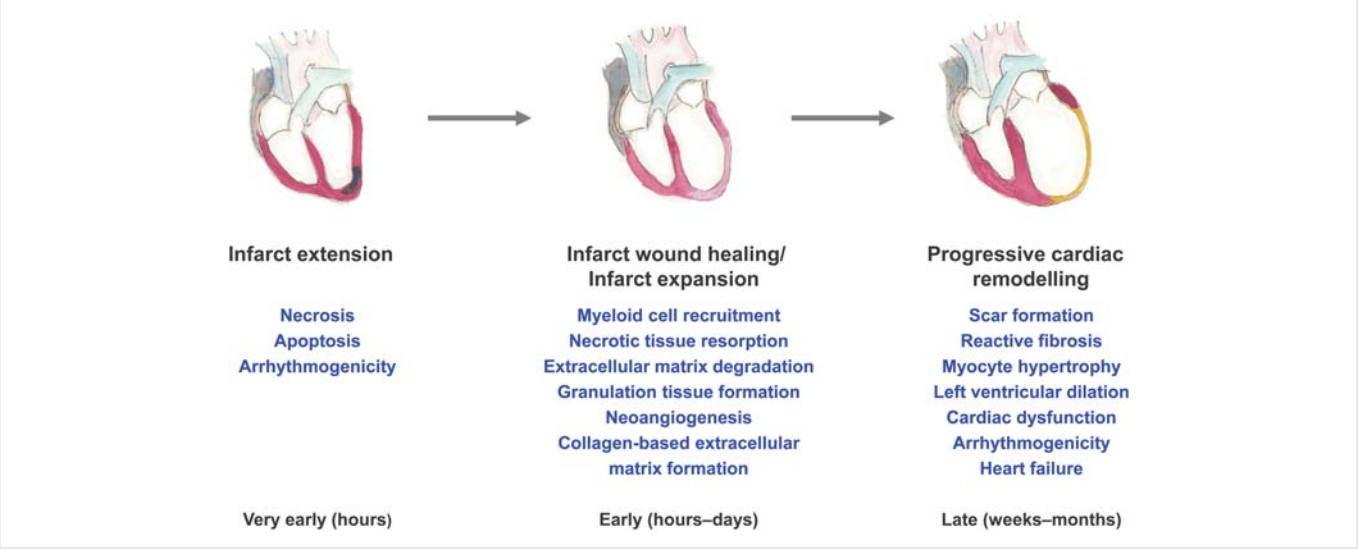

Figure 1 Ventricular enlargement in the early post-infarction phase is a consequence of thinning and dilation of the infarct zone (infarct expansion); in late phases, cardiac remodelling is characterized by architectural rearrangements of the surviving myocardium.

animal models of MI, to date we only partially understand the highly complex processes in the infarct and border zones where different subtypes of invading myeloid (and also lymphoid) cells interact in a time-dependent manner with the tissue *in situ* and stimulate wound healing processes.

The formation and maturation of new blood vessels for supplying the highly cellular granulation tissue with oxygen and nutrients is a crucial event in infarct healing and requires well-coordinated interactions between endothelial cells, pericytes, vascular smooth muscle cells, and the extracellular matrix in response to angiogenic mediators.[9,10] The vascularized granulation tissue promotes the migration of fibroblasts, cells critical for the formation of a collagen network as well as for the pathogenesis of cardiac fibrosis. In the healing wound, fibroblasts proliferate and phenotypically differentiate into contractile myofibroblasts acquiring smooth muscle features, which secrete large amounts of extracellular proteins in response to micro-environmental signals.[9,14,15] Deposition of collagen-based extracellular matrix leading to scar formation (reparative fibrosis) is a crucial stage of infarct repair and essential for structural integrity of the infarcted heart and prevention of LV rupture. Impaired and disorganized collagen matrix renders the scar less resistant to distension during overload, leads to an increase of wall stress, and predisposes to progressive infarct thinning and chamber dilation.[9,16] In contrast, fibroblast proliferation and differentiation to myofibroblasts in areas remote from the infarct site result in excessive deposition of extracellular matrix proteins (reactive fibrosis) and significant increase in myocardial stiffness, leading to relaxation abnormalities, arrhythmogenicity, progressive diastolic dysfunction, and heart failure. In addition, perivascular fibrosis impairs myocyte oxygen availability, reduces coronary reserve, and exacerbates myocardial ischaemia.[14] Finally, progressive myocardial fibrosis precipitates LV hypertrophy and systolic dysfunction. In ischaemic cardiomyopathy, fibrosis in the remote non-infarcted myocardium is a major determinant of progressive ventricular remodelling.[17] The current antifibrotic strategies, like ACE inhibition, angiotensin receptor antagonism, MR blockade, as well as HMG-CoA-reductase inhibition,[2,18,19] attenuate the development of progressive interstitial and perivascular fibrosis, beneficially modulating cardiac remodelling (for review, see Brown *et al.*[14]); however, more effective prevention of progressive remodelling and heart failure is mandatory.

Noteworthy, in addition to initial ischaemic injury and wound healing response, hibernating myocardium also (i.e. regions of viable myocardium with reduced baseline blood flow and abnormalities of contractile function) plays a significant role in modulating LV remodelling after ischaemia. Carluccio *et al.*[20] showed that in patients with hibernating myocardium, restored contractility of dyssynergic areas by revascularization reverted the alterations in LV volumes and shape, establishing a link between chronic dyssynergy and adverse LV remodelling.

Therapeutic options for the treatment of chronic ventricular remodelling in heart failure also include device-based therapies. Cardiac resynchronization therapy (CRT) with biventricular pacing is an established non-pharmacological adjunctive treatment for patients with symptomatic heart failure, reduced LV systolic ejection fraction (EF), and delayed ventricular conduction as manifested by a widened QRS complex (i.e. electrical dyssynchrony).[21] Several trials have demonstrated that CRT reverses cardiac remodelling and mortality and improves exercise capacity and cardiac performance.[21] The MADIT-CRT trial (Multicenter Automatic Defibrillator Implantation Trial: Cardiac Resynchronization Therapy)[22] highlighted the relationship between improvements in LV volumes and function with CRT and outcome benefit, emphasizing that remodelling indices may be the most accurate predictors of long-term morbidity and mortality in heart failure patients. However, a significant number of patients do not benefit from CRT. Extensive LV remodelling at baseline limits the benefits of CRT in terms of LV function improvement and incidence of cardiac events in patients with intraventricular dyssynchrony.[23] Recently, Sachse *et al.*[24] demonstrated that dyssynchronous heart failure is associated with structural remodelling of the transverse tubular system. Subcellular structures and function of myocytes impaired during heart failure are restored by CRT, suggesting that tubular system status can provide an early marker for the success of CRT.[24]

3. MicroRNAs as targets for prevention of remodelling

Targeting microRNAs (miRNAs), essential regulators of cellular gene programmes in cardiovascular disease, may prove to be a useful approach to prevent post-infarction remodelling (for review, see Bauersachs,[25] Small and Olson,[26] Thum *et al.*,[27] Gladka *et al.*,[28] and also Zhu and Fan,[29] this issue of *Cardiovascular Research*, pages 284–292). miRNA expression profiling identified numerous deregulated miRNAs during cardiac remodelling and failure.[30–32] We have shown a marked similarity between the miRNA expression pattern in human failing hearts and foetal hearts compared with adult normal heart tissue.[32] Thus, reactivation of the foetal miRNA programme seems to substantially contribute to alterations of gene expression, triggering pathological changes in the myocardium associated with progressive structural remodelling and failure. Comprehensive profiling of miRNAs and messenger RNAs in failing hearts from patients with or without biomechanical support showed cardiac miRNA signature to better reflect the actual functional status than messenger RNA signature.[33] As miRNAs and their respective target genes are enriched in a tissue/cell-specific manner, analysis of microRNAs in the heart has to focus not only on cardiomyocytes, but also on endothelial cells, smooth muscle cells, fibroblasts, and inflammatory cells[27,34] (for review, see Thum *et al.*[27] and Bauersachs and Thum[34]). The cross-talk between these cells appears to be a major determinant of myocardial remodelling; *Figure 2* summarizes miRNAs involved both early and late in cardiac healing and remodelling processes during/after myocardial ischaemia.

We have demonstrated that miR-21 and its target gene sprouty-1 are specifically enriched in fibroblasts, but not cardiomyocytes in the murine heart in response to pressure overload, and that an antagomir directed against miR-21 is able to prevent cardiac remodelling and failure.[35] Early after myocardial ischaemia reperfusion, miR-21 was specifically localized in the fibroblast-enriched infarct region and may regulate the expression of matrix metalloprotease-2 in the infarct area via its target PTEN (phosphatase and tensin homologue).[36] Up-regulation of miR-21 may be protective early after MI, as adenoviral overexpression of miR-21 reduced infarct size and left ventricular dilation.[37]

Targeting other miRNAs deregulated after cardiac ischaemia was associated with therapeutic benefit: miR-29 appeared to specifically be involved in fibrosis development, whereas miR-92a is an endothelial miR regulating angiogenesis.[38,39] In mice with ischaemia of the hind limb or MI, antagomir-92a stimulated angiogenesis and functional recovery.

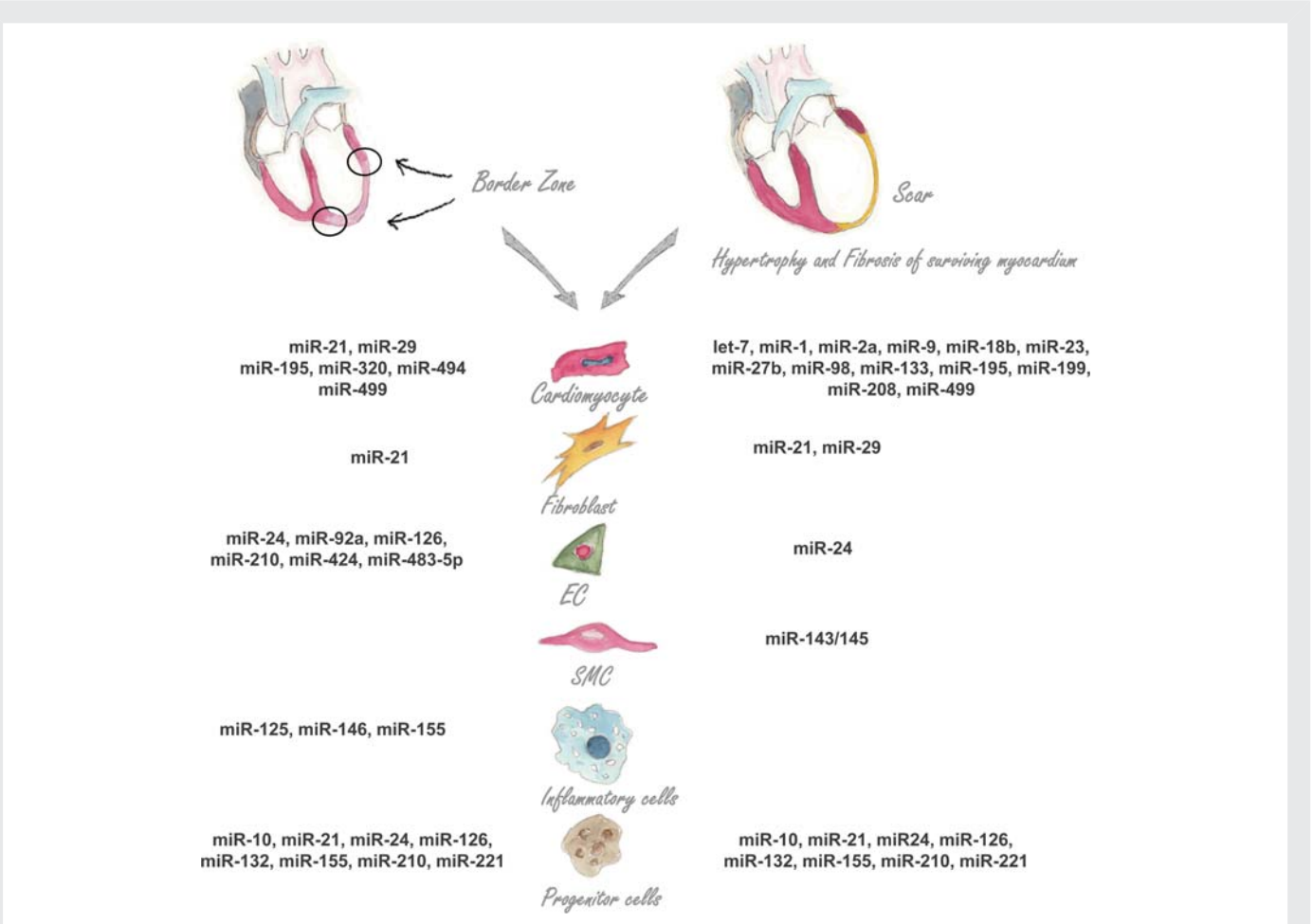

Figure 2 miRNAs as potential targets to modulate cardial healing and remodelling during/after myocardial ischaemia with putative assignment to cell types. (Left) miRNAs regulated early during hypoxia and healing processes mainly confined to the infarct and border zones; (right) miRNAs involved in the chronic remodelling process of hypertrophy and fibrosis of the surviving myocardium. See text for details. EC, endothelial cell; SMC, smooth muscle cell.

Recently, miR-24 was shown to be enriched in cardiac endothelial cells and considerably up-regulated after myocardial ischaemia. miR-24 induced endothelial cell apoptosis and inhibited angiogenesis.[40] These effects were mediated through targeting of the endothelium-enriched transcription factor GATA2 and the p21-activated kinase PAK4. Immediate treatment after MI with an antagomir against miR-24 improved cardiac function and attenuated LV remodelling in mice.

Given the crucial role of monocyte/macrophage invasion and polarization for wound healing after myocardial ischaemia (see below), miRNA regulation of myeloid cells may be critically involved in healing and remodelling post-MI; however, these investigations are in their infancy (see also *Figure 2*; for review, see Schroen and Heymans[41]). Furthermore, the enhancement of progenitor cell survival and engraftment by modulation of miRNAs was shown recently to improve cardiac repair.[42]

4. Cell and gene therapeutic strategies: from bench to bedside and back again

An impressive number of reviews provide comprehensive overviews on stem cell and gene therapeutic strategies that have been applied in preclinical and clinical studies targeting ischaemic wound healing and remodelling. We briefly highlight the current research efforts and future prospects.

4.1 Cell therapy after myocardial ischaemia

Experimental studies over the past decade have indicated that stem and progenitor cells may potentially improve functional recovery and remodelling after MI (*Figure 3*). The capacity of stem cells to differentiate and the discovery of cardiac resident stem cells[43–45] and of endogenous cardiac repair mechanisms[46,47] encouraged the translation of the stem cell therapeutic approach into clinical studies. Transplantation of skeletal myoblasts (SMs),[48] embryonic stem cells (ESCs),[49] bone marrow-derived cells (BMCs),[50] and mesenchymal stem cells (MSCs)[49] were employed to find the optimal cell type for cardiac repair. ESCs have the greatest capacity for cardiac cell differentiation and long-term cell survival of the various populations studied, but teratoma formation[51] and immunogenic and ethical issues have hampered their translation to clinical practice. Among the different cell sources, adipose tissue is raising increasing interest since adipose-derived stem cells (ASCs), besides their regenerative potential, are plentiful and relatively easily accessed, without oncological and immunological concerns.[52] APOLLO (ClinicalTrials.gov: NCT00442806) and PRECISE (ClinicalTrials.gov: NCT00426868) are ongoing clinical trials using ASCs on the basis of significant improvements on cardiac function and anatomy observed in animal studies.

To date, several trials have been performed in the settings of acute MI, ischaemic, and dilated cardiomyopathy (*Table 1*)[53–71] using mainly BMCs or SMs because of their easy availability and safety, although their capacity to transdifferentiate into cardiac muscle for myocardiogenesis is either lacking or minimal.

However, despite the magnitude of benefit demonstrated in animal studies, the clinical trials have shown only a modest or negligible improvement of heart function (*Table 1*), indicating that on the background of established interventional and pharmacological treatments, progenitor cell therapy in patients with MI provides only slight additional benefits, if any. Many of the clinical studies showing favourable effects of BMC therapy to date suffer from methodological limitations, i.e. lack of randomization and blinding, and use of methods other than magnetic resonance imaging, the gold standard for serial cardiac functional and structural evaluation. A meta-analysis of the trials performed ($n = 999$ patients) employing BMCs[72] showed only, though significant, an improvement of 3–4% of the LVEF and parameters of LV remodelling, likely imputable mainly to a favourable paracrine effect exerted by injected stem cell population[73] or to stimulation of new cardiomyocytes from endogenous progenitors.[74] Recently, similar results for LVEF were reported by Strauer and Steinhoff,[50] considering meta-analyses involving 2940 patients.

Inconsistent clinical efficacy of the cell therapy approaches may be attributable to the source and preparation of the cells used for cardiac

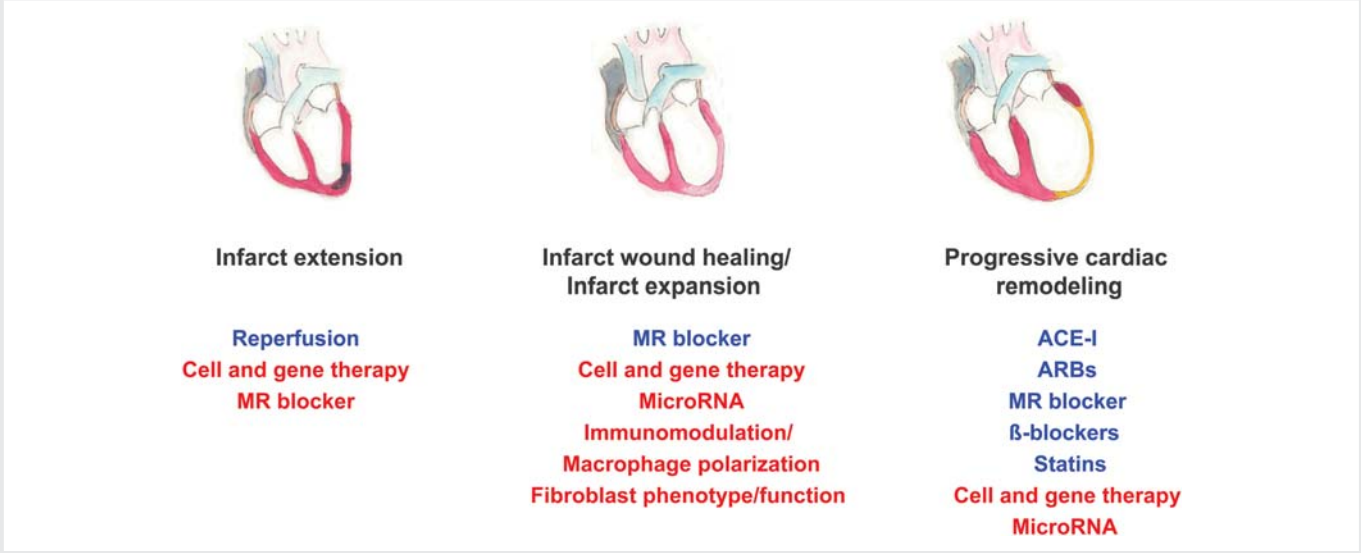

Figure 3 Established (blue), and emerging/novel (red) therapeutic targets to improve healing and remodelling after myocardial ischaemia. MR, mineralocorticoid receptor; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 1 Most relevant randomized controlled cell therapy trials in relation with LV remodelling

First author (year)	Study name (ref.)	Clinical scenario	*n*	Cell type	Primary outcome
Assmus (2002)	TOPCARE-AMI[53]	AMI	59	ProgC	↑ Global LVEF by 9% at 4 months, $P = 0.003$
Wollert (2004)	BOOST[54]	AMI	60	BMC	↑ Global LVEF by 6% at 6 months, effect mantained in patients with large infarcts
Lunde (2006)	ASTAMI[55]	AMI	97	BMC	↔ LVEF at 6 months
Janssens (2006)	LEUVEN-AMI[56]	AMI	66	BMC	↔ LVEF at 4 months; ↑ regional contractility and IS in patients with large infarcts
Schächinger (2006)	REPAIR-AMI[57]	AMI	187	BMC	↑ LVEF by 2.5% at 4 months, $P = 0.008$; ↔ LVEDV
Huikuri (2008)	FINCELL[58]	AMI	77	BMC	↑ LVEF by 5% at 6 months
Tendera (2009)	REGENT[59]	AMI	200	BMC	↔ LVEF or volumes; ↑ LVEDV and LVESV high-dose group
Hare (2009)	[60]	AMI	53	MSC	↔ Global LVEF at 6 months
Meyer (2009)	BOOST[61]	AMI	124	BMC	↔ LVEDV at any time point 5-year follow-up
Beitnes (2009)	ASTAMI[62]	AMI	100	BMC	↔ LVEDV at any time point 3-year follow-up
Hirsch (2011)	HEBE[63]	AMI	200	BMC	↔ Global LVEF at 4 months
Roncalli (2011)	BONAMI[64]	AMI	101	BMC	↑ Viability
Assmus (2006)	TOPCARE-CHD[65]	ICM	75	ProgC	↑ Global LVEF by 2.9% at 3 months, $P = 0.003$
Ang (2008)	[66]	ICM	63	BMC	↔ Global and regional LV function or IS
Menasché (2008)	MAGIC[67]	ICM	97	SM	↔ Global LVEF
Dib (2009)	CAuSMIC[68]	ICM	23	SM	↑ LVESD ($P = 0.07$) and LVEDD ($P = 0.07$) at 1 year
Duckers (2011)	SEISMIC[69]	ICM	40	SM	↔ Global LVEF at 6 months follow-up
Bolli (2011)	SCIPIO[70]	ICM	23	CSC	↑ LVEF by 8.2% at 4 months ($P = 0.001$), 12.3% at 1 year ($P = 0.0007$)
Seth (2010)	ABCD[71]	DCM	81	BMC	↑ LVESV by 17 mL, ↑ LVEF by 5.9% at 3-year follow-up, $P < 0.05$

AMI, acute myocardial infarction; ICM, ischaemic cardiomyopathy; DCM, dilated cardiomyopathy; LVEF, left ventricular ejection fraction; IS, infarct size; LVESV, left ventricular end-systolic volume; LVEDV, left ventricular end-diastolic volume; LVESD, left ventricular end-systolic diameter; LVEDD, left ventricular end-diastolic diameter; BMC, bone marrow-derived cell; CSC, cardiac stem cell; MSC, mesenchymal stem cell; ProgC, progenitor cell; SM, skeletal myoblast; ↑, improvement; ↔, no change; *n*, sample size.

repair, timing and mode of cell delivery and the lack of a complete understanding of the mechanisms of action. The mentioned aspects on stem cell therapy have been reviewed in detail recently by Malliaras and Marban,[75] and Lovell and Mathur.[76] As patients with normal LV function after MI have a rather good prognosis with only minor risk of adverse remodelling, cell therapy studies need to focus on patients with reduced EF early post-MI. In order to clarify the effect of BMC therapy on prognosis in patients with MI, the BAMI trial [effect of intracoronary reinfusion of bone marrow-derived mononuclear cells on all-cause mortality in ST elevation MI (STEMI), supported by the European Community] will include 3000 patients with STEMI and an EF of <45% in a randomized, controlled, open-label Phase III study to receive intracoronary infusion of BMC 5–8 days after successfully reperfused acute MI.

New approaches to cardiac regeneration appear promising. In a recent trial (SCIPIO, Phase I, ClinicalTrials.gov: NCT00474461), patients' own cardiac stem cells (c-kit+) were for the first time administered in humans with striking results.[70] EF improved by 8.2 and 12.3% at 4 and 12 months, respectively. Of note, in the CADUCEUS trial (ClinicalTrials.gov: NCT00893360) testing the safety of autologous cardiosphere-derived cells, scar size expressed as per cent of LV mass shrank markedly (by 30–47%) compared with control after 1 year (Late-Breaking Clinical Trial Abstracts: *Circulation* 2011;**124**:2365–2374). Increasing experimental evidence indicates that not single but combined cell therapy strategies might be able to overcome the complex issue of cardiac repair, closely resembling endogenous biological mechanisms.[77] Inducible pluripotent stem (iPS) cells, ESC-like cells generated by reprogramming adult somatic cells,[78] represent a great potential for heart regeneration: besides avoiding ethical controversies and immunogenic issues associated with the use of ESCs, iPS cells are able to undergo directed differentiation to the three most important cellular components of the heart, i.e. cardiomyocytes, vascular endothelial cells, and vascular smooth cells. Probably, once the viral delivery methods for pluripotency genes in somatic cells have been replaced by non-viral alternatives, cell replacement therapies using cardiomyocytes and other derivatives of iPS cells in combination with emerging tools like cell priming, tissue engineering, and bionanotechnology could be successfully employed in the regeneration process.[77]

4.2 Gene therapy after myocardial ischaemia

Essentially, gene therapy-based treatment for myocardial ischaemia involves the delivery of genes encoding therapeutic proteins carried by a vector to the ischaemic tissue or cardiac cells. Countless gene therapy preclinical studies and clinical trials have been conducted to evaluate the efficiency and safety of different therapeutic genes, vector systems, and delivery technologies on ischaemic heart diseases (for recent excellent reviews, see refs[79–84] and 'Special Section: Cardiovascular Gene Therapy'; *J Mol Cell Cardiol* **50**:742–812).

Myocardial ischaemia gene therapy approaches aimed at activating pro-angiogenic and pro-survival pathways, through the modulation of growth factors as well as proteins involved in apoptosis and oxidative stress, have shown reduced ischaemic and apoptotic cell death, enhanced revascularization of ischaemic tissue, and mobilization of stem cells for cardiac repair. Striking improvements in myocardial blood flow, performance, and remodelling have been reported in animal models of ischaemia and infarction.[81] Unfortunately, the proof of significant therapeutic benefits in the clinical setting remains to be provided. Although several non-controlled Phase I

clinical trials of pro-angiogenic gene therapy for ischaemic myocardial disease have demonstrated enhanced angiogenesis and clinical improvement,[83] limited evidence of efficacy and of a satisfactory outcome (success or improvement) resulted from placebo-controlled Phase II/III trials so far.[84] These disappointing results underscore the strong confounding placebo response/effect of gene therapeutic interventions, emphasizing the imperative of randomized, double-blinded, placebo-controlled trials. The lack of therapeutic effects is primarily the result of low efficiency of gene transfer and expression. Encouraging, the clinical safety and tolerability of angiogenic gene transfer was favourable, with no significant side effects (increased inflammation and angiogenesis in non-target organs, tumorigenesis, atherogenesis, plaque destabilization, diabetes, or other diseases) even in long-term follow-up.[85,86]

In preclinical studies of ischaemic heart failure, genetic manipulation of proteins involved in cardiomyocyte calcium handling[87] and strategies targeting members of the β-adrenergic signalling pathway have yielded highly promising beneficial structural and functional effects. The recent successful completion of the Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) trial[86] not only highlighted that SERCA2a gene delivery could be a potential breakthrough for patients with severe heart failure, but also offer renewed hope of treating heart disease via gene therapy. This small Phase II randomized, double-blind study demonstrated that restoring SERCA2a enzyme levels by intracoronary adeno-associated virus 1 (AAV1)-mediated gene delivery provides sustained improvements in heart failure symptoms, exercise tolerance, serum biomarkers, adverse cardiovascular events, as well as cardiac function and remodelling. Future larger trials will be critical in determining the potential clinical benefit of this promising approach for advanced heart failure.

Several gene therapy trials for coronary heart disease are currently underway worldwide (http://www.clinicaltrials.gov/), the majority testing therapeutic blood vessel growth. Overall, therapeutic angiogenesis by gene transfer could be a promising therapy for patients with chronic ischaemic heart disease not amenable to percutaneous revascularization or bypass surgery but may also represent a crucial adjunctive therapeutic intervention to improve infarct wound healing by promoting the growth of new blood vessels early post-infarction. The potential of this therapeutic approach lies in cardiac-specific, highly efficient gene transfer of biologically potent, safe, and pro-angiogenic growth factors directly to the injured ischaemic myocardium.[88] Of note, the CUPID trials demonstrated success using an AAV1 (ideally suited for cardiac delivery) as the vector, administered in a single dose directly to the heart by intracoronary infusion during a routine outpatient minimally invasive cardiac catheterization procedure. The current research efforts are aimed at improving AAV vectors by modifying the viral capsid proteins and/or tissue-specific regulatory elements to target expression of therapeutic genes specifically to the myocardium.[89]

The concomitant and/or sequential administration of multiple genes encoding angiogenic growth factors may be needed to achieve therapeutic angiogenesis, as the formation of a functional blood vessel leading to revascularization of ischaemic tissues involves an interplay between multiple growth factors at various time points.[83] In addition, novel gene constructs have been developed which allow genes to be switched on and off depending on cellular milieu, avoiding unrestricted protein synthesis or inhibition.[81]

Combining cell therapy with gene therapy is a novel promising approach to generate clinically relevant stem cells for autologous cell-based therapies. The ongoing Enhanced Angiogenic Cell Therapy in Acute Myocardial Infarction (ENACT-AMI) study, a Phase IIb, double-blind, parallel, randomized placebo-controlled trial,[90] investigates the efficacy of cell therapy in patients with LV dysfunction after acute MI, using transplantation of autologous progenitor cells overexpressing endothelial nitric oxide synthase.

5. Modulation of the inflammatory and fibrotic response: from bench to bedside

5.1 Mineralocorticoid receptor and wound healing after myocardial ischaemia

MR blockade reduces morbidity and mortality in patients with LV dysfunction and heart failure after MI.[91,92] Attenuation of LV dilation and excessive extracellular matrix turnover appear to be essential mechanisms of MR antagonism in chronic ischaemic heart failure.[93,94] Animal studies provided important insights into the mechanisms underlying cardioprotection by MR blockade in ischaemic heart failure and also strong evidence that more favourable effects on cardiac remodelling can be achieved by immediate initiation of MR-blocking therapy postinfarction.[13,95]

Immediate MR inhibition in rats with MI enhanced infarct neovascularization and reduced early LV dilation and dysfunction by modulation of macrophage recruitment and polarization at the site of injury. MR inhibition accelerated macrophage infiltration and promoted alternative M2 activation of macrophages with the production of factor XIIIa that drive healing responses and angiogenesis.[13] Based on this first observation highlighting the importance of MR for macrophage polarization as well as for wound healing and tissue repair after myocardial ischaemia, a recent study identified MR to be a critical regulator of macrophage polarization, as myeloid-specific MR ablation induced an M2 macrophage phenotype.[96] The effect on macrophage polarization and infarct wound healing may explain the effectiveness of the selective MR blocker eplerenone to reduce mortality in patients following acute MI as early as 30 days after randomization.[97] In the Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS), earlier MR antagonism after MI was associated with more favourable outcomes compared with later initiation.[97,98] The scar thickening observed in rats with MI treated with eplerenone may decrease wall stress and prevent infarct expansion as well as remodelling in non-infarcted sites. A reduction in early ventricular remodelling may improve the homogeneity of ventricular conduction and hence reduce the likelihood for life-threatening arrhythmias.

The Impact of Eplerenone on Cardiovascular Outcomes in Patients Post Myocardial Infarction (REMINDER) study (ClinicalTrials.gov Identifier NCT01176968) is testing the effect of immediate MR inhibition on cardiovascular mortality and morbidity in a broader population of patients with acute STEMI undergoing primary percutanous intervention.

New advances concerning the cell-specific role of the MR for ventricular remodelling post-infarction and heart failure progression come from mice with cardiomyocyte-restricted inactivation of the MR gene.[95] These animals displayed improved infarct healing and attenuated

adverse cardiac remodelling and contractile dysfunction. In the late post-MI phase, myocyte hypertrophy, reactive fibrosis, and oxidative stress in the surviving LV myocardium were prevented, while in the early phase, cardiomyocyte-specific MR deletion reduced myocyte apoptosis and infarct expansion and enhanced infarct neovessel formation. Rapid induction of nuclear factor-κB (NF-κB) activation after ischaemia in cardiomyocytes lacking MR, associated with accelerated recruitment of inflammatory cells (neutrophils and monocytes) to the injury site, appears to be the primary mechanism for improved infarct healing and remodelling in cardiomyocyte-specific MR knockout mice.[95]

NF-κB is a key signalling component for early inflammatory activation and recruitment of inflammatory cells after MI.[7] Increased recruitment of inflammatory cells, especially alternatively activated M2 macrophages, during MI healing leads to enhanced neovascularization, reduction in infarct thinning, and improved heart function in mice unable to locally regenerate corticosterone due to 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) deficiency.[99] Exogenous glucocorticoids have been shown to inhibit NF-κB activation and inflammatory cell infiltration and impair infarct wound healing.[7] Moreover, in the setting of MI and oxidative stress, MR may be activated by glucocorticoids.[100,101] Thus, regulation of corticosteroid activity by selective inhibitors of 11β-HSD1 (currently in Phase II clinical trials for the treatment of diabetes) may provide an alternative therapeutic approach for the amelioration of post-infarction healing and remodelling.

5.2 Monocytes/macrophages and wound healing after myocardial ischaemia

Over the last decade, the pivotal role of monocyte/macrophages as regulators and effectors of infarct wound healing has been recognized, indicating a potential therapeutic/preventive target for cardiac remodelling after myocardial ischaemia.[10,102–104] Depletion of monocytes[105] as well as defective macrophage recruitment/activation[106] impairs healing and promotes adverse LV remodelling after myocardial injury. In contrast, stimulation of macrophage infiltration[107] or injection of activated macrophages[108] into the ischaemic myocardium improved LV dysfunction and remodelling, by promoting myocardial healing and repair. However, tight control and timely repression of monocyte recruitment in the ischaemic myocardium is required for optimal infarct wound healing.[11] Kempf *et al.* recently showed that growth differentiation factor-15 (GDF-15), a member of the transforming growth factor (TGF)-β cytokine superfamily, is induced in the infarcted myocardium and plays a key role in controlling inflammatory cell recruitment, by interfering with chemokine-triggered leucocyte β_2 integrin activation. GDF-15 acts as an anti-inflammatory cytokine that represses monocyte recruitment into the healing myocardium, limiting inflammatory tissue damage and promoting survival. Loss of GDF-15 led to increased incidence of LV rupture after MI.[11]

Ingestion of apoptotic cells by macrophages actively suppresses the release of proinflammatory cytokines and induces the secretion of anti-inflammatory mediators, resulting in accelerated resolution of inflammation.[109] Macrophages recognize apoptotic cells via different mechanisms, including recognition of exposed phosphatidylserine. An innovative approach to improve MI repair is based on exogenous administration of defined phosphatidylserine-presenting liposomes as apoptotic-mimicking particles.[110] Immunomodulation of recruited cardiac macrophages by iv administration of phosphatidylserine-presenting liposomes post-infarction promoted angiogenesis and prevented cardiac remodelling. The effect of a 'non-specific' but broad immunomodulatory treatment, using autologous apoptotic leucocytes in a broad population of patients with heart failure, has been tested in the ACCLAIM trial, showing some benefits, however, only in patients without a previous history of MI and with New York Heart Association class II heart failure.[111]

Recently, distinct monocyte subsets with functional properties of inflammatory M1 or alternatively activated M2 macrophages, also known as wound healing macrophages, were shown to be sequentially mobilized during infarct wound healing.[112] Modulation of the ratio or the recruitment timing of the two subsets (e.g. by targeting the chemokine receptors such as CCR2 and CX3CR1) could be a novel therapeutic strategy for improving myocardial repair and remodelling.[102,113] In mice, monocyte subsets can be distinguished on the basis of monocyte chemoattractant protein-1 receptor (CCR2) and the fractalkine receptor (CX3CR1) expression, and the presence of the Ly6C antigen. $Ly6C^{high}$ monocytes express high amounts of the surface protein Ly6C and CCR2, while $Ly6C^{low}$ monocytes highly express CX3CR1. The expression of CCR2 and CX3CR1 are crucial for the recruitment of monocyte subsets into ischaemic tissues. The healing myocardium modulates its chemokine expression profile over time, and sequentially and actively recruits $Ly6C^{high}$ monocytes via CCR2, and $Ly6C^{low}$ monocytes preferentially via CX_3CR1. $Ly6C^{high}$ monocytes dominate the inflammatory phase of infarct healing and exhibit phagocytic and inflammatory functions, whereas $Ly6C^{low}$ monocytes accumulate in the injured myocardium later, to promote healing via revascularization, myofibroblast accumulation, and collagen deposition.[112] Silencing of CCR2 reduced the number of inflammatory $Ly6C^{high}$ monocytes and ischaemia reperfusion injury.[113] Of interest, $CD14^+CD16^-$ ($Ly6C^{high}$ analogs) and $CD14^+CD16^+$ (mostly overlapping with the $Ly6C^{low}$ subpopulation) are sequentially mobilized in patients with acute MI, and the magnitude of $CD14^+CD16^-$ monocyte mobilization is associated with the impairment of myocardial recovery and adverse LV remodelling.[114]

However, the developmental relationship between monocyte subsets and macrophage polarization into phenotypically and functionally distinct cells need further studies. It remains unclear whether distinct monocyte subsets mature into resident monocytes in the blood or polarize to different macrophage phenotypes at the injury site. Macrophages have remarkable plasticity that allows them to phenotypically polarize in response to microenvironmental signals to mount specific M1 or M2 functional programmes.[104,115–117] Emerging data indicate that biphasic regulation of macrophage phenotypes at the site of ischaemic injury characterizes wound healing after myocardial ischaemia.[12] Classically, proinflammatory activated (M1) macrophages, which are a major source of pro-inflammatory cytokines, dominate the initial inflammatory phase and change their activation profile to alternatively activated (M2) macrophages during the proliferative and scar tissue formation phase as the infarct wound matures.[12] Genetic ablation of the class A scavenger receptor promoted macrophage polarization towards an M1 phenotype after MI, with the consequence of sustained inflammation, functional deterioration, and adverse LV dilation.[118] Conversely, MR inhibition, as mentioned above, promoted the switch of macrophages to an M2 activation state during infarct wound healing, resulting in improved wound repair, angiogenesis, and remodelling.[13]

The current evidence suggests that the importance of numbers and/or density of infiltrated macrophages is secondary to dynamic changes in the macrophage polarization state in the infarct wound for the maintenance or resolution of the inflammatory processes.

Modulation of macrophage polarization at the site of ischaemic injury, identifying factors and mechanisms able to modify the balance between the M1 and M2 states, might prove to be an innovative and effective target to enhance myocardial repair and minimize remodelling post-infarction.

Clinical trials attempting to modulate the inflammatory response as well as proinflammatory cytokine pathways in ischaemic heart failure have been largely disappointing.[4,7] The cytokine cascade after myocardial ischaemia allows the myocardium to respond rapidly to tissue injury.[7,10] Although prolonged expression of inflammatory cytokine can lead to myocardial damage and adverse LV remodelling, a limited short-term/transient up-regulation of stress-activated cytokines within the first days after MI appears to play a protective and coordinating role for infarct wound healing. Of note, cytokines may exert different effects in acute and subacute phases of MI, with profound implications for therapies aimed to prevent adverse remodelling.[119,120] Transient up-regulation of STAT3 (the major downstream signalling molecule of IL-6) after myocardial ischaemia exerts cardioprotective effects. STAT3-deficient mice showed enhanced susceptibility to myocardial ischaemia/reperfusion injury with increased cardiac apoptosis and infarct sizes.[121] However, sustained IL-6-dependent and gp130-mediated STAT3 activation in subacute infarction promoted enhanced inflammation, adverse remodelling, high rupture rates, and heart failure.[119]

Moreover, as the inflammatory response during infarct healing involves a complex network of cytokine interactions, an innovative approach should employ combined inhibition/modulation of different cytokines. Therapeutic targeting of inflammation through the time modulation of macrophage polarization and consequently of their cytokine secretion profile appears to be a promising field of research following myocardial ischaemia.

5.3 Cardiac (myo)fibroblasts phenotype/function as a target for wound healing after myocardial ischaemia

During cardiac healing/remodelling fibroblasts are activated to differentiate into myofibroblasts, cells with proliferative, contractile, migratory and secretory properties. New promising therapeutic approaches to post-infarction remodelling specifically target factors that modulate fibroblast phenotype/function in the infarcted myocardium and simultaneously prevent myofibroblast differentiation in the remote non-infarcted myocardium.[14,15,122–124]

Wnt/frizzled proteins are involved in the proliferation, migration, and differentiation of cardiac fibroblasts.[125] Frizzled 1,2 receptors are present in myofibroblasts and are up-regulated in the infarcted myocardium. Immediate inhibition of frizzled 1,2 signalling after MI increased the number of myofibroblasts in the infarcted area, reducing infarct expansion, LV dysfunction, and heart failure-related mortality.[126] However, alterations in myofibroblast function and infiltration into the ischaemic myocardium may be associated with impaired wound/myofibroblast contraction leading to adverse remodelling. The chemokine interferon-γ-inducible protein (IP)-10 modulates fibroblast phenotype and function exerting antifibrotic effects.[127] Bujak *et al*. identified an interferon-γ-IP-10-mediated pathway that plays a key role in the fibrotic reparative response after MI, through mechanisms that involve changes in myofibroblast functional activation resulting in perturbation wound contraction. Genetic ablation of IP-10 substantially increased myofibroblast density, enhanced collagen accumulation in the infarct and border zone, impaired the mechanical properties of the healing wound, and was associated with enhanced expansion of the scar and early adverse dilation.[127]

Melchior-Becker *et al*.[128] recently showed that deficiency of biglycan causes cardiac fibroblasts to differentiate into pro-proliferative myofibroblasts due to increased sensitivity to endogenous TGF-β/Smad2 signalling. In mice lacking biglycan, a distorted and fragile collagen scar[129] as well as increased myofibroblastic response and myofibroblast contraction of the scar[128] led to infarct thinning, cardiac dilation, and dysfunction and a higher incidence of cardiac rupture after MI.

Of note, activation of the TGF-β/Smad3 pathway in the infarct border zone is critically involved in the pathogenesis of cardiac fibrosis and adverse remodelling after myocardial ischaemia. Smad3 loss results in accumulation of abundant but functionally defective fibroblasts that exhibit impaired myofibroblast transdifferentiation, reduced migratory potential, and suppressed expression of fibrosis-associated genes, leading to attenuation of reactive fibrosis, ventricular remodelling, and diastolic dysfunction.[124]

Cardiac fibroblasts, beyond being a major component of the heart, are able to 'detect' danger signals and enhance the inflammatory response after ischaemia. A recent study showed that inflammasome activation in cardiac resident fibroblast plays an essential role in inflammatory response and subsequent injury after ischaemia/reperfusion.[130] Cardiac fibroblasts are involved in a variety of cell–cell interactions, with the other fibroblasts, cardiomyocytes, and endothelial cells, under the coordination of the cytokine network. Targeting this complex cell–cell signalling might be a further option for the therapeutic approach to post-infarction remodelling.

6. Conclusion

Better therapies are needed to reduce the still unresolved burden of adverse cardiac remodelling leading to unacceptable morbidity and mortality especially after large and/or recurrent MI. Strategies addressing myocardial regeneration and pathophysiological mechanisms of infarct wound healing hold promise to prevent progressive dilation and failure. However, the highly complex healing processes post-MI deserve in-depth pathophysiological understanding, especially regarding endogenous cellular infiltration and wound maturation. Fascinating novel therapeutic approaches are on the horizon including elaborate progenitor cell therapy, miRNA modulation, and interference with the inflammatory and fibrotic response. Despite exciting results in animal models of myocardial ischaemia, all these promising approaches will have to prove their superiority in addition to the current standard of care in rigorous clinical trials.

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