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Post-infarct cardiac injury, protection and repair: roles of non-cardiomyocyte multicellular and acellular components

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Following myocardial infarction (MI), cardiomyocytes and infarct size are the focus of our attention when evaluating the extent of cardiac injury, efficacy of therapies or success in repairing the damaged heart by stem cell therapy. Numerous interventions have been shown by pre-clinical studies to be effective in limiting infarct size, and yet clinical trials designed accordingly have yielded disappointing outcomes. The ultimate goal of cardiac protection is to limit the adverse cardiac remodeling. Accumulating studies have revealed that post-infarct remodeling can be attenuated without infarct size limitation. To reconcile this, one needs to appreciate the significance of various cellular and acellular myocardial components that, like cardiomyocytes, undergo significant damage and dysfunction, which impact the ultimate cardiac injury and remodelling. Microvascular injury following ischemia-reperfusion may influence infarct size and promote inflammation. Myocardial injury evokes innate immunity with massive inflammatory infiltration that, although essential for the healing process, exacerbates myocardial injury and damage to extracellular matrix leading to dilative remodeling. It is also important to consider the multiple non-cardiomyocyte components in evaluating therapeutic efficacy. Current research indicates the pivotal role of these components in achieving cardiac regeneration by cell therapy. This review summarizes findings in this field, highlights a broad consideration of therapeutic targets, and recommends cardiac remodeling as the ultimate target.

myocardial infarction, ventricular remodelling, infarct size, inflammation, extracellular matrix, microvascular damage, autonomic nerves

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INTRODUCTION

Cardiac ischemia leads to significant injury to the myocardium including irreversible loss of cardiomyocytes. Reperfusion process, although being critical in salvage of the ischemic but viable myocardium, is associated with further injury (Hausenloy et al., 2016, Hausenloy et al., 2017). Cardiac protection and repair following MI have been dominant topics to pre-clinical and clinical research over several decades. Myocardial infarction (MI) with or without reperfusion is followed by inflammatory and fibrotic processes that are characterized by orchestrated and dynamic changes at the molecular, histological and geometric levels. These serial post-infarct events are critical in determining the extent of acute and chronic ventricular remodeling and dysfunction (Frangogiannis, 2015). Accumulating studies have shown that, in addition to infarct size, the degree of ventricular remodeling is also dependent on ischemic damage to other cell populations and acellular components of the myocardium, which needs to be appreciated when assessing cardiac injury, protective interventions and repair.

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LIMITING INFARCT SIZE OR CARDIOMYOCYTE REGENERATION AS THERAPEUTIC TARGETS

In patients with transmural or ST-elevation MI (STEMI), timely reperfusion has been well established as an effective intervention to limit the transmural degree and the mass of MI (Heusch and Gersh, 2017). Over the last three decades, major effort and resource have been devoted to research on post-ischemic cardioprotection, in particular, limiting infarct size by pharmacological or non-drug interventions. The current pre-clinical literature on this topic, however, is fraught with controversies (Lefer and Bolli, 2011; Jones et al., 2015). Pre-clinical studies have shown numerous cardioprotective therapies being effective in limiting infarct size (Hausenloy et al., 2016; Hausenloy et al., 2017). However, the infarct size-limiting effect of these interventions are not reproducible across different animal species, only with exception of the ischemic pre-conditioning (Jones et al., 2015). Furthermore, in the last few decades almost all clinical trials designed based on the pre-clinical findings have failed leaving the clinical translation of infarct size-limiting therapies being illusive (Kloner, 2013; Hausenloy et al., 2016; Hausenloy et al., 2017). Indeed, questions have been raised challenging the concept of limiting infarct size as the principal of cardioprotective therapy to improve clinical outcomes (Heusch and Rassaf, 2016). Another active research filed is stem cell therapy of the infarct heart targeting regeneration of working heart muscle. Different types of stem cells have been tested *in vivo* with certain degree of benefits, which occur with a limited degree of myogenesis irrespective of cell types tested. This outcome casts doubt on the feasibility of regenerating working myocardium (Heusch and Rassaf, 2016; Heusch and Gersh, 2017), but indicates the operation of paracrine mechanisms by introduced stem cells (Hodgkinson et al., 2016).

Meanwhile, accumulating studies have indicated that attenuation of ventricular remodeling and dysfunction can be achieved without reduction in infarct size. Thus, limiting ventricular remodeling rather than infarct size is worthwhile to be considered as the primary and ultimate therapeutic goal. This review will summarize the research findings in this field focusing on the significance of non-cardiomyocyte cellular populations including inflammatory cells, vascular endothelial cells and extracellular matrix (ECM).

CELLULAR AND ACELLULAR COMPONENTS OF THE MYOCARDIUM AND THEIR FUNCTIONALITY

To reconcile the infarct size-independent therapeutic benefits, one needs to appreciate the complex microstructur of the myocardium containing multicellular and acellular components each with unique functionalities. Occupying the largest volume of the myocardium, cardiomyocytes constantly perform dynamic contraction and relaxation. The ECM forms a fine and complex network architecture surrounding individual cardiomyocytes and muscle bundles as well as other cellular components of the myocardium. In addition to transmitting force generated by beating cardiomyocytes evenly across the entire wall, ECM also the major determinant of myocardial stiffness and tensile strength (Figure 1). Each cardiomyocyte is supported by several capillary vessels. The basic function of microvessels lies in two aspects: (i) providing nutritional blood flow to meet the need of high energy expenditure of cardiomyocytes, and (ii) acting as a barrier to prevent blood components from free access to tissue cells (Zhu, 2014). Microvessels ensure adequate and workload-dependent changes in myocardial blood perfusion and are pivotal in adaptive remodeling under diseased settings. Pericytes are smooth muscle-like cells that wrap around microvessels regulating microcirculation and barrier function of capillaries, as well as interacting with immune cells and stem cells in physiological and pathological settings (Katare et al., 2011; Avolio et al., 2015). Another independent micorvasculature is lymphatic system, which regulates tissue fluid generation/return and regulates regional immune function by transporting immune cells mainly lymphatic cells (Figure 1). The sympathetic and parasympathetic nerves form another components and our current knowledge on their functionality is largely limited to their regulation of chronotropy, inotropy and lusitrophy.

In the healthy heart, cardiomyocytes account for approximately 25% of total cellular population outnumbered by vascular endothelial cells (approximately 50%), while fibroblasts form another 15% of total cells (Pinto et al., 2016). Pronounced changes occur in cardiac cellular populations following MI, most notably massive accumulation of inflammatory cells particularly neutrophils and monocytes, and subsequent increase in fibroblasts in the infarct as well as remote regions (Frangogiannis, 2015). Regional extra-vascular accumulation of platelets has also been demonstrated (Du et al., 2011; Liu et al., 2011). Intense innate immune response proceeds the formation of granular tissues and the proliferation and activation of fibroblasts. These changes significantly impact the regional and dynamic changes the architecture of infarct wall during early and chronic phases after MI.

DAMAGE TO BLOOD MICROVESSELS

Following restoration of blood flow of the culprit coronary artery, tissue blood perfusion to part of the jeopardized myocardium remains compromised, a clinical situation

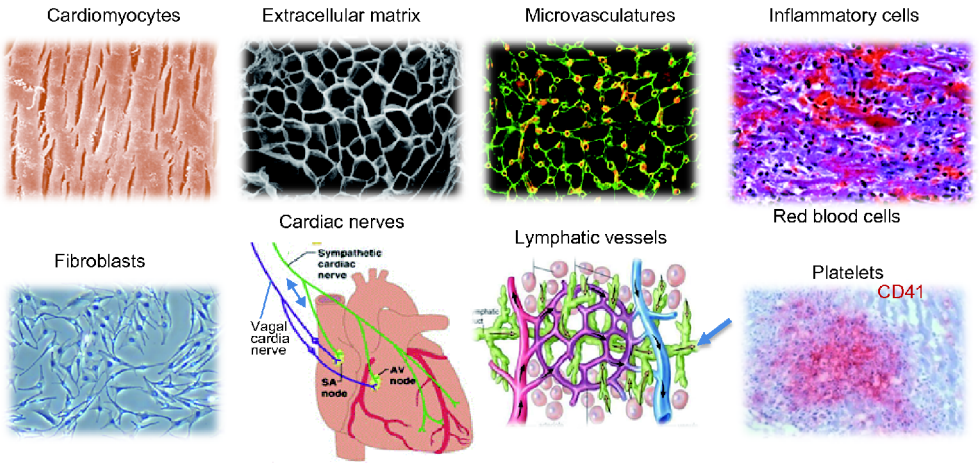

Figure 1 Various cellular and acellular components of the myocardium and changes in the cellular types following MI. Cardiomyocytes, fibroblasts, microvasculatures, lymphatic vessels and extracellular matrix (ECM) form major acellular and acellular structures of the myocardium. Autonomic nerves innervating the heart also impact cardiac function as well as responses to MI. It is also important to appreciate the dramatic changes in cellular populations within the infarct myocardium with pronounced increases in inflammatory cells, platelets and subsequently fibroblasts along the course of MI.

called "no-reflow" phenomenon or microvascular obstruction (MVO). MVO occurs in approximately 40% to 80% of cases (Ndrepepa et al., 2010; Eitel et al., 2011; Hamirani et al., 2014; Carrick et al., 2016). Numerous clinical studies have shown that presence of MVO, diagnosed by cardiac imaging techniques, including cardiac magnetic resonance (CMR), is associated with increased risk of major adverse cardiac events, larger infarct size, more pronounced ventricular remodeling, and poor survival (Ndrepepa et al., 2010; Eitel et al., 2011). In the clinical setting, MVO is believed to be due to blockade-from-inside, most likely by atherothrombotic debris entering into coronary blood stream during primary percultaneous coronary intervention (PCI) (Kloner, 2011). Blockade of capillary blood flow could also be caused by inflammatory cell adhesion to endothelial cells, intravascular platelet aggregation, endothelial cell swelling or vasoconstriction of arterioles (Kloner, 2011). Clinical studies have revealed that of all patients exhibiting MVO, MVO persists for several days in about 50% of cases and that the sustained form of MVO is associated with adverse clinical outcomes (Funaro et al., 2011). The reason(s) responsible for persistency of MVO remains unclear. Owing to our limited understanding of the risk factors and absence of effective therapy, MVO is regarded as a major hurdle in achieving salvage of ischemic myocardium by PCI.

Microvasculature also has barrier function by which plasma proteins, ions and event water pass through single layer capillary wall in a tightly controlled manner (Zhu, 2014). This function is pivotal to tissue health and achieved through inter-cellular tight junctions and adherent junctions (Hirase and Node, 2012). Myocardial ischemia and inflammatory signalling are known to disintegrate endothelial junctions via intracellular signalling resulting in hyper-permeability or microvascular leakage (MVL) (Gao et al., 2017). Thus, MVL likely occurs via mechanisms distinct from that for MVO (Figure 2). We recently studied the feature of MVL following reperfused MI in mice. Using a permeability tracer, we identified the presence of MVL that involves approximately 75% of the ischemic myocardium, much more extensive than that of infarct size (47%) or MVO (36%) determined under the similar ischemia-reperfusion conditions (Gao et al., 2017). The severity of MVL is in proportion to the duration of both ischemia and reperfusion, and correlated to the degree of acute LV dilatation and decline in ejection fraction. Importantly, we demonstrated, for the first time, that MVL is able to be imaged by CMR (Gao et al., 2017). We speculate that presence of MVL would facilitate regional oedema and inflammatory infiltration, which could exacerbate or sustain MVO by the mechanism of "blockade-from-outside" (Figure 2). Indeed, clinical CMR studies have indicated the loss of microvascular integrity manifested as myocardial oedema and intramyocardial hemorrhage, which are associated with adverse clinical outcomes independent of MVO (Eitel et al., 2011; Hamirani et al., 2014; Carrick et al., 2016). Further study is warranted to investigate therapeutic interventions that could minimize the extent of MVL as well as the sustained form of MVO. The capability of CMR imaging of both MVO and MVL would certainly facilitate research in this field.

INJURY AND EARLY RESPONSES OF FIBROBLASTS TO ISCHEMIA-REPERFUSION

Cell culture studies have investigated the fate of fibroblasts and cardiomyocytes subjected to hypoxia-reoxygenation simulating ischemia-reperfusion. In cultured neonatal cardiomyocytes, cell death becomes evident following hypoxia

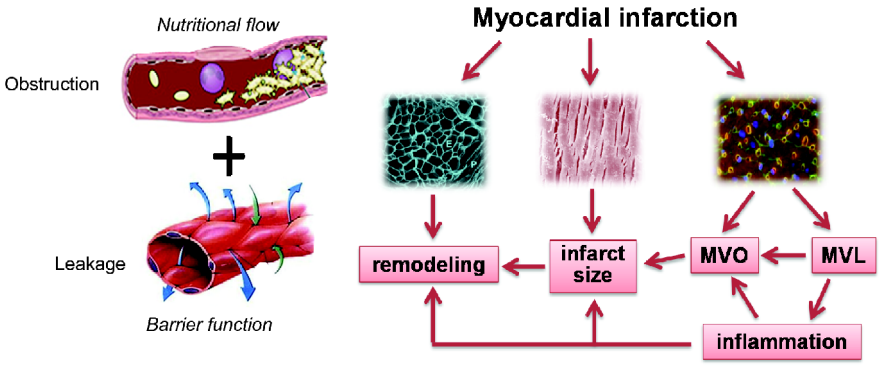

Figure 2 Basic functionality of microvasculatures of the myocardium and changes following MI. Microvessels deliver nutritional blood flow to the myocardium while keep tissue health by its barrier function. Reperfused MI leads to microvascular damage that impairs both pivotal function, manifested as MVO and MVL.

over 1 h (Portal et al., 2013), whereas fibroblasts are apparently more resistant to hypoxia with cell death occurring over 8 h after exposure to hypoxia (Vivar et al., 2013). Reoxygenation similarly induced apoptotic cell death of both types of cells (Portal et al., 2013; Vivar et al., 2013). Interestingly, cardiomyocytes co-cultured with fibroblasts are protected under simulated ischemia-reperfusion through paracrine mechanisms that involve tissue inhibitor of metalloproteinase, phosphoinositide 3-kinase (PI3K)/Akt and ERK1/2 (Abrial et al., 2014). On the other hand, fibroblasts also contribute to regional inflammatory response through activation of inflammasome, by which production of cytokines such as interleukin-1 (IL-1) is enhanced (Kawaguchi et al., 2011). During the sub-acute and chronic phases, proliferation and activation of residential fibroblasts or fibroblasts derived from other sources, such as endothelial-mesenchymal transition, are critical for the formation of granular tissues and fibrotic wound healing, but also development of interstitial fibrosis within the remote myocardium (Frangogiannis, 2015).

DAMAGE TO EXTRACELLULAR MATRIX AND RELATED COMPLICATIONS

MI is associated with dense infiltration by inflammatory cells, particularly neutrophils and monocytes. These cells express at high levels of proteinases such as matrix metalloproteinases (MMP) and myeloperoxidase, enzymes that degrade ECM proteins. Studies using strains of mice with specific gene deletion have shown that accumulation and activation of these enzymes, e.g. MMP-2, MMP-8, MMP-9 and myeloperoxidase, are the major mechanism responsible for breakdown of existing collagen networks in the infarct region (Gao et al., 2012). We demonstrated in mice breakdown of fibril or cross-linked collagen during 2–4 days after MI that was temporarily associated with a dramatic reduction in the tensile strength of the infarcted ventricular wall and the onset of cardiac rupture during 3–5 days after MI (Gao et al., 2005; Fang et al., 2007; Gao et al., 2012). Interventions such as gene deletion of MMP-2, MMP-9, myeloperoxidase or a cytokine macrophage-migration inhibitory factor (MIF), or treatment with MMP inhibitors, are effective in reducing the risk of wall rupture, a hard index of post-infarct ECM damage (Heymans et al., 1999; Askari et al., 2003; Matsumura et al., 2005; Fang et al., 2007; White et al., 2014). In addition to inflammatory cells, there is extra-vascular accumulation of platelets or even formation of intra-myocardial thrombus, changes associated with activation of circulating platelets and increased platelet-monocyte conjugation (Du et al., 2011; Liu et al., 2011; Gao et al., 2016). The pro-inflammatory action of platelets is largely through conjugating to and activating monocytes thereby promoting myocardial monocyte infiltration (Gawaz et al., 2005). We explored the role of platelets in post-MI inflammatory response, ventricular remodeling and cardiac rupture. A brief period of treatment of mice with anti-platelet drugs such as P2Y12 antagonists or CD41 antibody to induce thrombocytopenia, effectively prevented cardiac rupture acutely and ameliorated ventricular remodeling chronically (Du et al., 2011; Liu et al., 2011). Platelet-monocyte conjugation of peripheral blood was simultaneously reduced by the interventions tested. These findings highlight the feasibility of attenuating ECM damage as a consequence of acute inflammation as therapeutic approach to limit acute ventricular expansion and related complications including rupture and dilative remodeling.

Pre-clinical and clinical studies testing anti-inflammatory drugs in the setting of MI have failed to show any additional benefit to the current standard therapy and some revealed impaired healing with resultant complications (Seropian et al., 2014). It should be noted that inflammatory signalling and macrophages are critical for fibrotic healing and repairing process (Nahrendorf and Swirski, 2013; Aurora et al.,

2014; Frangogiannis, 2015). The timing of anti-inflammatory interventions is therefore critically related to final outcomes. In several mouse strains with permanent deletion of genes such as MMP-2, MMP-9, plasminogen system, myeloperoxidase or MIF, mice subjected to chronic MI showed an impaired fibrotic healing, worsening of ventricular dilatation with adverse functional and survival outcomes (Gao et al., 2012). In contrast, anti-inflammatory interventions, including inhibitors of MMPs, MIF or platelet P2Y12 or platelet depletion, given to mice only within the first 2 days after MI, were protective measured by the degree of acute infarct expansion, infarct size, risk of cardiac rupture and chronic ventricular remodelling, without detectable adverse effects (Fang et al., 2007; Du et al., 2011; Liu et al., 2011; Gao et al., 2012; Gao et al., 2016). The mouse model of MI exhibits intense inflammatory response followed a fibrotic healing that becomes largely completed by 7 to 14 days. Prolonged drug treatment targeting key inflammatory molecules such as MMPs leads to adverse consequences evidenced by more severe infarct wall thinning, poor fibrotic healing, increased risk of delayed onset of cardiac rupture and dilative remodelling (Iyer et al., 2016). Interestingly, clinical trials testing anti-MMP drugs such as doxycycline, given within the first 7 days after MI, were beneficial in limiting ventricular dilatation and improving cardiac function (Cerisano et al., 2017).

DAMAGE TO LYMPHATIC VESSEL SYSTEM

Lymphatic vessels regulate composition and volume of interstitial fluid and regional inflammation (Karkkainen et al., 2001; Nakamura and Rockson, 2008; Huggenberger et al., 2011). Interstitial fluid is generated from blood capillaries containing proteins and metabolites. Absorption of interstitial fluid back to the circulation via lymphatic system is primarily important for the healthy and function of the myocardium. Impaired lymphatic flow in diseased settings would lead to accumulation of excessive fluid and proteins in the interstitium. Experimental obstruction of cardiac lymphatics results in myocardial oedema, interstitial fibrosis and coronary vascular pathology (Solti et al., 1994; Nakamura and Rockson, 2008), as well as systolic dysfunction (Laine and Allen, 1991). There have been limited studies on changes in lymphatic vessels along the course of MI. During the early phase of MI, cardiac lymphatic flow increased significantly, albeit this is usually insufficient to alleviate regional oedema (Nakamura and Rockson, 2008). Using immunohistochemistry, Ishikawa et al. studied heart tissues from autopsied patients suffered from acute or chronic MI, and observed loss of lymphatic vessels during the early phase, followed by lympha-angiogenesis together with the formation of granular tissues in the sub-acute and chronic phases, likely due to enhanced expression by viable cardiomyocytes of vascular endothelial growth factor (VEGF)-C that binds to lymphatic vessel-specific VEGF-receptor-3 (VEGFR-3) (Ishikawa et al., 2007). A recent study in mice observed significant regional lympha-angiogenesis measured by a sustained upregulation of VEGFR-3 as a lymphatic vessel specific marker with its peak at day-4 after MI (Klotz et al., 2015). Immunological staining of VEGFR-3 revealed increase in the size and density of lymphatic vessels at the infarct region. Treatment with VEFG-C during the first week of MI further stimulated lympha-angiogenesis together with a significant reduction in infarct size and sustained improvement in LV ejection fraction (Klotz et al., 2015). Further studies are warranted to investigate the role of lymphatic vessels in the regulation of regional inflammation and tissue oedema, and potential interactions with microvascular damage, especially MVL.

INFLAMMATORY SIGNALLING EXACERBATES MYOCARDIAL INJURY

Clearance of necrotic tissues is achieved through breakdown of infarcted tissues and phagocytosis by inflammatory cells, which is essential for wound healing and scar formation. Such a dynamic process impacts ventricular remodeling at acute and chronic phases. The nature of inflammatory response following MI is the innate immunity triggered by release of danger associated molecular patterns (DAMPs). DAMPs refer to a complex mixture of molecules released from damaged tissues or cells, and are potent in activating toll-like receptors (TLR) and Nod-like receptors (NLR), receptors known to be critical in mediating innate immunity. DAMPs bind to TLRs and NLRs localize on inflammatory cells, endothelial cells, fibroblasts or platelets (Timmers et al., 2012). Activation of these receptors triggers adaptor proteins such as nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPK), leading to transcriptional activation of pro-inflammatory cytokines and chemokines (Hulsmans et al., 2016; Nahrendorf and Swirski, 2016). β_2-Adrenergic receptors (AR) is the major subtype of β-ARs highly expressed in immune cells regulating a variety of cellular function (Padro and Sanders, 2014). Genetic knockout of β_2-AR in immune cells blunted leukocyte infiltration in response to MI and impaired fibrotic healing leading severe infarct expansion and an exceedingly high fatality owing to a delayed onset of cardiac rupture (Grisanti et al., 2016).

Increasing evidence indicates that post-MI inflammation could exacerbate the ischemic myocardial injury through a mechanism of inflammatory cell death or programmed necrosis (necroptosis), which forms a potential therapeutic target (Figure 3). Studies have shown that inactivation of

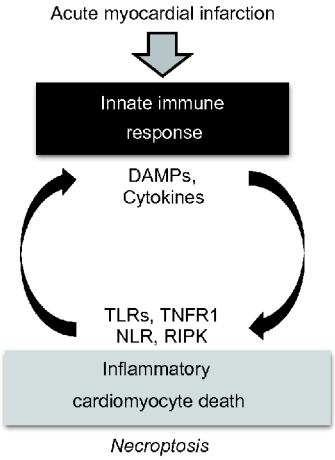

Figure 3 Inflammatory signalling exacerbates myocardial injury forming vicious cycle. PRRs, pattern recognition receptors; TNFR1, tumour necrosis factor receptor-1; RIPK, receptor-interacting protein kinase. RIPK1 is upstream trigger of necrosome; RIPK3 is the key molecule in necroptosis. Necroptosis refers to programmed form of necrosis.

pro-inflammatory molecules such as MIF (Gao et al., 2011), IL-6 (Jong et al., 2016), IL-1α (Lugrin et al., 2015) or TNFα (Sun et al., 2004), suppressed inflammatory response as well as the extent of myocardial injury, albeit some studies have also revealed insufficient fibrotic healing owing to attenuation of proceeding inflammation (Gao et al., 2012). Studies have also demonstrated that the spleen is a pivotal organ contributing to post-MI innate immune response following MI by splenic release of significant amount of pro-inflammatory monocytes and platelets (Swirski et al., 2009; Nahrendorf and Swirski, 2013; Gao et al., 2016; Nahrendorf and Swirski, 2016). Removal of the spleen immediately prior to MI is able to significantly reduce cardiac inflammatory infiltration and infarct size (Goltz et al., 2015; Gao et al., 2016), finding further implying adverse consequence of intense inflammation after MI.

DELAYED REPERFUSION CONFERS CARDIAC PROTECTION WITHOUT CHANGE IN INFARCT SIZE

Cardiac ischemia leads to a wave-front extension of myocardial necrosis from the endocardial to epicardial layers (Reimer et al., 1993). It is usually hold that in human patients, myocardial necrosis is completed within approximately 6 h after coronary artery occlusion. Hence myocardial salvage is expected if reperfusion commences within 6 h. However, an meta-analysis of clinical studies on STEMI patients revealed that delayed reperfusion, i.e. during 12 to 24 h post-MI, remains to be beneficial judged by survival, LV ejection fraction and ventricular remodelling (Takemura et al., 2009). Clinical trials have been conducted to test benefits by primary PCI-mediated reperfusion during 24 h to 28 days after the onset of MI. The outcomes are inconsistent for reperfusion achieved during 12–24 h but clearly lack of effect when reperfusion occurred at a mean of 8 days post-MI (Takemura et al., 2009).

Typically, compared to a reperfused MI, MI induced by permanent coronary artery occlusion involving a similar size of area-at-risk leads to more pronounced dilative remodeling and dysfunction. In human patients with STEMI, the incidence of cardiac rupture has undergone dramatic decline from 7%–10% prior to 1990s to current approximately 1% (Gao et al., 2012). This period has also witnessed the establishment of pharmacological and device-based reperfusion therapies as the clinical routines (Gao et al., 2012). In mouse model of MI-induced cardiac rupture, we observed that reperfusion *per se* effectively abolished the onset of rupture (Gao et al., 2005; Gao et al., 2012). Such protection is also evident in mice subjected to ischemia for 4 h followed by reperfusion. A prolonged ischemia up to 4 h in mice would result in a transmural infarction similar to that seen by permanent coronary artery occlusion and yet, rupture is prevented. Nakagawa et al. studied in rats the effect of reperfusion after 24-hour coronary artery occlusion (Nakagawa et al., 2008). Despite a comparable infarct size to that of non-reperfused MI group, during 1 to 4 weeks after MI, ventricular dilatation was significantly suppressed while LV fractional shortening improved in reperfused MI group (Nakagawa et al., 2008). Histological examination revealed thickening of the transmural ventricular wall indicating an improved regional wall stress. The benefits of late reperfusion are likely attributable to protection of other cellular populations and acellular component ECM.

CARDIAC SYMPATHETIC AND PARASYMPATHETIC NERVES

Cardiac autonomic nerves have long been regarded as the regulatory machinery of cardiac function. MI is known to interfere with efferent conduction of cardiac sympathetic and parasympathetic nerves (Inoue and Zipes, 1988; Du et al., 1998). Impaired efferent parasympathetic activity following MI is indicated by reduced heart rate variability or baroreflex sensitivity that bears prognostic significance (Inoue and Zipes, 1988; Schwartz et al., 1988; Casolo et al., 1992). We previously showed in rat model of chromic MI that attenuated efferent transmission of vagal nerves was reversed by the use of losartan suggesting that this phenomenon is at least in part mediated by the inhibitory pre-synaptic angiotensin-type 1 receptors (Du et al., 1998).

Recent studies have revealed influence of both sympathetic and parasympathetic neuronal limbs in regulating cardiac remodeling or repair. Studies conducted in different laboratory species such as rats (Li et al., 2004; Li et al., 2015;

Agarwal et al., 2016), rabbits (Uemura et al., 2010) or guinea pigs (Beaumont et al., 2015) have consistently shown that chronic stimulation of vagal nerves attenuated the extent of dilative remodelling and improved LV ejection fraction. The potential mechanisms involve anti-inflammatory action and heart rate reduction by activation of the parasympathetic nerves. Using sympathetic neuron-specific expression of a fluorescence protein (tdTOMATO), White et al. found, in neonatal mice, regional proliferation of cardiac sympathetic nerves following apical amputation (White et al., 2015). They further showed that, unlike a complete regeneration of neonatal mouse hearts at 4 weeks after apical amputation, damage to sympathetic nerves by treatment with 6-hydroxydopamine abolished regenerative capability of neonatal hearts, instead, formation of fibrotic scar was observed following apical amputation (White et al., 2015). This finding implies a pivotal role of the sympathetic nerves in neonatal cardiac regeneration and a new strategic approach for adult cardiac repair.

DISTINCT FEATURES OF ISCHEMIA-REPERFUSION INJURY TO DIFFERENT COMPONENTS OF MYOCARDIUM

Following MI, injury occurs to cardiomyocytes as well as to other cell types and acellular components. Damage to these components and subsequent resolution or changes in functionality are critical in determining the overall myocardial damage and ultimate ventricular remodeling. As discussed previously, salvage of the ischemic myocardium in human patients is achievable when reperfusion commences within 6 h. However, reversible and irreversible injuries to other cell types and ECM likely possess different time-course, which remain to be defined. For instance, fibroblasts are able to survive much longer than cardiomyocytes in this setting. While the onset of MVO may occur shortly after PCI, it may last for several days (Reffelmann et al., 2003; Eitel et al., 2011; Gao et al., 2017). MVL is not only dependent on the ischemic duration, but also on the reperfusion duration with its development reaching its maximum after reperfusion for 48 h in mice (Gao et al., 2017). Similarly, damage to the existing ECM in the ischemic myocardium parallel the establishment of regional inflammation within a few days post-MI (Fang et al., 2007, Spinale et al., 2016). Following initial loss, the lymphatic vessels show sustained proliferative growth that is associated with limited infarct size (Ishikawa et al., 2007; Klotz et al., 2015). Therefore, interventions could be given aimed at protecting different components of the myocardium even though the time-window for limiting infarct size has elapsed. In this regard, acute LV dilative remodelling is progressing over the first several days apparently determined not only by the infarct size, but also by damage to other myocardial structures.

IMPLICATIONS OF CELL-BASED THERAPY FOR MYOCARDIAL INFARCTION

Stem cell therapy is proposed to repair the infarcted heart by regeneration of working cardiomyocytes in replacement of scar formation. Despite enormous research effort, many hurdles remain in achieving cardiac regenerative repair (Heusch and Gersh, 2017). Adult mammalian heart has a limited ability to regenerate as their intrinsic stem cells are unable to differentiate into cardiac specific cells (Porrello et al., 2011). Furthermore, rigid fibrotic tissues associated with MI act as a barrier to introduced stem cells in general and impacts on their viability as well as their ability to proliferate, migrate to sites of damage and integrate with resident cardiac cells (Ramkisoensing et al., 2014). Another important reason is the insufficient attention given to the impact of non-cardiomyocyte cell populations, ECM and inflammation on the outcome of cell-based therapy. Recent effort has been given to address the changes in transcriptional profiles of non-cardiomyocyte cell populations in relation to the capability of cardiac regeneration (Quaife-Ryan et al., 2017). Similarly, Kanazawa et al. reported in swine model of reperfused MI that intra-coronary delivery of cardiosphere-derived cells not only reduced infarct size by 27%, but also limited to a similar scale the size of ischemic myocardium with MVO implying a casual relation (Kanazawa et al., 2015). Increasing number of studies have indicated the significance of certain types of inflammatory cells, e.g. macrophages, in cardiac repair and myocardial regeneration. Adult mammals including human exhibit limited regenerative capacity following MI, the neonatal mouse, however, is capable of regenerating injured myocardium (Porrello et al., 2011). Current studies have shown that such capability is critically dependent on several cardiomcyocyte-independent factors, including regionally originated macrophages (Aurora et al., 2014), angiogenesis (Malek Mohammadi et al., 2017), sympathetic reinnervation (White et al., 2015) and ECM-localized signalling protein periostin (Chen et al., 2017). In the setting of chronic MI, however, regional hyper-innervation around the infarcted region has been observed and activation of cardiac sympathetic nerves is able to trigger ventricular arrhythmias, action that is dependent on the infarct size (Du et al., 1998; White et al., 2015; Yu et al., 2016).

In the context of cardiac repair, stem cells or progenitor cells are introduced into damaged myocardial for the aim of cardiomyocyte regeneration. However, paracrine actions of the introduced cells with improved healing are generally agreed as the major reason for the benefits by cell therapy independent of myogenesis (Hodgkinson et al., 2016). Re-

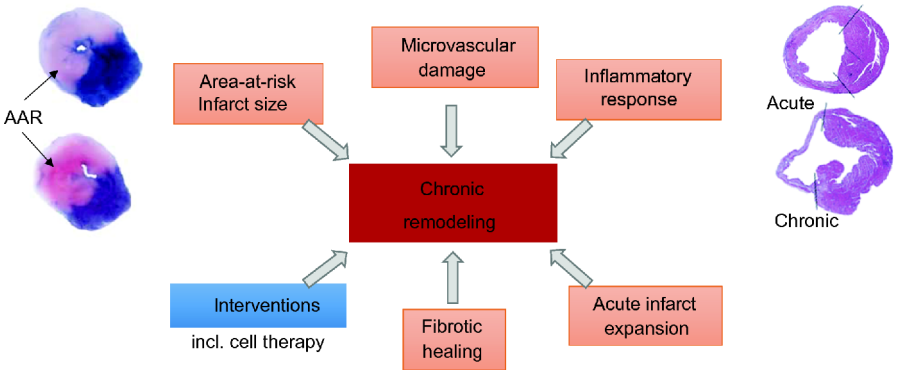

Figure 4 Factors to be considered in the evaluation of myocardial injury and efficacy of therapeutic interventions. Ventricular remodeling is determined not only by area-at-risk (AAR) or infarct size, but also by injury to other tissue components and subsequent inflammatory and healing processes. In this regard, remodelling is recommended as the ultimate therapeutic target.

cently, matrix-focused strategies have been undertaken to increase stem cell homing and survival as well as neovascularity (Ramkisoensing et al., 2014; Guyette et al., 2016). Studies have shown that three-dimensional matrix architecture with adequate fibre orientation and stiffness are critical for myogenic differentiation and interactions of newly differentiated cardiomyocytes with native cells (Segers and Lee, 2011; Taylor et al., 2017). It has been successful in regenerating a new heart by re-seeding stem cells to an acellular heart with preserved three-dimensional ECM scaffold (Guyette et al., 2016). This finding indicates the significance of such ECM scaffold in facilitating myogenic differentiation and promoting cell-cell and cell-matrix interactions.

CONCLUSION

Whereas infarct size is a determinant for the clinical outcomes and long-term prognosis after MI, ultimately, ventricular remodeling *per se* should be taken as the primary and final target in cardiac protection and repair (Figure 4). As discussed above, in addition to infarct size, other factors, including inflammatory response, microvascular injury, ECM damage and fibrotic healing, also influence the extent of cardiac remodeling (Figure 4). Infarct expansion refers to acute stretch of the infarcted region without extension of infarct mass. Such morphological change results in acute enlargement of the infarct segment that impacts significantly the degree of dilative remodeling in the chronic phase, when the architecture of stretched infarct segment becoming permanent by scar formation. There has been discrepancy between findings from *in vitro* hypoxia-reoxygenation model and *in vivo* ischemia/reperfusion models. For example, angiopoitein-like protein 4 reduces infarct size following ischemia-reperfusion *in vivo* but does not prevent cardiomyocyte death *in vitro* (Galaup et al., 2012), indicating that other cell types are key to this cardioprotective effect. Likewise, factors that suppress cardiomyocyte cell death in culture may not be able to reduce infarct size *in vivo*, implicating a role of non-cardiomyocyte factors that counterbalance such action.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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