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REVIEW ARTICLE

Molecular and cellular pathophysiology of circulating cardiomyocyte-specific cell free DNA (cfDNA): Biomarkers of heart failure and potential therapeutic targets

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Abstract Pathological cardiac damage during heart failure is associated with cell death and damage associated molecular patterns (DAMPs) release which triggers a viscous cycle of sterile inflammation to mediate maladaptive cardiac tissue remodelling during the progression to heart failure. DAMPs like cytokines, chemokines, and nuclear or mitochondrial genomic fragments are released in the pathological myocardium. Interestingly, circulating or cytosolic DNA fragments can play a role in the disease by interaction with nucleic acid sensors expressed in cardiomyocyte and non-myocyte neighbouring cells. The circulating cell free DNA (cfDNA) fragments have been clinically reported as markers for various diseases including cardiovascular pathophysiology. Such cfDNA within the DAMP pool can mediate intra- and inter-cellular signalling cascade to upregulate transcriptional expression of inflammatory mediators and trigger oxidative stress within cells. The cellular role of such genomic equivalents varying with chronic or acute stress might be correlated with the cell death forms encountered in myocardium during disease progression. Thus, cfDNA can be phenotypically correlated as a critical player towards upregulation of pathological processes like interstitial fibrosis, cardiomyocyte contractile dysfunction and cell death. Herein, we review the association of cfDNA with heart failure and analyse their potential usage as novel and effective therapeutic targets towards augmentation of cardiac function.

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Introduction

Cardiovascular pathophysiology has been associated with various forms of cell death among which apoptosis, necrosis and autophagy of cardiomyocytes are predominant.[1] The heterogeneous cardiac interstitium is composed of 30%–40% cardiomyocytes embedded within an extracellular matrix (ECM) scaffold along with fibroblasts, macrophages and other non-myocytes.[2] During pathological conditions endogenous damage associated molecular patterns (DAMPs) like cytokines, chemokines, or circulating endogenous nucleic acids *viz*. cell free DNA (cfDNA) or free RNA are released from myocardium as "danger signals".[3,4] Further, the myocardial debris comprising of cfDNA from dead or injured cardiomyocytes reportedly trigger intercellular communication among cardiac resident cells (CRCs) leading to fibrotic scar formation and ECM remodelling which are the hallmark manifestations of maladaptive cardiac remodelling.[1]

Patients with heart failure (HF), like chronic pathologies, show significant elevated blood cfDNA levels (~1,500 ng/mL) compared to the healthy control groups (<10–50 ng/mL).[5,6] The cfDNA mediates intercellular communication by nucleic acid sensors associated with cell membranes or those localized on membranes of intracellular compartments.[7] Interestingly, the presence of such cfDNA within tissue microenvironment might trigger necrosis, necroptosis and pyroptosis, *vis-à-vis* aggravation of inflammation.[6,8] Various DNA sensors like pattern recognition receptors (*viz*. Toll-like receptors), receptor for advanced glycation end products (RAGE) and absent in melanoma 2 (AIM2) have been found to be instrumental in cfDNA-mediated intrinsic inflammatory gene expression during chronic pathologies.[7,9]

During HF, cardiomyocytes, fibroblasts, resident macrophages or infiltrated immune cells of the myocardium show DNA sensor activation.[10] Clinical reports have confirmed association of circulating cfDNA with cardiac pathophysiology along with augmented inflammation.[5,11] The complex DAMP-mediated intercellular cross-talk originating from cardiomyocytes post-myocardial injury on one hand modulates cardiac metabolism, contractile function and fibrosis, while on the other hand aggravates chronic inflammation and cell death.[3] Thus, the autocrine and paracrine interactions between cardiac resident cells trigger systolic and diastolic dysfunction by geometrical and biomechanical maladaptive cardiac remodelling.

Biology of cfDNA during pathologies

Nucleic acids, known to be associated with inheritance in intact cells, were generally not expected to be circulating in body fluids. However, the surprising discovery of extracellular nucleic acids floating in human plasma by Mandel and Métais in 1948,[12] later characterized as tumor-derived double stranded DNA fraction,[13] opened the gates of a new cancer biomarker that may be used to detect mutations present in the genome of tumor cells.[14] The biological features of cfDNA described till date largely correspond to its source, release mechanisms and fragment size, wherein, DNA fragmentation is dependent upon nucleosome wrapping and ranges from as small as 100 bp to ~30,000 bp.[6,15] Reportedly, fragments <100 bp have been described as mitochondrial DNA.[16] Longer DNA fragments are a product of necrotic cell death, while apoptotic DNA fragments might be much shorter (~140–200 bp).[6] Generally, cfDNA enters in the blood stream through active and passive release mechanisms: *active mechanisms* involve spontaneous release of cfDNA (nuclear and mitochondrial fragmented DNA) in free or encapsulated form,[15,17] while *passive mechanisms* involve cfDNA release by cell injury/death pathways like apoptosis, necrosis, etc.[17,18]

In this review, we would explore the pathological aspect of cfDNA released in myocardium during chronic HF and the subsequent inter-cellular cross-talk leading to cell death or fibrosis. The scientific literature survey explores role of fragmented circulating mitochondrial DNA, or nuclear DNA towards modulating innate immune response; however, the overall mechanistic role of cfDNA fragments towards progression of cardiac pathophysiology has been elusive.

Cardiomyocyte pathophysiology and heart failure

Pathophysiological heart failure is characterized by reduced cardiac output, caused due to increased fibrosis, cardiomyocyte death and loss of contractility.[19,20] Structurally, cardiomyocytes perform the majority of contractile functions in the heart and fibroblasts constitute the majority of cell mass, while macrophages and endothelial cells are other resident cells of myocardium.[21] Cardiomyocytes are the first target and responders during myocardial exposure to damaging mechanical impacts such as hemodynamic stress, pressure overload, and volume overload.[22] In order to strive, cardiomyocytes initially respond by structural and functional remodelling which is characterised by increased cell size and mass as an adaptive mechanism—referred to *hypertrophy*.[22,23] Persisting mechanical stress by biochemical stressors in cardiomyocyte triggers cell death pathways including autophagy, apoptosis and necrosis[24] thereby releasing inflammatory DAMP molecules and triggering the *pathological* form of hypertrophy[3,25] (Fig. 1A). The intercellular communication by DAMPs triggers positive feedback within myocardial resident cells leading to an inflammatory burst.

Role of cfDNA as mediators of cardiac inflammation

Myocardial DAMPs are released from cardiomyocyte in the "*inflammatory phase*" of cardiac pathophysiology.[26] DAMPs, via autocrine action, trigger cardiomyocyte hypertrophy.[27] On the other hand, DAMPs, via paracrine action, stimulate fibroblast proliferation, migration and trans-differentiation to myofibroblasts to increase ECM turnover.[28,29] Remarkably, both cells further secrete fibrotic and pro-inflammatory factors[3,30] (Fig. 1B). The inability to resolve persistent inflammatory stimulus activate the molecular motifs of innate and adaptive immune system associated with myocardial damage and unrestored tissue homeostasis.[31] Thereby, stereotypical activation by DAMPs and infiltration within myocardium of lymphocytes, neutrophils, dendritic cells (DCs), monocytes and monocyte derived macrophages contribute towards pathological cardiac remodelling due to chronic and/or acute

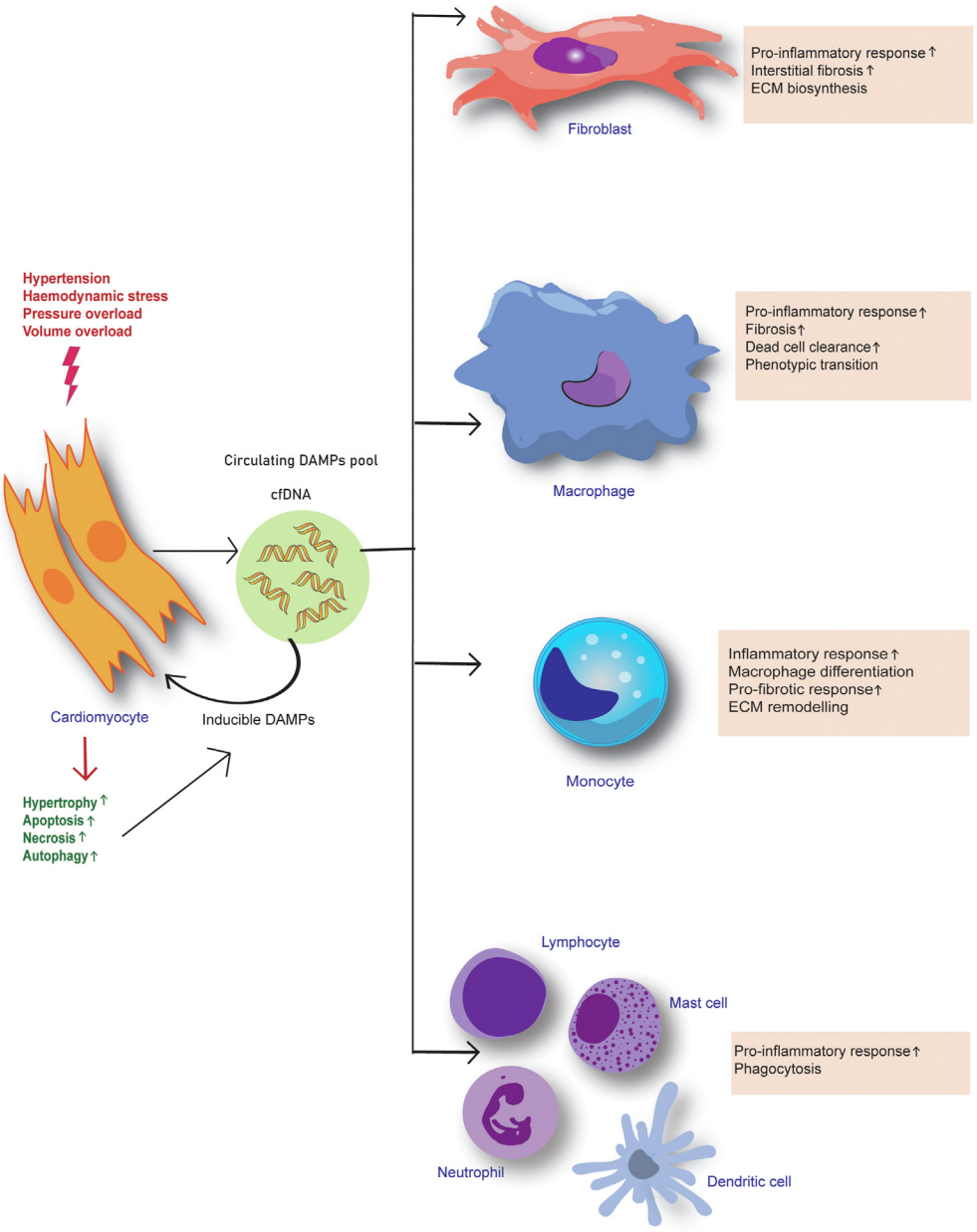

Figure 1 Significance of cardiac resident cells (CRCs). **(A)** Cardiomyocytes. **(B)** Fibroblasts. **(C)** Macrophages. **(D)** Monocytes. **(E)** Other immune cells (neutrophils, lymphocytes, mast cells and dendritic cells). Cardiomyocytes injured due to hypertension, hemodynamic stress, pressure and volume overload mediates development of pathological cardiac hypertrophy during progression to heart failure. Non-myocyte CRCs co-operatively interact with cardiomyocytes to inflame the heart and undergo maladaptive remodelling by triggering interstitial fibrosis and cardiomyocyte cell death mechanisms.

inflammation[30,32] (Fig. 1C—E). In consequence, the inflammation augments interstitial tissue fibrosis and cardiomyocyte death correlated with reduced cardiac function during HF.[3,33]

Principally, the circulating nucleic acids within the DAMP pool can facilitate inter- and intra-cellular communications, however, most clinical studies of cfDNA release during HF focussed on identification of circulating DNA-based signatures as biomarkers.[13,34] Such findings are critical towards management of the disease; nevertheless, circulating DNA structure, release mechanisms and its role as DAMPs in cellular signalling remains to be precisely understood before clinical implementation towards management. Recent reports have shown upregulated serum cfDNA levels in patients with cardiac angina and MI compared to healthy individuals.[35,36] Some studies also identified significant upregulation of a cardiomyocyte-specific unmethylated circulating FAM101A locus in HF patients compared to control subjects.[37] However, a mechanistic role of such cfDNA fragments in cardiovascular inflammation and associated pathology is yet elusive. The subsequent section would discuss the circulating cfDNA release mechanisms and sketch an intelligible concept map of inter- and intracellular cfDNA-mediated signalling during maladaptive cardiac remodelling.

Circulating cfDNA release mechanisms during pathology

Injured cardiomyocytes, during apoptosis or necrosis, passively release fragmented DNA by DNaseI-mediated digestion during pathology in multiple structural forms *viz.* particulate assemblies (exosomes, microparticles, or apoptotic bodies) or macromolecular structures (nucleosomes, virtosomes, DNA traps or DNA-serum protein complexes).[6,15] Such particulate assemblies are formed as a result of the highly electrostatic DNA structure. Auto-condensation results in macromolecular complexes or vesicle internalization, thereby masking them from nucleases and increasing their half-life in circulation.[6]

Membrane bound structures in circulation

Microvesicles, exosomes or apoptotic bodies protects the encapsulated DNA from nuclease-mediated digestion and can induce inter-cellular communication among CRCs during cardiac pathophysiology. Microvesicles are heterogenous extracellular bodies of 100—1000 nm in diameter that originate by outward budding and fission of the plasma membrane due to high shear during chronic cardiac pathology.[38] Nucleic acid-containing microvesicles or exosomes derived from norepinephrine treated cardiomyocytes reportedly modulates metabolic event in myocardium.[39] Exosomes are 30—100 nm homologous bodies formed from endosomal membrane of multi-vesicular bodies (MVB).[38] Exosomes are released by caveolin-mediated or clathrin-mediated endocytosis and also due to intracellular Ca^{2+} overloading during cardiomyocyte hypertrophy[40] (Fig. 2A). Apoptotic bodies measuring 1—5 μm are formed by membrane bulging of apoptotic cells that encapsulate part of the cytoplasm.[41] Active removal of such apoptotic bodies limits inflammation, however, inefficient ingestion by phagocytes or increased rate of apoptosis can lead to secondary necrosis[42,43] (Fig. 2B).

Macromolecular structures in circulation

The virtosomes are nucleic acid—lipoprotein complexes released from living cells in controlled energy-dependent

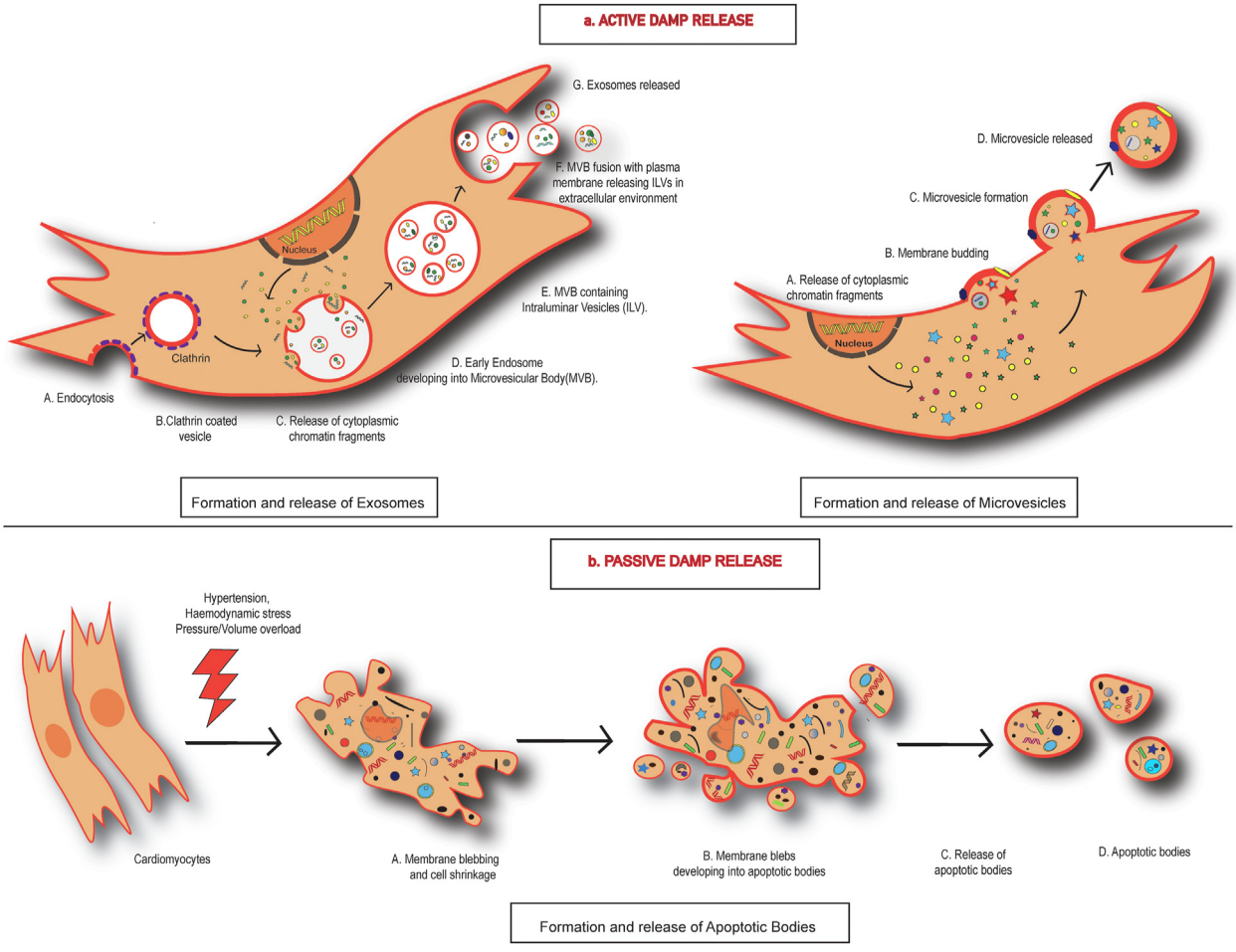

Figure 2 Schematic representation of DAMP release mechanism in CRCs. **(A)** Active release of DAMPs — Exosome and micro vesicle (MVs) released as extracellular bodies containing DAMPs. Exosomal release involves clathrin-mediated endocytic mechanisms, the cytoplasmic chromatin fragments contained within intraluminar vesicles (ILV) are released as exosomes from the multivesicular bodies (MVB) (1—7). Microvesicles involve release of cytoplasmic chromatin fragments that undergo membrane budding and release. **(B)** Passive release mechanism — Cardiomyocytes subjected to pathological damage undergo apoptosis, in the process apoptotic bodies containing cytoplasmic contents are released (1—4).

steps and associated with intercellular signalling by permeating cell membranes to enter the cell cytosol.[6] These complexes in circulation help to carry cfDNA across organs and perhaps might be of significance across organ to organ circulating DNA transport *viz.* cardio–renal axis.[44] Neutrophil extracellular DNA traps (NETs) comprising of decondensed chromatin, histone proteins, and granular proteins have been reported in myocardial ischemia due to its pro-thrombotic roles.[6] Excessive production of NETs may trigger chronic inflammation, monocyte differentiation or macrophage polarisation[45] in cardiovascular diseases. Circulating nucleic acids in plasma and serum contain extracellular DNA in combination with various proteins like albumin or globulin or fibronectin from plasma. For instance, the presence of platelet mtDNA and leukocyte mtDNA within plasma has been correlated with HF risk.[6]

Biochemical signatures of cfDNA

The cellular outcome of circulating DNA-mediated signalling is dependent upon chemical signatures like DNA methylation or oxidation; however, in the cytosol fragmented DNA is recognized by RAGE or AIM2 in a sequence-independent manner.[46] Pathological oxidative stress-induced DNA oxidation activates the antioxidant defence mechanisms and acts as a major driver in the physiological mechanisms of stress associated adaptations, while cell death is responsible for releasing the fragmented DNA in the extracellular milieu.[6,9]

Methylation. Studies have investigated the methylation status of fragmented circulating cfDNA in pathological myocardium and suggested strong association with patient mortality.[34,47] Extracellular genomic DNA with methylation marks is poorly internalized by cells and is a weak stimulator of PRRs compared to unmethylated fragmented mtDNA.[6] Reportedly, mitochondrial DNA recognised by TLR9 contributes to atrial fibrillation, elevates blood pressure and aggravates vascular dysfunction in pressure overload-induced HF mice model.[48,49]

Oxidation. HF is generally associated with cell death due to oxidative stress wherein DNA nucleobases might get oxidised to varying degrees.[50] Further, during mitochondrial damage, the mitochondrial DNA gets exposed to oxidative damage, wherein C8 gets oxidised to form 8-oxo-7, 8-dihydro-2-deoxyguanosine (8-oxodG).[51] Thus, biochemical estimation of the ratio of 8-oxodG to non-oxidised guanosine has been directly correlated with reactive oxygen species (ROS) generation and reported as a marker for longitudinal monitoring of HF.[51] Interestingly, oxidised mtDNA fragments might be of immense clinical importance in mediating signalling within myocardium during progression of HF due to its association with mitochondrial electron transport chain.[52]

Cellular receptors for sensing cfDNA during pathologies

Intercellular communication by cfDNA is facilitated by nucleic acid sensors, reportedly expressed in cardiomyocytes, vascular cells, fibroblasts, and immune cells of the myocardium.[53] The sensors are generally membrane associated or cytosolic in terms of cellular localization.[54]

Toll-like receptor 9 (TLR9). TLR9 is a nucleic acid receptor distributed in endolysosomal compartments which accelerates inflammatory responses during HF.[55] TLR9 activation occurs by binding of unmethylated CpG motif of DNA with the C-terminal of TLR9.[7,56]

In the cytosol, TLR9 priming with DNA initiates their intracytoplasmic redistribution from endoplasmic reticulum through proteolytic cleavage of the TLR9 ectodomain to endosomal compartments.[7,56,57] In essence, canonical TLR9 activation upregulates downstream pro-inflammatory signalling cascade through myeloid differentiation primary response protein (MyD88), thereby triggering cytokine production. This might lead to cardiomyocyte apoptosis, interstitial fibrosis and mature scar formation within the injured pathological myocardium[48,56,58] (Fig. 3A). The intracellular localization of nucleic acid-TLR complex limits the recognition of nucleic acids derived from dying cells of the pathological tissue.[54,59] Thus, endogenously compartmentalized TLR9 is not involved in the sensing of the circulating cfDNA found in the extracellular space. TLR9 interaction with extracellular DNA is facilitated by shuttling of RAGE across cell membranes that recognises HMGB1-bound DNA-immune complexes.[57] (see Fig. 3)

Receptor for advanced glycation end products (RAGE). RAGE is a transmembrane receptor of the immunoglobin superfamily reportedly upregulated in the injured myocardium.[60] RAGE has been implicated in multiple studies to reduce cardiac function.[60] The DNA bound to HMGB1 are recognised by positively charged surface of the RAGE ectodomain through the V–C1 tandem domain.[61]

RAGE binds to multiple negatively charged ligands, predominantly, HMGB1, S100/calgranulin family of proinflammatory polypeptides, β-sheet fibrils and amyloid-β peptide.[61] Reportedly, Tfam-bound mtDNA also associates with RAGE towards amplifying TNFα.[62]

S100 are small acidic RAGE-interacting proteins,[63] like S100B, which in post infarcted myocardium induces myocyte apoptosis.[64] On the other hand, S100A8/A9, during LPS exposure, reduces cardiomyocyte contractility and activates innate immunity during progression to HF.[65] The direct activation of S100 proteins by cytosolic DNA has not been characterised yet.

The HMGB1, a nuclear DNA binding protein, is significantly upregulated during myocardial ischemia/reperfusion injury.[66] HMGB1, released by necrotic and apoptotic cells, binds to extracellular DNA and gets endocytosed within cells by RAGE to reach the cognate cytoplasmic DNA sensors like TLR9, AIM2 or cGAS-STING and sustain inflammatory response.[57,66–68]

Interestingly, RAGE, TLR9 and HMGB1 form an interesting trio towards signal transduction by circulating DNA; wherein therapeutic strategies towards blockade of these signals might have clinical significance in cardiac dysfunction.

cGAS-STING. cGMP-AMP synthase (cGAS) in association with the receptor stimulator of interferon genes (STING) sense cytosolic DNA or oxidised mtDNA to trigger IFN stimulatory genes (ISG).[69,70] Interestingly, the footprint of the highly basic cGAS domain on DNA is ~10 bp.[71] Cytosolic DNA binding proteins like HU protein, mitochondrial

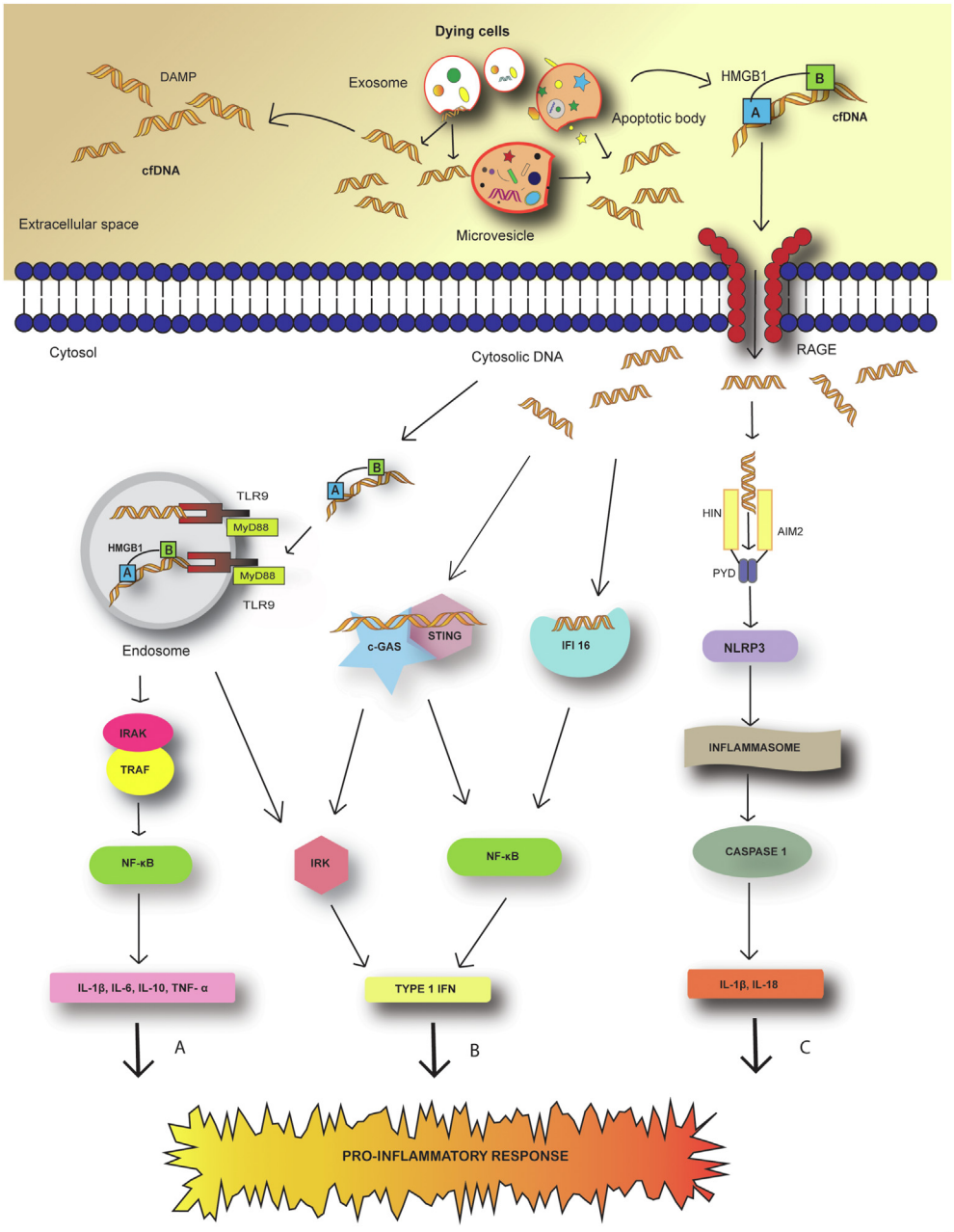

Figure 3 A schematic diagram of cell-free DNA (cfDNA) induced proinflammatory signalling pathways and secretion of interleukins, interferons etc. in cardiac cells. Inflammatory response is initiated when cf-DNA herein acting as DAMP binds nucleic acid receptors. Membrane bound or free cfDNA have been shown to interact with HMGB1 towards RAGE binding and co-operatively transduce various cytosolic signalling pathways, like TLR9, cGAS-STING and AIM2. **(A)** TLR9 binding to cf-DNA induces NF-kB mediated upregulation of proinflammatory interleukins. **(B)** Cytoplasmic cGAS-STING, IFI-16 mediated cf-DNA sensing initiate type I IFN response. **(C)** Cytosolic AIM2-cfDNA binding activates NLRP3 inflammasome. Activated inflammasomes can cleave caspases. Cleaved caspases induce IL-1B, Il-18 induction, leading to inflammatory responses.

transcription factor A (TFAM), or HMGB1 facilitate binding and activation of cGAS dimers by DNA.[67] Activated cGAS generates 2´3´-cGAMP, which is detected by an ER-localized adaptor, STING.[72] STING interacts with an IκB kinase (IKK) namely TANK binding kinase 1 (TBK1) and enables phosphorylation mediated dimerization of IFN regulatory factor 3 (IRF-3)[72]; concomitantly, IRF-3 translocate to nucleus and activates interferon- β (IFNβ) expression[71] (Fig. 3B).

An upregulated expression of cGAS-STING occurs in human hypertrophic heart and mouse model of pressure overload hypertrophy[10,73]; consistently, aortic banding induced cardiac hypertrophy was attenuated by STING

knockout.[73] Thereby establishing cGAS-STING as an important sensor of fragmented DNA during pathophysiology.

Absent in Melanoma 2. AIM2 and the Aim2-like receptors (ALRs) are structurally defined by two domains; the HIN domain and the pyrin domain (PYD); involved with sensing of cytoplasmic DNA.[74] The HIN domain is critical for DNA recognition while the PYD domain is involved with apoptosis or inflammation.[74] Reportedly, AIM2 has been associated with inflammasome activation by activation of caspase-1[75](Fig. 3C). Also, AIM2 promotes chronic inflammation in HF.[7,9,76] Other ALR family members *viz*. IFI16 have also been studied to activate the inflammasome.[75]

DNA-dependent activator of IFN-regulatory factor (DAI, also called Z-DNA binding protein-1, ZBP1) recognises cytosolic DNA,[77] leucine-rich repeat interacting protein-1 (Lrrfip1) induces IFN production via a β-catenin-dependent pathway,[78] Ku70 acts as a cytosolic DNA sensor to trigger ISGs and DExD/H box helicases *viz*. DHX9 detects CpG-A DNA to express interferon stimulatory genes (ISGs) and DHX36 detect CpG-B DNA to express pro-inflammatory genes (TNFα, IL-6, etc)[7,9] (Fig. 3).

Intercellular communication by cfDNA

The circulating DNA fragments of both nuclear and mitochondrial DNA origin are released as DAMPs into cardiac interstitium during HF.[79] DAMPs such as fragmented genomic equivalents released from dying cardiomyocytes induce inflammatory signalling by activating fibroblasts, macrophages and other immune cells.[30,32] Depending on the fragment size and chemical nature of circulating DNA, the cellular response varies, *viz*. unmethylated cfDNA and oxidised DNA trigger proinflammatory cytokines by TLR9-MyD88-NF-kB signalling cascade and stimulate oxidative stress by downstream NADPH oxidase 4 (NOX4).[9,56,57] Oxidised fragmented mtDNA also showed cytoplasmic activation of STING and AIM2 sensor pathways.[52]

During HF ROS upregulation occurs by NADPH oxidases or Xanthine Oxidase; while antioxidant systems balance the redox level.[80] However, an upregulated redox microenvironment activates "NACHT, LRR and pyrin domain-containing protein" 3 (NLRP3); concurrently, TLR9 stimulation is a priming step for NLRP3 activation.[52] TLR9 activation by circulating cfDNA leads to cardiac dysfunction and points towards a possible link between redox imbalance and circulating DNA fragments, thereby triggering apoptosis and pathological hypertrophy.[81] Comparable to ROS intrinsic cytosolic fragmented DNA from cardiomyocyte under hypoxic stress activated TLR9 by a non-canonical stress tolerance pathway, and modulated energy substrate utilisation and downregulated sarco/endoplasmic reticulum Ca^{2+} ATPase pump 2 (SERCA2) along with reduced mitochondrial ATP levels.[82–84] Intriguingly, unmethylated FAM101A fragmented DNA within myocardium were positively correlated with cardiac troponin levels and higher apoptosis in cardiomyocytes of acute MI patients.[34,83] Recently, Tian et al (2019) showed concomitant release of HMGB1 and cfDNA during ischemia-reperfusion and depletion of either TLR9 or RAGE attenuated myocardial infarction.[85] Taken together, the reports reflect a possible dose-dependent activation of TLR9 by circulating cfDNA; wherein, acute doses elicit the canonical inflammatory pathway, whereas, chronic doses trigger non-canonical stress tolerance pathways. Further research is warranted to reveal the possible role of such genomic or mitochondrial nucleic acid equivalents in TLR9 activation during progression of HF.

Thus, autocrine signalling of cardiomyocytes due to cfDNA might be instrumental towards upregulation of chemokines, type I IFNs and pro-inflammatory cytokines. The intercellular crosstalk initiated by cardiomyocytes with fibroblasts are associated with bidirectional release of various soluble factors.[86] In AngII stimulated cardiomyocytes co-cultured with fibroblasts STING upregulated transforming growth factor-beta 1 (TGF-β1) and resulted in cardiac stiffness and reduced cardiac function by collagen deposition.[73] Firstly, activated cardiac fibroblasts during cardiac injury sense DAMPs and trigger proliferation[86,87]; secondly, pro-fibrotic phenotype within resident fibroblasts releases pro-inflammatory molecules *viz*. IL-1β, IL-6, TNFα, etc. and remodels ECM proteins to aggravate interstitial fibrosis.[30,86]

During tissue injury, DAMP recognition by cardiac fibroblasts induces infiltration of immune cells within injured site by release of CXC chemokines to mediate neutrophil infiltration, CC chemokine-induces leukocyte infiltration and CCL2/CCL7 mediate $Ly6C^{high}$ monocyte recruitment.[32] Moreover, fibroblasts release proinflammatory molecules that can activate macrophage polarisation and monocyte phenotype transition which are associated with increased ECM deposition.[28,30,32]

The macrophages play an active role towards remodelling of the adult myocardium after injury/stress either directly depositing collagen or indirectly by TGFβ/Smad mediated fibroblast activation and collagen deposition.[88] Tissue-resident cardiac macrophages can be divided into C—C chemokine receptor 2^- ($CCR2^-$) ($MHCII^{low}$ and $MHCII^{high}$) derived from foetal monocytes and yolk sac (embryonic) and $CCR2^+$ subsets derived from adult hematopoietic lineages, respectively During tissue injury, $CCR2^-$ resident macrophages adopt an inflammatory role; promoting recruitment of monocytes ($CCR2^+$ $MHCII^{low}$L-$y6C^{high}$) from circulation that differentiate to inflammatory M1 monocyte-derived macrophage populations.[89] Macrophages like $MHCII^{low}$ are efficient phagocytic cells entrusted for removal of myocardial debris after tissue damage. The autophagic flux within macrophage regulates efferocytosis and thus circulating cfDNA fragments might upregulate cGAS-STING or inflammasome pathway triggering an autophagic dysfunction within myocardium.[90,91] Predictably, circulating cfDNA fragments can mediate progression to HF during pathological insults to heart by interstitial fibrosis and cardiomyocyte cell death, which is characterised by contractile dysfunction and reduced cardiac function (Fig. 4).

Circulating cfDNA scavenging strategies

The nucleic acid binding polymers inhibit activation of the receptors by binding to circulating cfDNA molecules. Hexadimethrine bromide (HDMBr), Polyphosphoramidate polymer (PPA-DPA), poly-L-lysine, protamine sulfate, polyamidoamine dendrimer, 1,4-diaminobutane core-PAMAM-G3 (PAMAM-G3) and β-cyclodextrin-containing polycation (CDP) can specifically

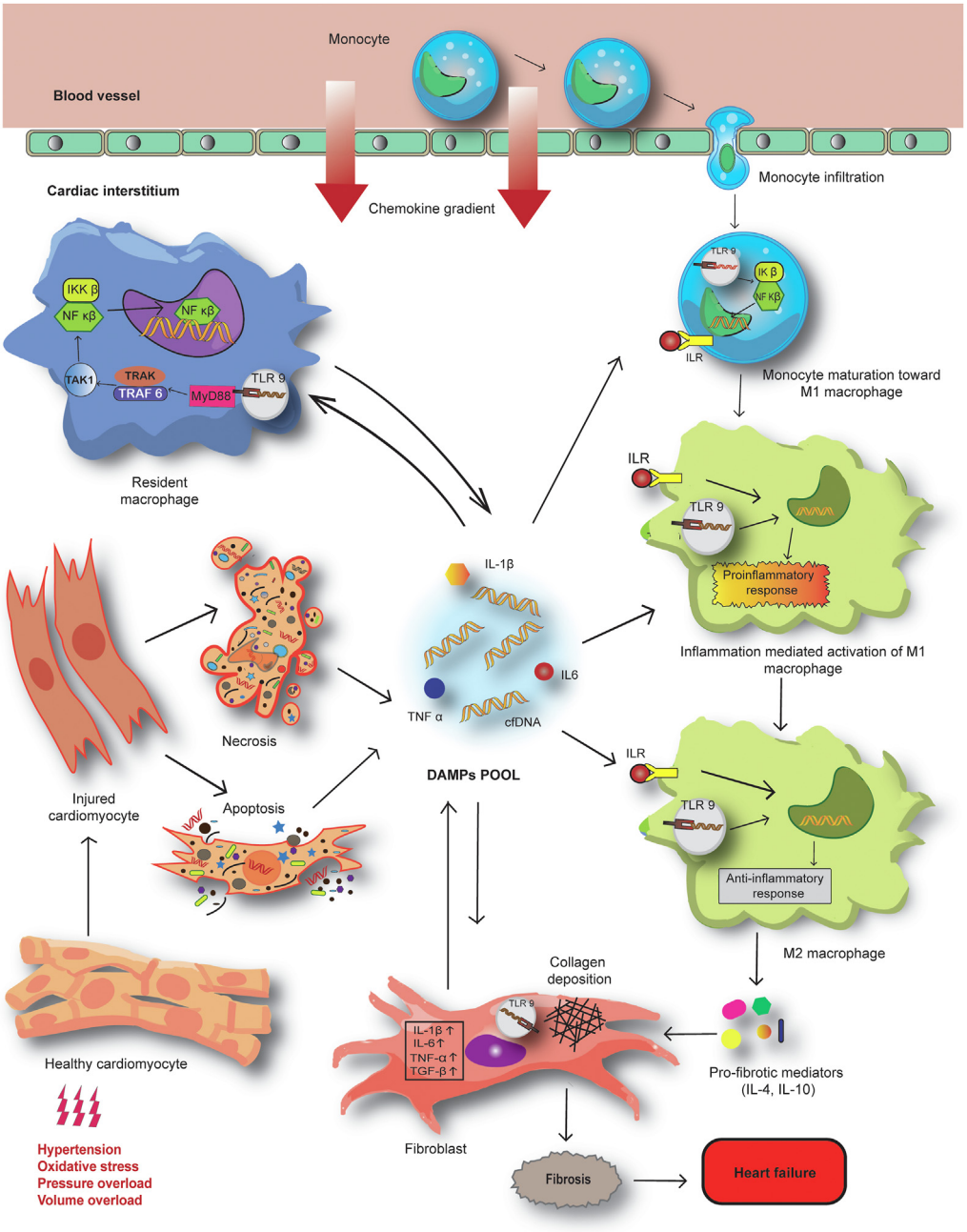

Figure 4 Cellular crosstalk between cardiomyocyte-macrophage-fibroblast at the core of heart failure in myocardium. Hypertension, hemodynamic stress, pressure and volume overload injure cardiomyocytes, resulting in cellular death pathways *viz.* necrosis, apoptosis, etc. Cellular debris released due to cell death mechanism contribute to myocardial DAMP pool, including nuclear and mitochondrial genomic fragments and associated structures. DAMPs activate fibroblasts which produces TGFβ, TNFα and inflammatory interleukins. Pro-fibrotic activation of fibroblast is also associated with excess collagen deposition leading to fibrosis and extracellular matrix remodelling. Sensing of DAMPs like cfDNA by resident macrophages also induces NF-kB mediated pro-inflammatory response. Increasing chemokine gradient further recruits innate immune cells into the damaged microenvironment. Maturation of monocytes into inflammatory subtype (M1) induces a proinflammatory response followed by transition into an anti-inflammatory phenotype (M2), essential to resolve myocardial scar by collagen deposition from fibroblasts. Cumulatively, the activation of nucleic acid receptors by cytosolic genomic equivalents are critical towards myocardial inflammation. The pathological outcome of myocyte apoptosis and cardiac fibrosis in an inflammatory milieu involves cardiac dysfunction and progression towards heart failure.

scavenge pro-inflammatory nucleic acids, which is partially dependent upon electrostatic interaction between oppositely charged molecules.[92] Hexadimethrine bromide (HDMBr) administered at a dose of 1—4 mg/kg t murine models showed neutralization of mtDNA and rescue from multiple organ dysfunction syndrome (MODS).[93] The cationic third-generation PAMAM dendrimer (PAMAM-G3) inhibits carotid artery injury in a mice model, reduce wound contraction and less scarring

compared to untreated mice.[94,95] However, usage of such polycations might be limited to pre-clinical applications due to their soluble nature, cellular internalisation and cytotoxic side effects. In this regard, usage of a minimally toxic cationic nanofiber may be considered as an alternative approach to limit inflammation.

Potential therapeutic opportunities

The circulating DNA is recognised by receptors that trigger inflammatory responses. From another perspective, pharmacological neutralisation of nucleic acid sensors has developed as an attractive novel drug target, yet, very few are presently available. The mixed TLR 7/9 antagonist ODN-2088 significantly ameliorated post-intimal thickening and reduced macrophage infiltration in mouse model of post-interventional myocardial remodelling.[96] Recent data indicate Aspirin to be an inhibitor of cGAS signalling; C-176 and C-178 inhibits STING pathway or various inflammasome inhibitors like Parthenolide, VX-765, etc.[97,98] Pre-clinical usage of such antagonistic pharmacological agents towards cardiovascular disorders calls for thorough knowledge of the receptor kinetics and biology, moreover, the intrinsic biological differences between humans and murine complements have remained evasive with respect to DNA sensors mediating inflammation.

Conclusion and future perspectives

From an immunological perspective the heart neither secretes inflammatory cytokines nor is as active like the skin, gut, lungs, or the liver. However, a variety of physical and chemical stresses can lead to mechanical damage and activation of cardiac innate and adaptive immune responses.[99] Future research directed towards deciphering circulating DNA mediated regulation of inflammatory signalling would be interesting from a therapeutic viewpoint. Pertinent to the cardiac muscle, inflammation mediates aggravation of cardiac function by triggering fibrosis and cell death. A thorough understanding of the nucleic acid sensor activation and the concomitant cell signalling mechanism would unravel mysteries that circulating cfDNA hold towards HF progression. Evidently, complementary information from various tissue pathological models will accelerate the understanding of the circulating DNA mediated response and the therapeutic value of the research towards development of clinical strategies for amelioration of circulating cfDNA mediated inflammatory signalling.

The prognostic and predictive value of cfDNA with respect to cardiovascular diseases was established by the pioneering works of Yokokowa et al and Zemmour et al.[34,37] The circulating cfDNA sensors were hardwired to recognise viral, bacterial or foreign nucleic acids.[100] Circulating cfDNA are released during unusual pathophysiological conditions such as excessive cellular stress and apoptosis into the cytosolic compartments or to extracellular spaces.[17] Circulating cfDNA present either in vesicular form or in association with proteins sometimes leads to the failure in self-discrimination by the sensors.[6] Thus, pathologies like chronic kidney disease, cardiac pathology, diabetes, etc. involved in release of circulating cfDNA might develop pro-inflammatory responses characteristic of autoimmune diseases. However, limited knowledge of cfDNA mediated signalling during chronic pathology has resulted in fewer translation to therapeutic application; thereby, opening a broad scope for future research with pre-clinical models. Putatively scavenging of the circulating cfDNA from the tissue of origin and blocking DNA sensors in the responder cells might be effective towards mitigating the inflammatory effect of the circulating cfDNA.

Author contributions

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript.

Conflict of interests

The authors declare no conflict of interests.

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