Chapter

The Cellular Stress Response Interactome and Extracellular Matrix Cross-Talk during Fibrosis: A Stressed Extra-Matrix Affair

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Abstract

Diverse internal and external pathologic stimuli can trigger cellular stress response pathways (CSRPs) that are usually counteracted by intrinsic homeostatic machinery, which responds to stress by initiating complex signaling mechanisms to eliminate either the stressor or the damaged cells. There is growing evidence that CSRPs can have context-dependent homeostatic or pathologic functions that may result in tissue fibrosis under persistence of stress. CSRPs can drive intercellular communications through exosomes (trafficking and secretory pathway determinants) secreted in response to stress-induced proteostasis rebalancing. The injured tissue environment upon sensing the stress turns on a precisely orchestrated network of immune responses by regulating cytokine-chemokine production, recruitment of immune cells, and modulating fibrogenic niche and extracellular matrix (ECM) cross-talk during fibrotic pathologies like cardiac fibrosis, liver fibrosis, laryngotracheal stenosis, systemic scleroderma, interstitial lung disease and inflammatory bowel disease. Immunostimulatory RNAs (like double stranded RNAs) generated through deregulated RNA processing pathways along with RNA binding proteins (RBPs) of RNA helicase (RNA sensors) family are emerging as important components of immune response pathways during sterile inflammation. The paradigm-shift in RNA metabolism associated interactome has begun to offer new therapeutic windows by unravelling the novel RBPs and splicing factors in context of developmental and fibrotic pathways. We would like to review emerging regulatory nodes and their interaction with CSRPs, and tissue remodeling with major focus on cardiac fibrosis, and inflammatory responses underlying upper airway fibrosis.

Keywords: extracellular matrix, homeostasis, tissue repair, fibrosis, cellular stress, RNA binding proteins, RNA interactome

1. Introduction

Fibrosis is an inherent reparative response invoked to restore tissue integrity following a pathologic insult, which metamorphoses into a devastating pathology culminating in scars due to self-catenating heralding inflammatory loops. Regeneration is a fundamental biological process initiated to orderly replace the damaged tissues, however, deregulation of chronic inflammation, growth factor-receptor cross-talk, intra-intercellular communication, and various extracellular matrix proteins eventually result in an aberrant wound healing response marked by fibrosis or scarring. Excessive scarring can obliterate tissue architecture, culminating in organ failure and death. Therefore, lack of coordination and synchronization in molecular and cellular events that guide *"regeneration"* results in *"degeneration"* of the affected organ. Fibroproliferative disorders are widely occurring and include pulmonary fibrosis, systemic sclerosis, liver cirrhosis, cardiovascular disease, progressive kidney disease, corneal scarring, proliferative vitreoretinopathy and posterior capsular opacification. Aberrant tissue remodeling marked by fibrosis is also implicated in cancer metastasis and chronic graft rejection in transplant recipients. The obvious deprecating impacts of fibrosis are enormous deterrents to patients; moreover, the disease has failed to meet the required treatments till date. Lack of availability of desired therapeutic interventions is majorly due to an incomplete understanding of the mechanism of the disease, therefore, gaining insights into the mechanistic pathways of fibrosis would facilitate improved therapeutic approaches to target novel mediators besides the cryptic or altered ECM components as previously reported by our group [1–5].

TGF-β is a central node in driving fibrotic pathways and other diseases, however, targeting TGF-β pathways has not met desirable clinical success, perhaps due to the incomplete mechanistic information on its role in development and pathology. Therefore, gaining insights into regulation of TGF-β during normal development and pathology could facilitate recognition of alternate target-pathways that may spare or minimally perturb the role of TGF-β in physiology. An interesting and relatively less explored theme is involvement and regulation of TGF-β isoforms in fetal wound healing that is marked by absence of scar. Intriguingly, there are common mediators and unifying pathways that underlie tissue repair, homeostasis and fibrosis in diverse organs; therefore, harnessing the potential non-fibrotic themes from scarless wound healing might add to the understanding of challenging fibrotic disorders. A systematic and meticulous re-assessment and re-evaluation of the role of mediators of scarlessly healing wounds might offer a reasonably potential tool to be manipulated to prevent fibrosis and decode the invisible lines dividing the "homeostasis-tissue repair-fibrosis continuum".

Interestingly, there are few physiological paradigms where wounds heal scarlessly or with minimal scarring, for instance the wounds in the early gestation fetus and in the oral mucosa of mammals heal without scar [6] the transition from scarless to scarred healing occurs in humans during late weeks of gestation [7]. A recent study showed that dermal fibroblasts with a scarring phenotype when transplanted into oral mucosa ended up generating more scar-like connective tissue compared with oral mucosal fibroblasts transplanted into the dermis [8]. The oral mucosal fibroblasts were shown to possess a higher baseline production capacity of several ECM-associated proteins than the skin fibroblasts, except type III collagen, which could be possibly attributed to a more favorable wound healing in oral mucosa [9]. Healthy endometrium heals scarlessly and is suggestive of regenerative healing and can be paralleled to fetal-like scarless healing responses that are also seen in the

buccal mucosa of the oral cavity. Endometrial repair involves highly orchestrated cross-stalk in stromal, epithelial, vascular, and immune cells and presents a remarkable epitome of healing that involves over 400 cycles of resolution of inflammation, angiogenesis, tissue remodeling, and formation of new tissue without any residual scarring [10]. Recently, neutrophil gelatinase-associated lipocalin, follistatin like-1, chemokine ligand-20, and secretory leukocyte protease inhibitor were identified as important signatures in menstrual fluid that were proposed to facilitate scar-free repair [11]. Endometrial stromal cells were shown to exhibit distinct phenotypic and immunomodulatory profiles and displayed lack of HLA class II that was proposed to drive their physiological roles in tissue repair and immune tolerance during pregnancy [12].

Psoriasis represents a unique form of "scarless-like or hyper-regenerative" wound healing marked by nonscarring, inflammatory, and hyperproliferative tissue repair responses. An amazing aspect about the psoriatic lesions is the fact that with appropriate therapy the complex skin lesions can be reverted back to healthy appearing skin, with little if any evidence of altered changes in the epidermis and dermis. Psoriatic plaques are exciting conundrums as they do not go to fibrosis even amidst heralding auto-inflammatory loops [13]. An interesting common mediator oncofetal fibronectin extra domain B (Fn-EDB), has been reported to be prominent in psoriatic lesions and wound healing in fetal tissue [14]. Interestingly, psoriatic plaques despite being vulnerable to infections, do not tend to get infected because of the presence of massive antimicrobial peptides like LL37. Psoriasis pathogenesis involves strong polymorphonuclear neutrophil (PMN) infiltration and high levels of the PMN associated antimicrobial peptide, LL37. Psoriasis is marked by self-reactive inflammatory loops of innate immune responses, which trigger subsequent adaptive immune responses against autoantigens like LL-37, ADAMTSL5, and HNRNPA1, with LL-37 and HNRNPA1 having RNA-binding properties. The phenomenon of self-RNA sensing by nucleic acid sensors [15–17] is central to autoinflammatory and autoimmune diseases like psoriasis, however, the role of RNA-binding proteins LL37 and HNRNPA1 (the proven autoantigens) in contributing to inflammatory loops remains largely unexplored. In a psoriatic mice model study excessive polyamine generation was shown to facilitate self-RNA sensing by immune cells [18] independent of RBPs, however, a recent study has implicated the role of neutrophil extracellular trap (NET)-associated RNA and LL37 (RBP) in self-amplifying inflammation in psoriasis [19]. Herster et al., highlight an unappreciated yet potential axis involving neutrophils, LL37 (RBP-like) and surprisingly, RNA that are abundant in psoriatic as opposed to healthy skin; suggesting a novel role of NET-derived RNA-RBP (LL37) complexes in self-propagating inflammatory loops. Host defence peptides or antimicrobial peptides like LL37 can have immunomodulatory protective [20] or pathological roles. The dual roles are proposed to be linked to post-translational modifications of peptides by citrullination or carbamylation that may depend on the disease context and result in altered ability of antimicrobial peptides to bind nucleic acids, thereby compromising their immunomodulatory potential (reviewed in [21]). Since RNA binding proteins (like LL37 and HNRNPA1) are emerging as potential molecules that can rewire inflammatory circuits depending on the pathological context, and several RBPs are also known to regulate developmental and fibrotic pathways by interacting with spliceosome machinery and acting as trans- regulators of RNA processing machinery [22], we would like to discuss their role in driving ECM remodeling in context of cardiac fibrosis with particular focus on RBM20, a cardiac-specific RBP that is emerging as a global regulator of cardiac development and disease.

2. Cardiac stress induced wound healing, repair and fibrosis: patch up, break up or a stressed extra-matrix affair

Plethora of extrinsic or intrinsic stressors (persistent hypertension, myocardial infarction, neurohormonal deregulation, hypoxia, ischemia-reperfusion, pressure over load, drug toxicity, mechanical stretch, radiation, etc.) can result in fibrosis and heart failure. The cardiac myocardium is a mosaic of diverse cell types with overrepresentation of cardiomyocytes and fibroblasts, and moderately populating endothelial cells, vessel smooth muscle cells, and immune cells like tissue resident macrophages. The absolute proportions of the cellular components are not determined; however, lineage tracing studies have identified the above inmates in the myocardium of healthy heart. Cardiac wound healing following a stress-induced injury (resulting in development of contraction bands, mitochondrial calcification and membrane disruption) involves a sequalae of pro-inflammatory, anti-inflammatory, and reparative events and majorly resulting in cardiomyocyte death and functional decline. Concomitantly, the neighboring fibroblasts in the myocardial niche act as central nodes to drive aberrant wound healing, ECM (extracellular matrix) remodeling and fibrosis. The continuum of tissue homeostasis, repair, and fibrosis is not appreciably understood, however, the inherent plasticity of fibroblasts and existence of pro- and anti- inflammatory, and pro-fibrogenic polarizing fibroblast phenotypes suggests a cardinal role of these cells in cardiac regeneration and repair. The most challenging aspect of cardiac remodeling is the recreation and restoration of qualitatively (structurally and functionally), and quantitatively compliant ECM, which is pro-regenerative or anti-fibrotic. It is known that extensive ECM deposition in the myocardial infarct can result in arrhythmias, however repopulating the native ECM following destruction is an absolute requirement for maintaining optimal tension in a highly contractile organ like heart. Fibrosis is projected to be the major player behind compromised myocardial compliance resulting in altered contraction-coupling events, reduced ventricular filling, decreased cardiac output and arrhythmias. End-stage heart failure and cardiac arrhythmia happen to associate with fibrosis, which is triggered as a compensatory response to counteract tissue damage, however, perpetuating cycles of stress and inflammation may result in decompensatory fibrosis and organ-failure during late-stage pathologies marked by recalcitrant accentuating cellular stress and catenating chronic inflammatory loops. Cardiac fibrosis is common in several cardiac diseases including atrial fibrillation, hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and heart failure with preserved ejection fraction. Aberrant extracellular matrix remodeling can be present in myocardial ischemia and infarction as seen in ischemic (imbalanced oxygen supply and demand) heart diseases caused by atherosclerosis of the epicardial coronary arteries, and nonischemic heart diseases like aortic stenosis, diabetic cardiomyopathy, hypertensive heart disease, and hypertrophic cardiomyopathy, in which myocardial interstitial fibrosis results in adverse ventricular remodeling. Hypertrophying of ventricular cardiomyocytes is an initial adaptive response to compensate for cardiac over load and restore normalization of cardiac output by balancing ventricular wall stress, however, persistence of stress/stressors eventually culminates in cardiomyocyte death, fibroblast activation into myofibroblast, deposition of aberrant ECM, interstitial fibrosis, and adverse cardiac remodeling [23, 24].

Fibroblasts are key players in the secretion, deposition, organization and regulation of ECM turnover, however, their phenotypic heterogeneity, functional diversity, and attendant signalling pathways that modulate fibrotic over regenerative repair in cardiac diseases remain largely indecisive [25–37]. The sphingosine 1-phosphate (S1P) signaling pathway is a hot spot in research for fibrotic diseases

involving lung, liver, and heart [29], and improved understanding of its cross-talk with fibroblasts is in progress as the attendant pathways are still controversial. Therefore, improved understanding of the molecular mechanisms and cross-talk underlying fibroblast activation and cardiomyocyte death are central for restoring cardiac homeostasis, designing novel regenerative approaches and developing anti-fibrotic therapies. Given the close association underlying tissue repair and fibrosis, it is intuitive that the balanced participation of common mediators can drive regeneration as opposed to skewed responses that may result in fibrosis. However, what are these checks and balances and how do they determine differential outcomes (homeostasis, repair or fibrosis) is the major challenge in the field of treating fibrosis or regenerating cardiac tissues *in vitro*. Delineating the subtle mechanisms and cellular, molecular, and extracellular matrix players implicated in regenerative and non-regenerative hearts can provide insights into the homeostasis-repair-fibrosis continuum, which remains the most vexing challenge in developing successful regenerative/anti-fibrotic approaches for fibrotic disorders. Further, the refractory response of cardiomyocytes to complete cell-cycle progression through mitosis limits their self-renewal, therefore, cardiomyogenic approaches to treat heart failure remain practically intractable [38–48].

3. Abortive cellular homeostasis rebalancing in cardiac fibrosis: a tragedy behind the broken heart

Cardiomyocytes being post-mitotic senesce with age and create cellular stress induced pool of biological waste over the course of ageing favoring late onset of fibrotic cardiomyopathies (acquired and inherited). Therefore, ageing and pro-fibrotic gene inheritance serve as additional "self-contained spontaneous stressors" that can impair the cellular homeostatic autophagic machinery, which is indispensable for cardiac tissue repair and proteostasis rebalancing [49–52]. Regulation of autophagy pathways are strongly implicated in liver, lung, heart, kidney and cystic fibrosis, which suggests that autophagy is a potential target for the treatment of chronic multiorgan fibrotic diseases involving aberrant extracellular remodeling [53]. The divergent homeostatic pathways converge to counter cellular stress or clear the stressor by intersecting and coordinating with precision, therefore, the intersection points, interacting partners and regulatory nodes are under extensive research. Molecular and cellular players driving homeostasis rebalancing in diverse diseases including fibrosis are continuing to emerge and recently reported for endoplasmic and mitochondrial stress pathway networks [54–56]. Mitochondria and endoplasmic reticulum (ER) in cardiomyocytes are overrepresented organelles to co-ordinate the increased metabolic demands and maintain active calcium stores for smooth flow. Endoplasmic reticulum and mitochondria quality control circuits are also integral to cardiac function, and deregulation of these pathways are strongly implicated in cardiac diseases including heart failure [57–63]. ECM remodeling is emerging to coincide with metabolic rewiring in cardiomyocytes and matrix-guided control of mitochondrial function in cardiomyocytes is seen as a potential therapeutic target in cardiac fibrosis, repair, regeneration and tissue engineering [64]. A recent trend in increasing ribosome profiling studies has stratified quality control checks that provide additional fidelity by clearance of defective messenger RNAs under ribosome associated quality control [65]. A genetic locus for cardiac hypertrophy that has been associated with alterations in stoichiometric translation rates of sarcomeric proteins has recently been defined [66]. Pro-fibrotic translationally regulated genes underlying cardiac fibrosis with proline (amino acid rich in collagen) codon usage promoted collagen synthesis emphasizing the importance

of translational rates that may be heightened in failing hearts that select for codon biasing for profibrotic genes [67].

Importantly, the stress pathways gradually converge, overlap and cross-talk with RNA metabolic, and sterile inflammatory pathways through processing, secretion, and regulation of DAMPS (danger associated molecular patterns) generated in response to heralding stress and inflammation. DAMPs include diverse endogenous host-derived molecules (extracellular ATP, histones, HMGB1 chaperons, etc.), which can be sensed by innate immune receptors [17] owing to their cellular/extracellular mis-localization, stress induced modification or overexpression related conformational anomalies. DAMPs are primarily released by damaged and dying cells to facilitate sterile inflammation, which is important for tissue repair and regeneration, however, if left unchecked they can result in of numerous inflammatory diseases, metabolic disorders, neurodegenerative diseases, autoimmune diseases, cancer and fibrosis. Recently TGF-β has been proposed as an inducible DAMP that activates mechanotransducing pathways resulting in self-perpetuating loops leading to activation of myofibroblasts in diverse pathologies including cardiac fibrosis. Sarcomere integrity and rhythmic efficiency amidst high protein turnover, multiple protein-protein interactions, cyclic contractions and relaxations, and diverse "stress-stimuli" such as pressure overload, metabolic alterations, oxidative stress, hypoxia, ischemia-reperfusion, mechanical stress etc. is remarkable. However, it is also highly vulnerable to succumbing to these multiple stressors that can result in generation of DAMPs leading to inflammation, fibrosis and heart failure. The myocardium cell types express DAMP- sensing receptors and are proficient to respond immediately to stress and damage. Therefore, efficient quality control mechanism regulating cardiac homeostasis are indispensable to sarcomere maintenance and dynamic adaptation to stress [68–72].

4. Inflammatory networking in cardiac fibrosis: a heart on (in) flame

It is becoming increasingly recognized that the regenerative ability is not completely reliant on genetic makeup, environmental conditions or evolutionary hierarchies but the nature and extent of the immune responses to cardiac injury equally play important role in governing regenerative and non-regenerative modes of wound healing [73]. Importantly, cellular stress pathways cross- talk with inflammatory pathways that actively participate in restoring tissue homeostasis and overactivation of the inflammatory mediators can result in cardiomyocyte death and fibrosis. Macrophages are crucial for tissue homeostasis, following injury, circulating monocytes give rise to proinflammatory macrophages through activation mediated by DAMPs or cytokine secretion. Macrophages may contribute to cardiac fibrotic remodelling through secretion of TNF-α, IL-1β, IL-10, TGF-β and growth factors [74]. Studies have shown that Gata6 expressing macrophages can regulate cardiac fibrosis [75], $CX3CR1^{+}$ and $CCR2^{+}$ resident macrophages may positively or negatively regulate cardiac fibrosis, respectively following injury [76, 77]. Mast cells exist in low density in heart tissue, however, following an injury mast cells infiltrate heart tissue [78]. DAMPs may trigger degranulation of mast cells that leads to the release of inflammatory mediators including tryptase, chymase, TNF-α, and IL-1β [79]. Tryptase and chymase activate a potent fibrogenic mediator i.e. TGF-β (that promote myofibroblast differentiation and collagen production). Mast cells also produce PDGF-A and FGF2, which positively regulate fibrosis [80, 81]. However, studies have shown that mast cells can also produce IL-10 (anti-inflammatory agent) that is a negative regulator of fibrosis [82, 83]. Dendritic cells (DCs) play important role in initiating an adaptive

immune response in post-injury cardiac remodelling. Studies have shown that DCs infiltrate cardiac tissue following an injury, specifically $CD11^+$ DCs (bone marrow derived) are held crucial for cardiac homeostasis. Deficiency of $CD11^+$ DCs following a cardiac injury may result in enhanced fibrosis [84]. Another study had shown that deletion of cardiac $CD103^+$ DCs resulted in increased fibrosis [85]. Adaptive T and B lymphocytes are also central to cardiac inflammation as B and T cells infiltrate cardiac tissue following injury. There are different subsets of T cells including: $CD4^+$, $CD8^+$, $CD73^+$ and Tregs. $CD4^+$ cells have been reported to produce proinflammatory and fibrotic cytokines like IFN-α, following injury [86], while CD73 expressing T cells reduced fibrosis [87]. B cells secreted proinflammatory cytokines like IL-1β, IL-6 and TNF have been positively associated with fibrosis [88]. Neutrophils are known to regulate fibrosis in context dependent manner, however, neutrophil-derived extracellular traps (NETs) are becoming increasingly implicated in fibrotic pathologies including cardiac fibrosis. NETs have recently gained attention in chronic inflammatory, autoimmune and fibrotic settings including cystic fibrosis, interstitial lung disease, thromboinflammation, hypertrophic cardiomyopathy and liver fibrosis (reviewed in [89–95]). Notably, NETs have been reported to be associated with *disease-specific* bioactive proteins loaded onto them [96]. Intriguingly, emerging clinical and experimental studies indicate that neutrophils are able to release intrinsically and qualitatively different NETs decorated with *disease-specific* bioactive proteins dictated by diseased inflammatory environment containing tissue factor, IL-1β, IL-17, and LL37, suggesting systemic inflammation driven transcriptional-reprogramming in circulating neutrophils, which triggers *de novo* expression of disease-specific protein fingerprints that are extracellularly delivered through generation of NETs [97] and references therein, these exciting findings implicate NETs as potential anti-fibrotic targets. The non-immune cells of myocardial niche also participate in inflammatory responses, e.g. cardiomyocytes can generate pro-inflammatory mediators leading to profibrotic TGF-β and IGF-1 signalling [98, 99]. Endothelial cells can serve as both positive and negative regulator of fibrosis by generating profibrotic mediators like TGF-β, FGFs, or endothelin-1 [100] and undergoing endothelial to mesenchymal transition [101]. Endothelial cells express HIF-1 (hypoxia inducible factor) that can have anti-fibrotic effects [102] endothelial CXC chemokine Interferon-gamma-inducible protein (IP)-10/CXCL10, is also an anti-fibrotic molecule [103, 104].

The key observations that reflected elevated circulating proinflammatory cytokines in heart failure with reduced ejection fractions pumped the research into exploring role of immune system in heart failure pathogenesis. If inflammation is the cause or result of heart failure is still debatable, however, the developments in understanding the roles of innate and adaptive immune cells in heart failure are in active progress to identify heart failure patients who can have a cardio-inflammatory phenotype and can receive prospective anti-inflammatory and immunomodulatory regimens. The CANTOS trial with anti- IL-1β antibody canakinumab indicated decreased hospitalization rates in certain group of heart failure patients [105], these findings have renewed the interest in decoding cardio-inflammatory pathways for therapeutic targeting. Sensing of DAMPs can trigger non-cellular and cellular effectors in including IL-1, IL-6, IL-8, TNF, chemokines, complement system, inflammasome assembly, and activation of neutrophils, monocytes, macrophage innate immune cells that further engage the adaptive immune arm to trigger inflammatory loops [106]. Leukocyte dependent regulation of cardiac fibrosis is an ongoing area; however, it stays controversial and warrants further studies to exploit leukocyte plasticity and heterogeneity in cardiac fibrosis therapeutics [107]. Recent demonstration of engineered T cells or the CAR T-cell therapy directed against activated fibroblast specific antigen has sparked new hopes to existing limited clinical

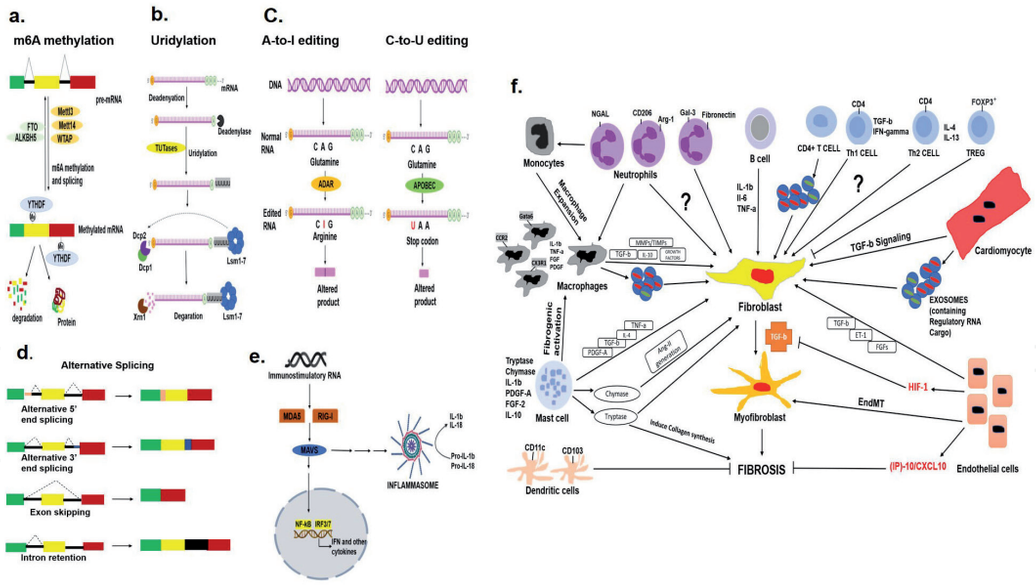

Figure 1.
RNA processing and modifications and their link to inflammation in cardiac fibrosis: (a) m6A Methylation, (b) Uridylation, (c) Editing, (d) Alternative Splicing, (e) Immunostimulatory RNA may result in Interferon and cytokines production. (f) Interaction between immune and non-immune cells during fibrotic remodeling. Diverse RNA processing pathways can result in generation of immunostimulatory RNAs that can trigger inflammatory cascade.

interventions and therapies in fibrotic heart failure [108, 109]. A recent study has demonstrated that macrophages expressing Mertk immune receptor in the heart supports cardiomyocyte health by phagocytosing exopher particles ejected from stressed cardiomyocytes harboring defective mitochondria. Mertk facilitated defective elimination of mitochondria from the myocardial tissue and prevented activation of the inflammasome, autophagy, metabolic stress, and ventricular dysfunction [110]. IL-11 signaling is also implicated in cardiac and cardiorenal fibrosis, however further studies will better indicate its precise role in driving pathogenesis [111]. A cross-talk between RNA processing pathways and immune- and non-immune cells through diverse mediators in context of cardiac fibrosis has been depicted in **Figure 1**.

5. Messenger RNA regulatory networks may modulate cellular stress and inflammation driving cardiac fibrosis: a message to the heart still in the outbox

The global co- and post- transcriptional mechanisms implicated in cardiac fibrosis are not well established, however, emerging studies are geared at extracting the subtle communications to identify intersection points between the cellular stress regulating pathways, regulatory non-coding RNAs, RNA metabolism intermediates/mediators and cardiac RNA binding proteins (RBFox, HuR, MBNL2, PUM2, QKI, CELF1, MBNL1, PTBP1) in context of cardiac diseases including fibrosis [66, 112–120]. A recent study implicated NUP155 subdomain hotspot with enriched allelic variants of the gene that suggests important role of RNA metabolism in cardiac disease and development [121].

Stem cell based regenerative approaches have found limited applicability in clinical translation to treat fibrosis. This ignited the research targeted at identifying cell- free secretory molecules that could not only have potential anti-fibrotic/regenerative potential to ameliorate fibrosis but characterization of these molecular

players would also facilitate understanding of mechanisms underlying pathogenesis of fibrosis. In this context, bioactive vesicles/exosomes have been extensively investigated to explore the role of regulatory RNAs (generated through RNA processing pathways) in delivering pro- or anti- fibrotic outcomes, besides non-coding RNAs are also extensively studied in context of fibrosis independent of their exosomal/vesicular loading [120–141]. The cardiac inflammatory circuits also tend to converge at the regulatory networks controlled by RNA processing, metabolism and surveillance pathways. Particularly, the post- transcriptional regulation of cytokines to stabilize mRNA, determine the strength of proinflammatory pathways. Altered expression of AU rich (ARE) or GU rich (GRE) elements in cytokine and cytokine pathway intermediate transcripts impairs mRNA decay and can result in heightened immune responses as seen in diseased states [142]. Immunostimulatory RNAs (like double stranded RNAs) generated through deregulated RNA processing pathways along with RNA binding proteins of RNA helicase (RNA sensors) family are emerging as important components of immune response pathways during sterile inflammation that involves DAMP sensing [15, 16]. Mitochondrial quality control pathways intersecting with the endosomal compartments and lysosomes are recently reported to favor generation and release of mitochondrial-derived vesicles in former condition [143], further offering discernible biologically stable lipid vesicles that may help investigate how secreted cargos can impact tissue repair and homeostasis or trigger fibrosis, and can be extended to establishment of liquid biopsies for studying the progression of fibrotic diseases or alternatively these vesicles may serve as therapeutic tools like exosome-derived non coding RNAs [121, 125, 127, 128, 138, 139, 141, 144] circulating microRNAs (miRNAs) and tissue resident miRNAs play paradoxical role both as anti-fibrotic [145–148] and pro-fibrotic [149–151].

6. Harnessing RNA metabolic pathways in cardiac development and disease: getting to core of the heart

The cardiac output is tightly tuned to the functional outputs of the cardiac transcriptome or faithful expression of cardiac-specific genes. It is becoming evident that cellular processes (that are linked to generation of RNA variants) including alternative splicing, RNA editing, epitranscriptomic modifications like methylation, and alternative polyadenylation [152] have a major role in shaping the cardiac adaptive responses [119]. The advent of high throughput NGS sequencing has revealed striking diversity in RNA species/variants/isoforms that have revolutionized the field of RNA biology by informing on the codes of burgeoning RNA inventory, which has now been exploited in context of functional relevance of the neo-RNA entities in context of physiological and pathological outcomes. Expanding information and identification of genetic markers for heterogeneous complex diseases like heart failure has made it appreciably evident that cardiac development and differentiation cues are under tight regulation of splicing events [153], and mis-splicing of certain genes like titin (TTN) that is implicated in contractility and mechanosensation can result in adverse cardiac extracellular remodeling and fibrosis. Interestingly, single cell RNA sequencing of cochlear hair cells recently documented unappreciated complexity in splicing diversity and isoform abundance underlying biology of hearing and deafness, and reported sorcin (a key player in cardiac excitation-contraction) as a top hit in cochlear outer hair cells [154]. These exciting findings reflect potential shared mechanosensory targets that could result in co-manifestation of heterogenous genetic disorders like heart failure and hearing loss, which involve electrical conductance and contraction events (it remains to be

explored if sorcin may have isoformic pattern of expression). It is already known that cardiac arrhythmias are a feature of Jervell and Lange-Nielsen syndrome (JLNS), an autosomal recessive disorder associated with congenital profound sensorineural hearing loss arising from homozygous or compound heterozygous mutations in either KCNQ1 or KCNE1 subunits of potassium ion channel conducting the slow component of the delayed rectifier current [155, 156].

Constitutive RNA splicing primarily involves the spliceosome machinery acting at the splice sites, however, alternative splicing may differ in its mechanism of action by engaging further cis- elements (regions within pre mRNA besides 5'/3' splice sites) like enhancers of exon/intron splicing [157]; the cis-elements are further acted upon by trans- acting modulators that constitute a family of RNA binding proteins (RBPs) with RNA binding motif (RBM), which further regulate negatively (repress) or positively (activate) the splice site selection. The activators include serine/arginine-domain–containing (SR) proteins and SR-related factors that dictate binding and assembly of the spliceosome complex and decide differential inclusion of exons in the mature transcript [158]. In contrast, the repressor trans-elements include the family of heterogenous nuclear ribonucleoproteins (hnRNPs) that tend to suppress splice site recognition [159, 160]. Therefore, alternative splicing events diversify the overall repository of functional and/or regulatory genes by inclusion/exclusion of exon, intron retention, alternative 5' or 3' splicing, and mutually exclusive exon utilization [119, 152], and at the same time splicing diversity may reflect pathological vulnerabilities dictated by the inherited variants that offer isoformic switching.

7. RBM20 interactome in cardiac development and disease: determining the soft- or hard- heartedness

Cardiac diseases including cardiomyopathy and arrhythmia are long known to be regulated by isoformic pattern of protein expression for genes including titin [161], CAMK2D, LDB3 and CACNA1C [162, 163]. Titin isoform-switching mechanisms at RNA (alternative splicing) and protein (post-translational modification) levels, which direct titin-based passive tension tuning remained largely elusive [164–169]. RNA binding protein RBM20, which is a splicing related factor was known to steer various aspects of cardiac function by regulating genes involved in biomechanics (TTN and TPM1), ion homeostasis and electrical activity (CAMK2D and CACNA1C) and signal transduction (CAMK2D and SPEN). Titin is the best exemplified target of RBM20 and TTN mutations are vastly implicated in cardiomyopathy [170, 171], and cardiovascular diseases [164, 172–174]. However, the mechanisms underlying alternative splicing of titin and role of thyroid hormone and insulin signaling in regulating it were nearly correlative [175–178] as the splice factors regulating alternative splicing in titin remained undetermined until early this decade. The role of post-transcriptional regulation in cardiac function and pathogenesis of human heart failure gained impetus following a pioneer study [179] on RNA binding motif (RBM20) protein, which has now emerged as a global regulator of cardiac alternative splicing isoformic switch in protein titin. RBM20 was found to be predominantly expressed in striated muscle, with maximum expression in the heart and its deficiency in rats was reported to resemble the pathophysiology of genetic dilated cardiomyopathy (fibrotic remodeling in heart). The RBM20-null rats exhibited increased subendocardial fibrosis with age and this effect was accompanied by electrical abnormalities and sudden death. The reduced activity of RBM20 (due to mutations/variants) resulted in altered isoform expression of genes central to biomechanics, electrophysiology and signal transduction culminating in

cardiomyopathy, fibrosis, arrhythmia and sudden death [179]. Following these seminal findings that delineated the role of RBM20 driven splicing in titin isoforms, the ribonucleoprotein RBM20 has now paralled the role of titin in regulating structural and functional characteristics of cardiac development and disease. Cardiac-specific splicing events are attributed to RBM20, and recent studies with defective RBM20 variants have been shown to be associated with cardiac transcript variants resulting in cardiomyopathy including DCM (dilated cardiomyopathy) [180–190].

Structurally, RBM20 is a 1227 amino acid long protein with a leucine-rich N-terminal domain, zinc finger (ZnF) domain 1, RNA recognition motif (RRM) followed by mutational hotspot arginine-serine (RS) rich domain, glutamate (E) rich and ZnF2 regions located towards C-terminal. RRM and RS regions, and phosphorylation within RS region, are reported to be crucial for nuclear localization [179, 191, 192], RRM is important for binding to "UCUU" RNA sequence that dictates RBM20 binding to target genes. Several mutations are reported in RS region (predominantly the RSRSP stretch, amino acids 634-638) that likely disrupt its nuclear localization and hence splicing of target genes like titin, subsequently resulting in adverse cardiac modelling and familial dilated cardiomyopathy with associated fibrosis [148, 183, 193–203]. Compared to nuclear localization regions, the structural components contributing to splicing activity of RBM20 towards its targets are not fully explored, the near C-terminal E region has shown to have some contributions to splicing though [179, 204]. RBM20 is known to interact with spliceosome complex subunits U1 and U2 (small nuclear ribonucleic particles) and U2 related proteins like U2AF6 and U2AF35 [205]. The inventory of RBM20 regulated cardiac pre-mRNAs is dynamic and includes the following validated genes in human and rat- titin (TTN), calcium voltage gated channel subunit α1 C (CACNA1C), calcium/calmodulin dependent protein kinase II delta and gamma (CAMK2D and CAMK2G), formin homology 2 domain containing 3 (FHOD3), Lim domain binding 3 (LDB3), Lim domain only protein 7 (LMO7), muscular-enriched A type laminin-interacting protein (MLIP), PDZ and LIM domain 3 (PDLIM3), reticulon 4 (RTN4), ryanodine receptor 2 (RyR2), SH3 domain containing kinase binding protein 1 (SH3KBP1), sorbin and SH3 domain containing protein (SORBS1), and triadin (TRDN) [153, 179, 205–208].

Titin is an integral sarcomere protein responsible for maintaining passive elasticity in heart, structurally it is organized into modular structure with immunoglobulin-like (Ig), fibronectin type III (FnIII), proline (P), glutamate (E), valine (V), and lysine (K) containing highly elastic I-band region. The N-terminal domain of titin anchors it to the sarcomeric Z-band and C-terminal domain embeds it into M-band. The A-band maintains rigidity during contraction by binding to myosin. Titin's structural integrity is central to normal cardiac function, maintaining passive tension and driving length-dependent activation/Frank-Starling effect. Besides providing mechanical properties, titin stretching also participates in cellular signaling that facilitates cardiomyocyte growth and might be implicated in chronic myocardium remodeling, hypertrophy and fibrosis. Cardiomyopathy patients with mutations in titin gene further demonstrate the contribution of titin to systolic and diastolic heart failure. Systolic dysfunction underlies dilated cardiomyopathy (dilation of left ventricle) and hypertrophic cardiomyopathy (myocardial hypertrophy and ventricular thickening). Diastolic dysfunction is a hallmark of restrictive cardiomyopathy with preserved contractile force, however, abnormal relaxation during diastole results in decreased cardiac output due to inappropriate ventricle filling. Truncation mutations in titin gene cause dilated cardiomyopathy through diverse pathways that involve haploinsufficiency, activation of mTOR energy sensor pathways and increased metabolic stress (recently reviewed in [209]). Human induced pluripotent stem cell (hiPSCs) culture models aimed at generating

cardiomyocytes from titin mutation carrying patients depict disorganized sarcomeric array, contraction disability and impaired force generation, however, similar extent of sarcomeric damage and myofibril contraction impairment has not been recapitulated in human studies or biopsied cardiomyocytes from titin variant patients. Therefore, it is alternatively proposed that titin variants may operate through creating a metabolic stress that could impair cardiac function independent of mutation sites by altering RNA metabolism pathways triggering non-sense mRNA decay (NMD) of abnormal titin variants and development of DCM phenotype. The cardiac metabolism could switch to branched chain amino acid pathway in place of fatty acid metabolism, deregulation of mTOR and autophagy pathways [210–218].

RBM20 cardiomyopathy has high penetrance and correlates with increased rates of heart failure, arrhythmias, and sudden cardiac death, new insights into RBM20 cardiomyopathy are extensively discussed recently [190]. Given the large size of titin (near 300kb) it is known to undergo extensive splicing events and yield several titin isoforms with cardiac N2B and N2BA to be the best characterized. N2B (shorter and stiff isoform) and N2BA (longer and pliant isoform) are adult cardiac isoforms of titin that regulate passive stiffness in the heart, and this is attributed to their structural dissimilarity in the highly elastic I-band region. Alternative splicing variants of titin during cardiac development keeps selecting for the shorter and stiffer isoform N2B in course of fetal to adult cardiac development, and physiologically N2B is overexpressed as compared to pliant N2BA isoform. However, aberrant expression patterns of titin isoforms resulting in altered ratios of N2B and N2BA are associated with cardiac diseases including cardiomyopathy with fibrosis and heart failure. RBM20 is shown to facilitate exon skipping events thereby selecting for shorter and stiffer forms of titin over development. In animal models, RBM20 homozygous mutations show increased ratio of N2BA/N2B (mirroring DCM phenotype), induced expression of RBM20 in RBM null mice decreases this ratio, however, intermediate effects (titin length, passive tension, sarcomere length) are seen in heterozygous mutations, indicating quantitative modulations of RBM20 as potential therapeutic approach to treat cardiac diseases [153, 179, 183, 194, 219]. RBM20 mutations in human patients result in severe inherited early onset DCM, manifesting even early on in younger patients with sudden death [190, 220]. Patient-specific stem cell based hiPSC culture models or CRISPR/CAS9 gene editing tools have been exploited to measure the effect of RBM20 point mutations in cardiomyocytes and alterations in sarcomere length, calcium handling, electrical coupling have been reported. The human iPSCs containing RBM20 mutations offer great tractable and tunable system to model cardiomyopathy *in vitro* and investigate potential signaling pathways contributing to the pro-fibrotic phenotypes [189, 221, 222]. The paradigm-shift in RNA metabolism associated interactome has begun to offer new therapeutic windows by unravelling the novel RNA binding proteins and splicing factors in context of cardiac development and fibrotic cardiomyopathies [119]. Biogenesis of regulatory non-coding RNAs i.e. microRNA, long noncoding RNA, and circular RNA, and their role in cardiac fibrosis, and RBM20 mediated alternative splicing of titin pre-mRNA is shown in **Figure 2**.

We briefly discuss the inflammatory networks in upper airway fibrotic diseases like laryngotracheal stenosis and subglottic stenosis that are relatively less explored in terms of mechanisms of fibrotic pathways, however, these pathologies need special attention as they might affect increasing number of patients given the current COVID-19 pandemic. Recent reports show that COVID-19 critically ill patients need mechanical ventilation, and many of these patients who need prolonged ventilation need surgical tracheostomy that is implicated in development of upper airway fibrosis.

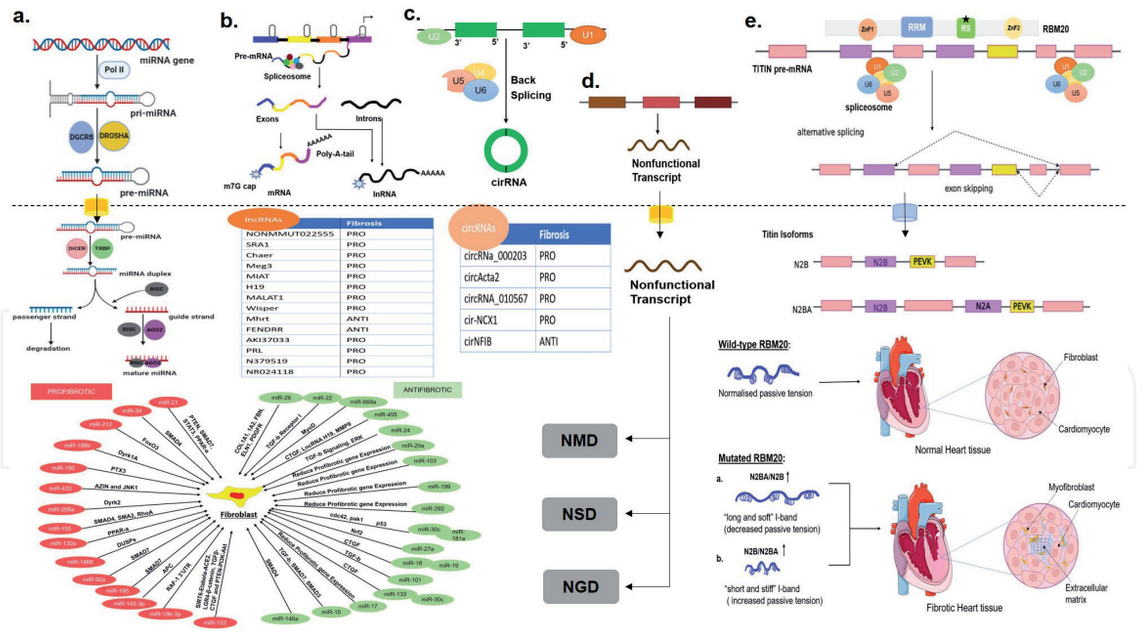

Figure 2.
Biogenesis of regulatory non-coding RNAs i.e. microRNA, long noncoding RNA and circular RNA and their role in cardiac fibrosis (a-c). Fate of aberrant RNA transcripts (d). RBM20 dependent splicing of Titin pre-mRNA resulting in formation of Titin isoforms which regulate cardiac development and fibrosis. Abbreviations: miRNA: microRNA; lnRNA: long non-coding RNA; cirRNA: circular RNA; NMD: nonsense-mediated decay; NSD: nonstop-mediated decay; NGD: no-go decay

8. Laryngotracheal stenosis: the pathogenesis and inflammatory pathways

Laryngotracheal stenosis (LTS) is an abnormal wound healing process of laryngotracheal mucosal inflammation, wound healing and scar formation. LTS is a fibrotic disease leading to pathologic narrowing of the larynx, subglottis, and trachea (the upper airway). There can be multiple etiologies to LTS, ranging from intubation injury (iatrogenic), radiation, autoimmune disease, to idiopathic [223]. The early stages of LTS are marked by dysphonia and communication difficulties that can develop into life-threatening progressive dyspnea leading to the airway compromise [224]. The most common form of LTS is the iatrogenic LTS (iLTS) caused by regional hypoxic and ischaemic pressure (stress) induced necrosis of the airway following prolonged intubation or tracheostomy [225]. The possible patho-physiology behind iatrogenic LTS is the surpassing of the pressure exerted by the cuff while prolonged intubations to that of the mucosal capillary perfusion pressure (approx. 35 mmHg), which results in ischemia, inflammation of the mucosa, and subsequent fibrotic strictures [226, 227]. The airway is primarily formed of 3 sets of cell types including epithelial cells, the fibroblasts, and the resident immune cells. The cross-talk of fibroblasts, immune cells and inflammatory cytokines participates in the development and propagation of LTS.

9. Inflammatory networks in the pathogenesis of LTS

TGFβ-SMAD2/3 cascade has been implicated in LTS and TGF-β antagonists have shown to attenuate fibrosis, however, TGF-β3 isoform has been reported to have antifibrotic response in LTS healing by significantly decreasing the inflammation and collagen deposition [228, 229], indicating opposing roles for isoforms of TGF-β. Hypoxia induced expression of IL-6 plays an important role in the pathogenesis of

LTS, the pressure exerted by the endotracheal tube cuff causes hypoxic and ischemic necrosis of the laryngotracheal mucosal tissue leading to inflammation and scarring marked by increased expression of IL-6, α-SMA & collagen, importantly IL-6 and myofibroblasts were also increased in an *ex-vivo* culture of healthy tracheal fibroblasts cultured under hypoxic conditions [230]. Possible role of B or T-cells in the formation of granulation tissue has also been been suggested [231]. Increased expression of profibrogenic Th2 cytokine IL-4 was seen in the brush biopsy samples of LTS scar and it stimulated fibroblast activation and excessive collagen formation in the LTS wound [225]. The expression of another Th2 cytokine IL-13 appeared to follow the same expression pattern of IL-4 and resulted in excessive fibrosis [232]. In contrast to Th2 cytokines, the Th1 cytokine IFN-γ inhibited fibrosis in LTS patients. Subsequent studies have shown the impact of IFN-γ on the LTS fibroblasts as significant decrease in levels of collagen and TGF-β expression was reported in the IFN-γ treated human LTS-derived fibroblasts compared to the untreated LTS-derived fibroblasts and normal laryngotracheal fibroblasts [226]. Dysregulated functioning of macrophages is related to fibroproliferative LTS [232], a prolonged cytokine signalling in the form of IL-4/IL-13 by Th2 cells can also contribute to impairment of macrophages by switching the non-fibroproliferative "classically activated" M1 macrophages into the fibroproliferative "alternatively activated" M2 macrophages [232]. Mice LTS model of chemical and mechanical injuries showed increased expression of M2 cell surface marker CD206 [224]. Inflammatory cytokine expression study in iLTS and autoimmune LTS patients demonstrated elevated levels of the macrophage growth factor granulocyte macrophage colony-stimulating factor (GM-CSF) and M2 cytokine IL-10 than that in controls [233]. Fibroblasts are the mesenchymal cells which are not terminally differentiated and rest in inactive state, under homeostasis. In their inactive but normal state they localise to the subepithelial layer of the airway tissue and provide for the biochemical and mechanical support to the tissue [234]. However, studies have reported increased ECM production and migration, and reduced contraction of iLTS fibroblasts [235], moreover, studies involving use of beta-aminopropionitrile (βAPN), an inhibitor of collagen cross-linking also demonstrated enhanced profibrotic features (overexpression of collagen I and II) in iLTS-derived fibroblasts [236], metabolically they exhibited enhanced glycolysis to oxidative phosphorylation ratio as see in proliferative cancer cells, justifying highly proliferative nature of iLTS-derived fibroblasts [237]. Emerging studies are reporting genetic link to LTS pathology, suggesting alternative treatment approaches to cure this fibrotic pathology. A functional single nucleotide polymorphism of TGF-β1 located in a negative regulatory element of its promoter was associated with the iatrogenic LTS. The data identified protective and susceptible genetic loci in patients undergoing endotracheal intubation. Another study, focused on 3 candidate genes encoding the innate immune receptor CD14, matrix metalloproteinase-1 (MMP-1), and the cytokine transforming growth factor β1 (TGF- β1). Reported association between MMP-1 and susceptibility to iLTS following intubation merits further investigation in a lager patient cohort [238].

10. Subglottic stenosis: a complex interplay between inflammation and fibrosis

Subglottic stenosis (SGS) is a relatively less explored fibrotic pathology in terms of mechanistic insights on inflammatory pathways. Researchers have gathered information to some extent by evaluating the changes that occur in the airway as a result of obliterative bronchiolitis [239], which shares the same fibrotic features of subglottic stenosis as studied in a murine model with emphasis on cytokines such

as IL-1β, TGF-β and prostaglandin PGE2 [240]. SGS is accompanied by an acute and an exaggerated inflammatory response that triggers a shift in the cellular and molecular components in the healing wound in favor of more fibroblastic etiology [235]. SGS has numerous potential etiologies of which the most common cause in adults and children alike is prolonged endotracheal intubation. With developments in intensive care and associated intubations, it is natural that iatrogenic stenosis will become a major factor affecting post ICU quality of life. Animal and human studies have shown an upregulation of inflammatory markers in stenotic tissues. Patient factors like increased BMI, diabetes mellitus and chronic laryngopharyngeal reflux have also been implicated as causative factors for development of subglottic stenosis [241]. Cytokines like IL-1β, IL -10, TNF α, IFN γ and GM-CSF have shown significant increase in subglottic stenosis specimens [226]. Enhanced expression of profibrotic growth factors and cytokines like TGF-β, PDGF, IL-1, and Prostaglandin E2 was seen in patients of healing laryngeal lesions [242]. Expression of matrix metalloproteinases (MMPs), α-SMA, SMADs, IL-1 continued to rise 3 weeks beyond the initial insult [231]. Therefore, it appears that SGS undergoes an aberrant healing response as cytokines corresponding to various stages of wound healing process including inflammation (IL-1, TGFβ), proliferation (SMADs, TGFβ), and maturation (MMPs, αSMAs) are reported to present in pathogenesis of SGS. Idiopathic subglottic stenosis (iSGS) also has an inflammatory network evident from various studies suggesting the role of γδ T cells in IL-17A dependent tissue inflammation and airway remodeling in iSGS. Further studies delineating the role of RNA biology pathways might open up viable therapeutic options for this devastating pathology.

11. Future perspectives and summary

We are encouraged to chase the role of RBPs in homeostasis-tissue-repair-fibrosis continuum based on our recent preliminary findings, where we report for the first time an *in vitro* model that rigorously recapitulates proteolytic stress (as encountered in fibrotic pathologies) induced stress granule (SG) biomolecular condensate-like proteome signatures [243]. Dynamic phase separated membraneless organelles including SGs are induced upon varied stress-stimuli (infectious or non-infectious) and are implicated in spatiotemporal control of various cellular functions including formation of signalling complexes, clustering of vesicles, sorting and trafficking of cargo [244]. Phase separated biomolecular condensates are becoming increasingly linked to developmental and pathological pathways [245–249]. We proposed proteases as novel stressors that can have diverse outcomes when present at varying concentrations (protease-antiprotease balance is crucial for driving tissue repair or fibrotic phenotypes). We observed heightened ribonucleoproteins (RNPs), spliceosome machinery, regulatory RNA generating proteins, and RNA binding proteins (RBPs) in our high-throughput proteomics data [243]. The formation of SGs like proteome was concomitant to translational halt in majority of proteins, sparing few essential cytoprotective proteins including exosome biogenesis and secretory pathway proteins that undergo synthesis despite stress environ. We hypothesize that the unique stress-associated proteins that represent "stress-essentialome" might get packaged and enriched into exosome vesicles in addition to the hitchhiking of SG regulatory RNAs, RBPs, RNPs and cytoprotective proteins onto the exosomal carriers leading to conglomeration of unique disease/stressor-specific cargos in exosome silos. Translational halt would result in polysome run off and dissociation of ribosome and translating mRNAs that would partition into the stress granules, therefore, RNA and polysome profiling of stressed cells in addition to exosome cargo profiling might offer valuable information on

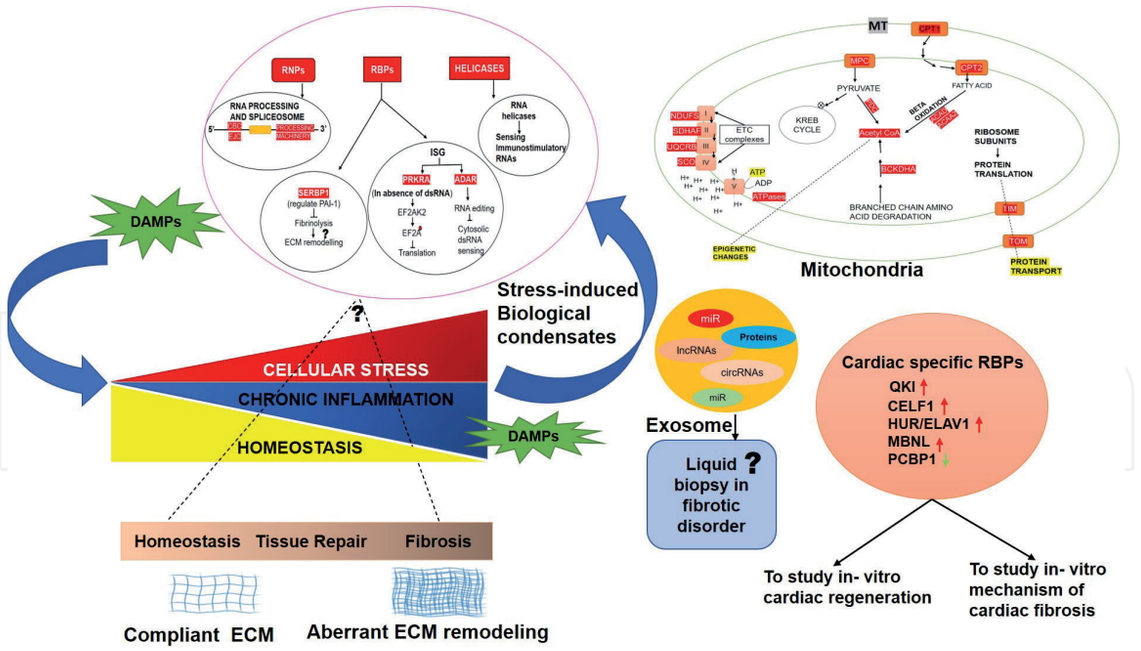

Figure 3.
Proposed model for cellular stress induced biological condensates that can regulate homeostasis, tissue repair and fibrosis. A cellular-stress induced reshaping of RNA processing machinery by generation of biomolecular condensates, and their coupling to mitochondrial and exosomal pathways. The stress-coupled exosomes are proposed to carry pathologic cargo which can be exploited to develop liquid biopsies in the context of fibrotic disorders. The expression of cardiac-specific RBPs in our model can be utilized to develop cardiac regenerative approaches in vitro or to study the role of RBPs in cardiac fibrosis. RNPs: ribonucleoproteins: RBPs: RNA binding proteins; CBC: cap binding complex; EJC: exon junction complex; SERBP1: SERPINE1 mRNA-binding protein 1; PAI-1: Plasminogen activator inhibitor-1; ISG: Interferon stimulated gene; PRKRA: Protein kinase, interferon-inducible double stranded RNA dependent activator; ADAR: Adenosine deaminases acting on RNA; EF2AK2: Eukaryotic Translation Initiation Factor 2-alpha Kinase 2; RED color: upregulated proteins; MT: mitochondrion; CPT: Carnitine palmitoyl-transferase; MPC: mitochondrial pyruvate carrier; PDC: Pyruvate dehydrogenase complex; ACAD: Acyl-CoA dehydrogenase; ACAA2: acetyl-Coenzyme A acyltransferase 2; BCKDHA: branched-chain alpha-keto acid dehydrogenase; NDUFS: NADH-ubiquinone oxidoreductase subunit; SDHAF: Succinate dehydrogenase complex assembly factor 1; UQCRB: Ubiquinol-cytochrome c reductase binding protein; SCO: synthesis of cytochrome c oxidase; TOM: translocase of the outer membrane; TIM: translocase of the inner membrane; ETC: electron transport chain. DAMPs- danger associated molecular patterns.

pathologic transcripts, associated regulatory RNAs (miRNAs, lncRNA) and translating ribosome composition besides the proteome signatures, thereby offering new dimensions to investigate stress-induced regenerative or fibrotic responses and the role of RNA processing machinery and RBPs in driving these triggers. It might offer identification of cell or tissue specific splicing factors and an opportunity to attempt rescuing splicing related alterations inherited in genome (pathogenic variants), to switch for native isoforms. In addition, stress-induced secretion of extracellular vesicles/exosomes could offer novel therapeutic opportunities by developing liquid biopsies in fibrotic diseases (a less explored area), which may yield meaningful information and help predict progression of the disease in suitable disease-specific *in vitro* models. Exosomes are relatively stable, can avoid background noise, can be easily detected from blood and their molecular analysis may decipher pathobiology of disease, fibrotic liver derived exosomes (in mice) showed increased CCN2; decreased Twist1, miR-214 than control mice [250]. Circulating exosomes from mice with alcohol related liver disease when transmitted to normal mice resulted in pro-inflammatory/fibrogenic liver phenotype [251]. MiR-125 has also been found to be upregulated in serum from patients with cirrhosis than controls [252]. Multiple studies *in-vitro* and in mice have revealed pathologic micro-RNAs associated with liver injury, liver fibrosis and liver malignancy [250]. However, major challenge in translating bench to bedside knowledge include reliable standardization and

characterization protocols, and validation in a well-characterized patient population. Therefore, we are interested in deciphering the molecular information stored in exosomes of patients with liver cirrhosis using standardized protocol and their correlation with key events like death, sepsis, organ failures. Our proposed model of stress- induced reshaping of RNA metabolic pathways that are coupled to mitochondrial alterations and exosome biosynthesis is shown in **Figure 3**.

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