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Nanomedicine has elegantly attempted to cure multiple gene polymorphisms and mutations in cardiovascular diseases using gene therapy techniques

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Abstract- The molecular mediators that induce MI, the loss of myocardial regeneration, and the development of heart disease are all being researched more thoroughly. It's intriguing to think about how genetic factors could be used to modulate these disease mediators. DNA, RNA, or proteins may both be included in this operation. However, direct delivery of these biomolecules is not always effective. Using gene therapy methods, nanomedicine has elegantly attempted to reverse many gene polymorphisms and defects in complex diseases.

The stability of these biomolecules, as well as their controlled release and passage through barriers to the operating site, can be aided by delivery systems. Precision-tailored vector delivery has been shown to reduce toxicity and improve drug availability. Currently, a variety of distribution systems are being evaluated, with the frontrunners incorporating security and conclusions being selected. New biological mediators, as well as the complex interactions within them, as well as their pharmacokinetic and pharmacodynamic profiles, will be discovered in the future. This opens the door to a more advanced delivery system that meets the biological requirements for maximum therapeutic efficacy

I. INTRODUCTION

The Encyclopedia of DNA Elements (ENCODE) and the Practical Annotation Of Mouse (FANTOM) consortia demonstrate that at least 80% of our genome is transcribed, encodes for less than 3% of protein-coding transcripts, and is mostly made up of noncoding RNAs (ncRNAs), which were previously dismissed as "genomic noise" and are classified at random based on transcript duration. Short ncRNAs include microRNAs (miRNAs), transfer RNAs, and small nucleolar RNAs, while longer RNAs include ribosomal RNAs, regular antisense transcripts, and other long noncoding RNAs (lncRNAs). The human genome now contains 2500 miRNAs and 50,000 long lncRNAs, approximately doubling the number of protein-coding transcripts from 25,000 to 50,000 (https://www.encodeproject.org/).

MiRNAs are a class of small single-stranded noncoding RNAs (ncRNAs) with an evolutionary conserved size of 19–25 nucleotides that regulate gene expression after transcription. Their primary mechanism of action is to inhibit gene expression by pairing complementary base pairs with sequences in protein-coding transcripts (1, resulting in translational repression or mRNA degradation. Seed sequences, which are nucleotides 2–8 at the miRNA's 5′ end, are required for identification (2). The primary sequence of only a small subset of lncRNAs has survived evolution, but the remainder of lncRNAs have tissue and cell type-specific expression, suggesting that their expression must be strongly regulated. LncRNAs perform a number of molecular functions, including scaffolding for protein-protein interactions, transcriptional activators or repressors, molecular decoys, and key platforms for the assembly of complex protein components (3).

II. ABNORMALITIES IN NCRNA AND CARDIOVASCULAR DISEASE

When ncRNAs and their targets are encoded in the genome, genetic rules apply. As a result, single nucleotide polymorphisms (SNPs) in the sequence of a target site can influence ncRNA regulation, making them potential candidates for functional variations. Variations in ncRNA genes may influence the expression level and functionality of the ncRNA, leading to differential regulation of their target genes. Genetic variants have the ability to affect MiRNA behavior in a number of ways. Transcription rate and miRNA expression levels may be affected by differences in miRNA promoter areas, host gene splice sites (for intronic miRNAs), or polycistronic, clustered miRNAs. Variants in the miRNA transcript affect the miRNA hairpin's binding affinity to biogenesis enzymes or accessory proteins, as well as processing precision and strand loading bias into RISC, resulting in canonical miRNA expression and target gene deregulation. Variants in the mature sequence can generate isomiRs, which can affect target specificity, while variants in target messenger RNAs can either destabilize or create

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new miRNA seed regions (4). SNPs within lncRNA loci can alter the expression profile of the lncRNA or its downstream target genes, subtly alter the transcript's characteristic 3D architecture and any of its functions associated with its ability to interact with other RNA molecules or proteins, or trigger mutations in micropeptides, provided that lncRNAs are less evolutionary conserved. Given the potential impact of ncRNA genetic variants on cellular functions and the importance of ncRNA-mediated gene regulation, it's no surprise that genetic variants have been linked to or associated with a variety of human diseases (5, 6, 7). In this part, we'll go through the different effects that genetic variations in human ncRNAs may have on cardiovascular disorders.

1.1 Variants in ncRNA target genes

The vast majority of typical variants related to miRNA aberrant functions in cardiovascular diseases have been found in the 3'UTR of ncRNA target genes.

One example is hereditary variants related to coronary artery disease (CAD), a leading cause of death linked to a number of risk factors, including genetic and environmental factors (8). The Ge laboratory was one of the first to investigate the effects of common SNPs in the 3'UTR gene regions on CAD susceptibility, using an in silico study and a luciferase assay to prove that the rs4846049 polymorphism in the MTHFR gene's 3'UTR area was related to an increased disease risk (9). When homocysteine plasma levels are high, the MTHFR gene is necessary for folate and homocysteine metabolism, and its dysfunction has been related to an increased cardiovascular risk (10). MiR-149's binding to the 3'UTR of MTHFR was affected by the rs4846049 mutation, which had a higher affinity for the T form than the wild type G allele. In a follow-up case–control study involving 654 affected Chinese Han patients, the same authors found that while the T allele was significantly associated with an increased risk of CAD, the homozygous TT condition was significantly associated with a decrease in HDL-C and apoA levels, both of which are protective against CAD. Since TT carriers' MTHFR protein levels were lower than GG carriers', but mRNA expression levels did not vary significantly, an increased decay of MTHFR or translational repression mediated by miRNA is thought to occur in the Chinese Han population (9).

TCF21, a vascular developmental transcription factor gene implicated in controlling cell fate decisions in developing human coronary artery smooth muscle cells (HCASMC), maps to 6q23.2, which has been compared to CAD in GWAS (11). The rs12190287 polymorphism is found in the 3'UTR region of the TCF21 gene (12). As shown by luciferase assays in HCASMC (12), this change disrupts the seed binding sequence for miR-224, which binds more strongly in the presence of the disease-associated C allele than in the presence of the protective G allele, resulting in post-transcriptional selective repression of the C allele of TCF21. MiR-224 and TCF21 have inverse expression levels in HCASMC, and miR-224 dysregulates TCF21 gene expression in response to atherosclerotic signaling pathways such as transforming growth factor and platelet-derived growth factor signaling in an allele-specific manner in response to atherosclerotic signaling pathways such as transforming growth factor and platelet-derived growth factor signaling (12). In vivo, the TCF21 protein and miR-224 were found in diseased human vessel walls, with inversely regulated expression in atherosclerotic lesions (12). These findings support a functional role for miR-224 in HCASMC, involving post-transcriptional TCF21 gene repression via preferential binding to the C rs12190287 variant, shedding light on the 6q23.2 locus's genetic risk for CAD (12).

Another variant found in the 3'UTR of a miRNA target gene related to CAD is rs3088442 in the SLC22A3 gene. This gene, which is part of the SLC22A3-LPAL2-LPA gene cluster in the 6q26-27 CAD danger locus (15), produces the organic cation transporter-3 transmembrane protein, which is required for drug and bioamine outward transport (13), (14). According to a case–control study in the Chinese population and an in silico analysis, the rs3088442 polymorphism provides a putative binding site for miR-147 in the SLC22A3 mRNA. SLC22A3 deficiency has been related to the inhibition of LPS-induced monocytic inflammatory response, which involves the inhibition of both NF-kB nuclear translocation and MAPK signaling cascades, according to transfection studies in THP-1 cells. As a result, the levels of pro-inflammatory mediators IL-6, IL-8, and MPC-1, as well as histamine synthase HDC expression, were diminished. The same effect is produced by exogenous histamine, a vasoactive autacoid that triggers immune and inflammatory responses. Treatment with miR-147 mimic or antagomir established the function of the SLC22A3 rs3088442 polymorphism in the reduced risk of CAD regulated by miR-147 (14). LPS stimulation induced miR-147 upregulation and decreased the expression of the protein carrying the rs3088442 version. The Encyclopedia of DNA Elements (ENCODE) and the Practical Annotation Of Mouse (FANTOM) consortia demonstrate that at least 80% of our genome is transcribed, encodes for less than 3% of protein-coding transcripts, and is mostly made up of noncoding RNAs (ncRNAs), which were previously dismissed as "genomic noise" and are classified at random based on transcript duration. Short ncRNAs include microRNAs (miRNAs), transfer RNAs, and small nucleolar RNAs, while longer RNAs include ribosomal RNAs, regular antisense transcripts, and other long noncoding RNAs (lncRNAs). The human genome now contains 2500 miRNAs and 50,000 long lncRNAs, approximately doubling the number of protein-coding transcripts from 25,000 to 50,000 (https://www.encodeproject.org/).

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the function of the SLC22A3 rs3088442 polymorphism in the reduced risk of CAD regulated by miR-147 (14). LPS stimulation induced miR-147 upregulation and decreased the expression of the protein carrying the rs3088442 version.

Functional genetic variations in the proximal promoter and 3′UTR of the Chromogranin A (CHGA) gene have been related to essential hypertension (16) and hypertensive end-organ harm (17). Given the lack of clarity surrounding the pathogenesis of hypertension, it is thought to be a polygenic disorder caused by a combination of environmental and genetic factors, which may account for up to 45 percent of inter-individual blood pressure discrepancies (18). Both in the central nervous system and the peripheral nervous system, CHGA regulates vasodilation and inflammation (19), as well as blood pressure homeostasis and catecholamine release (catestatin peptide) (20). Chga expression is higher in the adrenal glands and plasma of spontaneously hypertensive rats (SHR) and lower in the brainstem of spontaneously hypertensive rats (SHR) as compared to Wistar Kyoto control rats (WKY) (22). Due to the polymorphism's enhanced interaction with miR-22, goal resequencing showed the rs13449558 typical variance in the 3'UTR of the Chga locus in SHR, which has been linked to a decrease in luciferase signal in PC12 cells (22). MiR-22 antagomir therapy in SHR resulted in lower systolic and diastolic blood pressure, as well as an increase in brainstem CHGA protein and a decline in adrenal CHGA protein, which is interesting. This finding supports the function of rs13449558 in Chga dysregulation in brainstem cardiovascular control nuclei, ultimately contributing to the pathogenesis of hypertension in SHR and providing clues for the development of novel therapeutic approaches (22). CHGA is affected by differences of other genes. In humans, the ATP6V0A1 gene for the vacuolar H + ATPase subunit maps to 17q21, a positional candidate locus inside the catestatin linkage confidence interval (23). In the general population, the rs938671 polymorphism in the 3'UTR region of ATP6V0A1 has been related to catestatin processing from CHGA and blood pressure (24). This variant disrupts the hsa-miR-637 recognition motif, resulting in a decrease in ATP6V0A1 gene expression in PC12 cells, according to a combination of computational and in vitro functional methods (24). The rs938671 allele consistently inhibited vacuolar pH modulation in fluorescence monitoring studies of a CHGA/EGFP chimera in PC12 cells, and the ratio of CHGA precursor to its catestatin fragment was reduced after treatment with the V-ATPase inhibitor bafilomycin A1, which mimicked the increased pH caused by the C allele (24).

Similar to what was found in the CHGA gene, the rs7079 standard variant in the 3'UTR of the human angiotensinogen gene (AGT) modulates hypertension in a miRNA-dependent manner. The AGT gene has been related to coronary atherosclerosis (26), cardiac hypertrophy (27), and essential hypertension in Caucasian (28), Indian (29), and Japanese (30) populations. According to computational studies, both miR-31 and miR-584 will bind the sequence around the rs7079 region of the AGT gene when the C allele is involved, but not when the A variant is present. The expression of the C allele can be suppressed as a result of miR-31 and miR-584 binding. Transfection of miR-31 and miR-584 mimics decreased AGT mRNA levels in Hep3B and HEK293 cells, while luciferase assays in the presence of miR-31 and miR-584 mimics inhibited reporter gene function in the presence of the C variant but not the A allele in Hep3B and HEK293 cells. As a result, this repression was alleviated in the presence of anti mirs for miR-31 and miR-584 (25).

Long QT syndrome (LQTS) is an inherited cardiac condition that causes chronic ventricular repolarization, mainly due to ion channel abnormalities in the heart, which predisposes people to life-threatening arrhythmias (31). Long QT syndrome (LQTS), a hereditary cardiac disease characterized by excessive ventricular repolarization due to disruptions in cardiac ion channels, has also been attributed to variations in the 3'UTR regions of ncRNA target genes. The most common type, LQT1, is determined by mutations in the KCNQ1 gene, which encodes Kv7.1, the pore-forming subunit of the voltage-gated potassium channel and has been shown to be regulated, at least in part, by miR-1 or miR-133a (32), (33), (34). To see whether 3'UTR SNPs play a role in disease heterogeneity, the whole 3'UTR of the KCNQ1 gene was sequenced in 168 LQT1 patients (35).

Three SNPs (rs2519184, rs8234, and rs10798) were shown to have an allele-specific relationship with QT interval corrected for heart rate (QTc) and symptom frequency: Patients with the derived SNP variant on the mutant KCNQ1 allele had a shorter QTc and less symptoms, while people with the derived SNP variant on the wild-type KCNQ1 allele had a longer QTc and more symptoms. In luciferase reporter assays, each of the three SNPs reduces the expression of the reporter gene (35). These findings show that these functional variants may impair the protein balance extracted from the normal or mutated allele's expression; therefore, when the 3′ UTR SNPs are on the mutation-carrying allele, the disorder is less severe, however when they are on the stable allele, the phenotype is more severe.

GWAS revealed a large number of SNPs and genomic loci associated with cardiometabolic syndrome (CMS) in noncoding sequences, such as 3'UTR regions (36), (37). Hypertension, elevated fasting glucose, waist circumference, and triglycerides, as well as obesity, physical inactivity, insulin resistance, and poor HDL-C, are both contributing factors for type 2 diabetes and cardiovascular disease (38). SNPs discovered in the 3'UTR sequences of CMS genes are a starting point for potential studies into miRNA-mediated disease gene regulation. For eg, Ghanbari and colleagues used GWAS to find variants in miRNA-binding sites in subjects with different cardiometabolic phenotypes in order to select SNPs that were expected to be functional in their genomic loci (39). The co-expression of the miRNA and related target gene in relevant cardiometabolic tissues, such as blood and adipose tissue, and the allele-specific expression of the gene hosting the variation were prioritized based on the proven association with the phenotype, and the co-expression of the miRNA and related target gene in relevant cardiometabolic tissues, such as blood and adipose tissue. The effects of these variations on relative mRNA expression levels were measured using luciferase assays, which validated the putative correlations between selected miRNAs and genes hosting 10 selected SNPs while also determining the

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effects of these variations on relative mRNA expression levels. Indeed, if the main allele in the 3'UTR region inhibited miRNA-mRNA interaction, resulting in a significant reduction in luciferase function, the minor allele substitution decreased miRNA-mediated inhibition, and vice versa (39). The interaction of the studied miRNAs with the 3'UTR regions of the LPL (miR-136 and miR-410), FADS1 (miR-181a-2), HSD17B13 (miR-375), MKRN2 (miR-154), CDKN2B (miR-138-2-3p), and FN3KRP (miR-34a) genes resulted in decreased gene expression, which was greatly reduced by the minor allele The presence of an SNP can either completely interrupt the miRNA binding site, resulting in normal expression of the target gene (e.g., the rs1173092 minor allele in the miR-375-binding site in HSD17B13), or partially abrogate the miRNA-binding site, owing to the position of the mutated nucleotide in the target site or compensatory 3′ end pairing (e.g., the rs1173092 minor allele Two SNPs increased miRNA binding to the PVRL2 (miR-320e) and IKZF3 (miR-326) genes' 3'UTR regions, which is an unexpected result that may be explained by the SNP upgrading the original recognition site or establishing a new binding site (39). Atherosclerotic cerebral infarction (ACI) is a complicated syndrome exacerbated by a combination of factors like smoking, diabetes, a history of coronary heart disease, and a low level of HDL cholesterol (40-42). The MMP-9 gene has been related to the progression of early atherosclerosis, vascular remodeling, and arterial plaque breakdown, and its levels have been shown to be elevated in patients with atherosclerosis and acute ischemic stroke (43). Using MALDI-TOF mass spectrometry and in vitro functional experiments, the rs1056628 C allele in the 3'UTR of the MMP-9 gene was shown to be associated with an increased risk of ACI as compared to the A variant (40). rs1056628 is located in the miR-491-5p binding site, according to in silico predictions, and overexpression of this microRNA in human umbilical vein endothelial cells decreased MMP-9 protein expression without affecting relative mRNA levels. Overall, these results showed that miR-491-5p inhibits MMP-9 translation in the presence of the rs1056628 A allele, but not in the case of the rs1056628A > C conversion, which could affect miR-491-binding 5p's site and lead to MMP-9 gene overexpression (40).

Stroke is a coronary heart disease caused by a blockage of the blood supply to the brain (44). Angiopoietin-1, an angiogenic growth factor with anti-permeability and anti-inflammatory properties that regulates chronic or uncontrolled inflammation (45), is a key regulator in its pathogenesis. Because of this function of the gene, Chen et al. based their findings on the common variant rs2507800 in the miR-211 target site within the 3'UTR of the angiopoietin-1 gene (46). Centered on plasma angiopoietin-1 levels in stroke patients in different case–control association trials, the researchers discovered that the TT genotype was associated with a significantly lower risk of complete stroke, ischemic stroke, hemorrhagic stroke, and lacunar infarction than the AT and AA genotypes. Luciferase assays showed a significant reduction in reporter gene expression when A549 cells were co-transfected with a precursor of miR-211 and the 3'UTR carrying the A allele. These results suggest that the T allele of rs2507800 will defend against stroke by decreasing miR-211 binding and increasing angiopoietin-1 downregulation, rendering small vessels more prone to stroke danger and damage (46).

Surprisingly, a study led by Wang's team discovered a similarity between ischemic stroke and an SNP in the coding region of a lncRNA's target gene (47). Using expression quantitative loci study, a list of alternative genes whose expression may be influenced by genetic variations in ANRIL was discovered (47). In atherosclerotic lesions, CARD8, which encodes a caspase recruitment domain (CARD) member, is highly expressed (47), (48). In functional assays in HepG2 cells or human umbilical vein endothelial cells, the association between the CARD8 and ANRIL genes was confirmed, with knockdown of ANRIL expression lowering CARD8 expression and overexpression of ANRIL increasing CARD8 expression (47). Two case–control experiments performed in different Chinese populations find a clear association between the minor T allele of the rs2043211 variant in exon 5 of CARD8 and a protective function against ischemic stroke (47). More study is needed to establish how rs2043211 heterogeneity influences ischemic stroke progression and its connection to ANRIL, but current evidence indicates a connection between disease progression and the ANRIL-related CARD8 pathway (47).

These results, taken together, illustrate the role of common variants in ncRNA target genes in the susceptibility to and pathogenesis of cardiovascular diseases in a ncRNA-dependent manner, and may contribute to the creation of new selective therapeutic approaches.

1.2 ncRNA gene variants

Variations in genes encoding the actual coding regions of (pri/pre-) miRNAs and lncRNAs have only been found in a few cases, considering the fact that widespread variants in ncRNA target genes have been extensively defined and we can verify this observation for cardiovascular diseases.

The most common arrhythmia in the world, especially among the elderly, is atrial fibrillation (AF), which has a strong genetic component (49). AF, which is also a risk factor for ischemic stroke and thromboembolism, may cause or exacerbate heart damage (50). MiRNAs have been related to increased atrial arrhythmogenicity and AF and have been shown to play a crucial role in regulating atrial excitability (51). In this regard, irregular miRNA expression in cardiac tissue and blood has been observed in animal models and AF patients on a daily basis (52). In atrial tissue samples from patients with AF, altered levels of miR-1 and miR-133 have been discovered in various studies (53-55). Genetic screening of the MIR1-1 and MIR 1-2 genomes, which encode miR-1-1 and miR-1-2, and the MIR133A1, MIR133A2, and MIR133B genes, which encode miR-133a-1, miR-133a-2, and miR-133b, was conducted in 120 probands with suspected inherited AF. Eight of the ten variants were well-known, with minor allele levels in the AF population that were equivalent to those found in public databases (56). The 79 T > C substitution in the MIR133A2 gene has been discovered to modify the way the miRNA duplex is processed, allowing the usually damaged miR-133a-5p strand to clump together. Since two affected family members are genotype-negative, the presence of the MIR133A2 mutation in

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the proband may have a modifying effect by altering gene expression profiles in the atrium, according to the authors (56).

In a review (57), Roldan and colleagues looked at the prognostic effects of two adaptive MIR146A polymorphisms, rs2431697 and rs2910164, on adverse cardiovascular events (ACE) in 901 AF anticoagulated patients. TLR4 signaling stimulates NF-B, which drives immune responses such as the development of pro-inflammatory cytokines, exacerbating any disease (58). TLR4 stimulates NF-B, which drives immune responses such as pro-inflammatory cytokine production, exacerbating any disease. MiR-146a-5p is an important negative regulator of inflammation regulated by TLR4; signalling downstream TLR4 triggers NF-B, which drives immune responses such as pro-inflammatory cytokine production, exacerbating any disease. In AF patients, the homozygous rs2431697 TT genotype was related to ACE, suggesting that this miRSNP could be used as a prognostic marker (57). When TT monocytes were compared to CC monocytes, functional testing showed that TT monocytes had lower levels of miR-146a-5p. These results suggested that TT people have a higher inflammatory capacity when exposed to inflammatory stimuli, perhaps due to lower levels of miR-146a-5p (57).

Variants in ncRNA genes have also been linked to myocardial infarction prevention (MI). In a major case–control study using gene-based genome-wide tag SNPs, a haplotype block on chromosome 22q12.1 containing the standard variant rs2301523 and 5 other SNPs in linkage disequilibrium was found to be significantly associated with MI (59). The MIAT gene (also known as Gomafu) codes for a nuclear-retained lncRNA expressed in a number of neuronal cell types (60), and several of these mutations have been discovered there. MIAT binds to splicing factors and alters their availability to pre-mRNA, which has been shown to influence splicing efficiency (59), (60). In HEK293 cells, luciferase assays showed that the transcriptional role of MIAT was substantially higher in the presence of the rs2301523 minor G allele than in the A variant, most likely attributable to a weaker interaction of an unknown nuclear oligonucleotide with the G allele, enhancing the risk of MI. (59.) About the fact that the interacting proteins and effect of MIAT on the heart are still unknown, genetic and functional data sheds new light on the pathophysiology of MI and the importance of this lncRNA.

Regulatory variantsncRNA administrative region variations have been used in a number of forms.
The effect of a variant affecting the ANRIL binding site on CAD has been discovered in the same manner as it has been for stroke. In GWAS (61), (62), the ANRIL gene is found on 9p21, a site that has been related to CAD. This time span is adjacent to a genomic area that also includes the CDKN2A (CDKN2A) and 2B (CDKN2B) inhibitor genes (61), (62). Given the fact that the CDKN2A and CDKN2B genes encode for the negative cell proliferation regulators p16INK4a and p14ARF, respectively, and p15INK4b, ANRIL has been proposed to regulate senescence at the CDKN2A locus (63), (64). In vascular smooth muscle cells (VSMC), homozygous rs1333049 polymorphism in the 9p21 locus was related to lower mRNA and protein levels of p16INK4a, p15INK4b, and the long transcript of ANRIL in atherosclerotic lesions, particularly in cells expressing the CC genotype (65). rs1333049 has been linked to an increase in the cell proliferation marker PCNA levels, which is highest in VSMC carrying the CC genotype, as well as a decrease in immunostaining for p16INK4a or p15INK4b and a higher VSMC content in atherosclerotic plaques, based on the negative role of p16INK4a and p15INK4b in cell proliferation (65). These findings back up the theory that the 9p21 vulnerability allele lowers ANRIL long transcript expression while raising short transcript expression, potentially downregulating CDKN2A and CDKN2B expression (63).

In a case–control study of a Japanese cohort, researchers discovered a link between the rs1333049 risk allele and lower ANRIL expression, as well as CDKN2A and CDKN2B participation in aortic plaque (66). The knock-down of ANRIL in VSMC with siRNA revealed a connection between ANRIL and genetic susceptibility to atherosclerosis through CDKN2A/B regulation, which resulted in a decrease in CDKN2A expression and an increase in anti-proliferative CDKN2B gene expression, resulting in a decrease in viable VSMC (66). These results suggest that the rs1333049 common variant has an effect on ANRIL expression, which regulates CDKN2A/B gene expression in cell proliferation and, as a result, atherosclerosis progression. Further study is required to see whether these SNPs directly regulate ANRIL expression or whether they alter the binding site of ANRIL's repressor transcription factor STAT1 in APRIL's enhancer to learn if they relate to the disease. Alternatively, SNPs can induce exon skipping, causing ANRIL transcripts to be modulated, resulting in shorter, less effective splice variants or non-functional isoforms (64).

Delivery through the heart
Another challenge in successful cardiac rehabilitation is efficient cardiac delivery of biological macromolecules such as proteins, DNA, and mRNAs into the diseased environment. Depending on the delivery line, the amount of barriers they must overcome varies. The therapeutic must stay in the circulation for systemic delivery to the diseased tissue, in this case the infarcted area of the heart. At a local stage, which involves extravasation of blood from the circulation into the myocardium. For proteins that function by binding to receptors on the cell surface, such as growth factors, merely getting into the tissue might be enough.

Since DNA, mRNA, and certain proteins must arrive intracellularly to be successful, tissue localization is insufficient. Ses macromolecules must enter the cell through endocytosis and successfully exit before being degraded by endo-lysosomes because spontaneous membrane translocation is virtually impossible for them.

When therapeutics are administered locally, either through intramyocardial injection or application on the myocardium's surface, they provide

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direct entry to the diseased tissue, but intracellular activity may be a major barrier. Aside from these traditional roadblocks, cardiac repair therapies based on protein, DNA, or mRNA each have their own combination of advantages and disadvantages.

Many biological processes depend on proteins as the supreme workhorse. Direct protein delivery, as a result, is a very enticing technique. They do, however, have short half-lives, necessitate regular administration, and require different techniques for each protein. Gene therapy, on the other hand, is a game-changing technology for ensuring long-term speech, but effective myocardial therapy necessitates the right cars. Currently, there are two types of gene delivery carriers: viral and non-viral gene vectors. Nonviral gene distribution pathways have attractive properties when opposed to viral vectors. They frequently have drugs with similar characteristics, such as low immunogenicity, unrestricted DNA loading duration, low production costs, and low toxicity. Some non viral synthetic carriers, such as cationic polymers and ionizable lipid nanoparticles, have already shown early success in gene transfer from the cell membrane to the nucleus. One of the challenges with using plasmid DNA for gene therapy is that it needs to enter the nucleus to be expressed, which is challenging in terminally isolated non-proliferating organs like the heart. Long-lasting transient expression is provided by updated mRNA, which eliminates the need to return to the nucleus for translation.

IV. PROTEOMIC DELIVERY SYSTEMS

Proteins are the cell's workhorses, but they aren't often the perfect therapeutic weapon. The high cost of protein production, the complexity of producing them with the correct tertiary structure and posttranslational changes, and delivering them to the heart are all potential obstacles to using protein as a therapeutic directly. After localized and sustained angiogenic growth factor distribution into the ischemic myocardium, angiogenesis, cardiomyocyte viability and proliferation, complete cell apoptosis, and cardiac remodeling both increased. However, since clinical translation of basic protein distribution has failed, high doses of these proteins could be needed, which may lead to unnecessary side effects such as tumor growth promotion. This highlights the importance of proteins striking a fine balance between on- and off-target effects; proteins are also resistant to proteolysis and clear quickly. Researchers have been working to build delivery platforms that can secure and track the release of therapeutic proteins for cardiac regeneration to overcome these limitations.

2.1.1. Hydrogel-based protein distribution pathways

Hydrogels are an ideal candidate for medicinal applications due to their biocompatibility for intramyocardial injection. Because of the aqueous environment inside the gel, several proteins can adopt their desired conformation. Hydrogels in their normal form have been observed in a multitude of ways. VEGF is one of the most often used proteins for heart reconstruction because of its important role in angiogenesis. Despite this, VEGF is difficult to translate into the clinic due to its short half-life and potential side effects (88,89).

Promoting endogenous cardiomyocyte proliferation in the infarcted area's boundary zone can assist with cardiac rehabilitation after a MI. 6-Bromoindirubin-3-oxime (BIO) and Insulin growth factor-1 are two potential causes that can facilitate cardiomyocyte survival and proliferation (IGF-1). In a MI rat model, Fang et al. investigated the regulated delivery of BIO and IGF-1 in a hybrid gelatin-based hydrogel solution to facilitate cardiac repair. Cardiomyocyte proliferation and revascularization are seen through infarcted areas, resulting in increased heart output (30). Injection of an alginate hydrogel containing IGF-1 and HGF improved cardiomyocyte viability, reduced adverse cardiac remodeling, and improved cardiac output in a rat model of acute MI (92). Awada et al. encapsulated tissue inhibitor of metalloproteinases-3 (TIMP-3), fibroblast growth factor-2 (FGF-2) and stromal cell-derived factor-1 (SDF-1), which improved cardiac contractility while reducing ventricular dilation, inflammation, fibrosis, and extracellular matrix degradation in a rat model of myocardial infarction.

Although extracellular matrix-based hydrogels can be used for a number of therapeutic molecules such as microRNAs and Extracellular vesicles (94), exogenous natural hydrogels are controversial in clinical trials due to their immunogenicity and challenging quality control over physical properties and degradation rate. In this case, synthetic hydrogels could be a better option. To monitor opioid release and decay rates, the density of crosslinking and swelling can be controlled (95,96). One of the composite structures of natural and synthetic systems is hyaluronate modified with hydroxyethyl methacrylate for cross-linking (97). SDF-1 (stromal cell-derived factor-1) was mixed with a radical polymerization initiator and inserted intramyocardial in a rat MI model, where it gelled. The findings showed better ejection fraction, capillary mass, and contractility compared to the control sample.

Synthetic hydrogels have been shown to be effective in alleviating cardiac apoptosis, fibrosis, and infarcted area, as well as improving angiogenesis and cardiac function, using basic molecules like colchicine or complex structures like extracellular vesicles. ((98), (99), (100)) in various MI models, including rat and mouse. Surprisingly, the timing of administration can be critical when it comes to success. The hydrogel was injected one week after the rat MI model was developed (101), and it improved cardiac remodeling and performance. In addition to VEGF, simple fibroblast growth factor (bFGF) is a well-known angiogenic factor. This factor can be useful in the treatment of MI, but it has a short half-life. To encapsulate bFGF, researchers used a Dextran-grafted poly (E-caprolactone)-2-hydroxyethyl methacrylate/poly (N-isopropylacrylamide (Dex-PCL-HEMA/PNIPAAm) hydrogel. The effects on angiogenesis and heart function in a rat model of acute MI were investigated. This study used high mobility group box 1 protein, a nuclear protein that serves as a chromatin-binding agent. HMGB1 encapsulated in a Dex-PCL-HEMA/PNIPAAm hydrogel reduced cardiac remodeling and improved cardiac efficiency after a rat MI (102).

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Myeloid-derived growth factor (MYDGF), a newly discovered paracrine-acting protein, has been shown to be effective after MI (103). The viscoelastic mechanical properties of hydrogels make catheter distribution for injection very difficult. This is one of the most difficult aspects of getting hydrogels into the clinic. One approach for avoiding this is in situ gelations (97). The uniformity of such a delivery gel for other therapeutic molecules was shown by the incremental release of extracellular vesicles (104) and microRNAs (105).

2.2. Nano and microparticle-based protein delivery systems

Nanoparticles and microparticles can be constructed from a variety of non-immunogenic, easy-to-handle, and biodegradable materials (106). Their surfaces may be functionalized with antibodies, peptides, or small molecules to help them target. The use of nano- and microparticles to encapsulate and transport proteins stops extracellular metabolizing enzymes from destroying proteins, allows for continuous release, and targets specific areas with surface ligands, potentially improving therapeutic efficacy (107,108). Because of their huge surface size, nano- and microparticles decay rapidly. This higher biodegradability, as compared to hydrogels, has the potential to suppress systemic inflammatory responses within the tissue, assuming the depot lasts long enough to have therapeutic results. There is an almost infinite range of materials to choose from, similar to hydrogels. Synthetic polymers, on the other hand, are usually more well-characterized than their natural counterparts and can be tailored by manufacturing techniques (109,110). Synthetic polymers are normally better characterized than natural alternatives and may be tailored due to manufacturing processes, where natural materials may be expected to exhibit better biocompatibility.

As growth factor-mediated release formulations, several proteinaceous nano- and microparticles have been investigated. PlGF has been shown to boost heart function and facilitate angiogenesis ((111), (112), (113), (114). PlGF release tests in vitro showed an eight-week period of sustained PlGF release from the nanoparticles. Increased left ventricular function, vascular density, and anti-inflammatory cytokine IL-10 levels, as well as decreased scar development and proinflammatory cytokines TNF- and IL-6, were observed in a rat model of ischemic cardiomyopathy (115).

Until being injected into the ischemic heart of a rat, silk fibroin microspheres were entrapped in sodium alginate solutions, resulting in an injectable alginate hydrogel designed for controlled release of IGF-1. The silk fibroin microspheres allowed for continuous IGF-1 release, which resulted in a smaller infarcted area and improved cardiac function (116). When different proteins were combined within these microcapsules, similar findings were obtained in an ischemic heart mouse model and a rat model of chronic heart disease (117,118). Biocompatibility, safety profile, and regulatory clearance make poly (lactic-co-glycolic acid) (PLGA)-based nanoparticles attractive materials (109,119). Poly Lactic co-glycolic acid nanoparticles containing VEGF were grown. This approach allowed for continuous delivery of VEGF to the intended site for at least one month in a murine myocardial infarction model, with reduced infarct length, increased vascular density, and improved left ventricular contractile function, suggesting that this nanoparticle-mediated delivery technique increased VEGF therapeutic efficacy for ischemic heart disease (110).

Overproduction of superoxide has been shown to induce cardiomyocyte apoptosis and play a role in the development of ischemia/reperfusion injury (IR), which causes cardiomyocyte death and loss of heart function. Superoxide dismutase (SOD), an endogenous superoxide scavenger, improves cardiac function after a myocardial infarction. However, large animal trials have shown that this strategy is ineffective. This may be because SOD1 has a short half-life, so prolonged-release could be helpful. A delivery mechanism based on polyketal polymer was tested in a rat model of IR. Three days after IR, polymer-encapsulated SOD substantially reduced excess superoxide released in the ischemic core's boundary zones, while free SOD1 or empty particles did not (120).

Proteins with different properties and kinetics can also be encapsulated to make them behave the same way. Cardiac development and regeneration are linked to growth factors including fibroblast growth factor 1 (FGF1) and neuregulin-1 (NRG1). Due to rapid degradation, they have failed to provide therapeutic benefits in clinical trials. To render PLGA microparticles and PEG-PLGA microparticles, Pascual-Gil et al. merged ses components. Intra-myocardial injection of NRG1 or FGF1-encapsulated PLGA and PEG-PLGA microparticles caused similar improvements in ejection fraction and angiogenesis after being injected into the infarcted area in a rat myocardial infarction model (109). In terms of phagocytosis in the heart, there was no difference between PEGylated and non-PEGylated carriers. PEG tends to have little beneficial function against phagocytosis in this formulation. Related findings were observed in a porcine ischemia-reperfusion model and a rat myocardial infarction model using this method (121).

Regulated delivery of PDGF-BB injection with self-assembling peptide nanofibrous gels to the compromised myocardium for up to two weeks after ischemia/reperfusion in rats prevented cardiomyocyte apoptosis, decreased infarct length, and increased systolic myocardial function (122). Cell implantation is one approach for cardiac repair, but it has been shown that poor cell engraftment and survival compromise these procedures. After myocardial infarction surgery, sustained myocardial delivery of IGF-1 with self-assembling peptides and cardiomyocytes to rat myocardium for 28 days improved implanted cell survival and myocyte cross-sectional area, as well as systolic activity (123). Furthermore, combined treatment of IGF-1-tethered self-assembling peptide nanofibers and cardiac progenitor cells in the infarct boundary zone promotes cardiomyocyte survival and vessel development, reduces infarct length, and improves cardiac function in a rat myocardial infarction model (124). Encapsulation of microparticles with growth factors has failed to mitigate secondary operation complications in some cases despite

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successful angiogenesis with low systemic effects in patients undergoing bypass surgery (125).

SDF-1 is a well-researched chemokine that seems to be a promising candidate for promoting regeneration. SDF-1, on the other hand, is rapidly degraded by exopeptidases and matrix metalloproteinase-2. Segers et al. used S-SDF-1, a chemokine that is resistant to matrix metalloproteinase-2 and exopeptidase elimination while maintaining chemotactic bioactivity and developing less neurotoxin. Purified and tethered to RAD16-II self-assembling peptides to form nanofibers, which were found to improve capillary density and cardiac function 28 days after administration in a rat myocardial infarction model. These findings imply that inducing chemotaxis by local chemokine transmission may be a feasible regeneration strategy (126). Kim et al. combined PDGF and FGF-2 in the same hydrogels and discovered similar positive results in the same animal model (127), which was inspired by the findings above.

Despite the targeting ligands, intravenously administered liposomes delivered their payload to many organs following injections, potentially limiting their applicability (128).

2.3. Silicone scaffolds and cardiac patches as protein delivery systems

Materials used in cardiac applications must be capable of resisting cardiac contractile motion (129). Polymer scaffolds (section 2.3.1) This is especially true of scaffolds, which are three-dimensional matrices that integrate with the host cardiac tissue to aid pumping. Scaffolds must have elasticity and mechanical strength to cope with the heart's dynamic characteristics. They should be pre-loaded with stem cells to jumpstart the regenerative process, rather than just providing an environment that attracts cardiac cell ingrowth (130). Synthetic polymeric scaffolds may be constructed to follow particular physical requirements and packed with single or multiple cytokines or growth factors to increase cardiac integration (131). The scaffold provides initial physical protection, with a controlled and continuous release of bioactive factors causing tissue production and scaffold degradation ((132), (133), (134). The ability of cardiac tissue scaffolds to promote vascularization in order to enhance regionally (stem) cell survival and assist surrounding ischemic tissue is essential for their successful use (135,136). In a rat model of ischemia, transmission of VEGF from a biopolymer augments vessel ingrowth into the scaffolds, despite the fact that the long-term effect of angiogenesis inside scaffolds after the VEGF supply is exhausted has not been thoroughly studied (136).

SDF-1 has long been known to play a role in angiogenic processes after myocardial ischemia, and its receptor, CXC chemokine receptor 4 (CXCR4), has been shown to play a key role in promoting angiogenesis after MI (137). The cardiac output was analyzed after a myocardial infarction and treatment with a polyurethane scaffold loaded with SDF-1-transfected cells. Two weeks after the rats' myocardial infarction, sowed scaffolds were embedded epicardially or cells were injected into the myocardium. Both groups experienced comparable changes in systolic function, with no major differences, meaning that scaffolding is not always equivalent to simple local injection. Surprisingly, infarction size was reduced while angiogenesis was not increased in two SDF-1 cohorts, meaning that the mechanism of action might not be as predicted (138).

In addition to vascularization, the inflammatory response that occurs during ischemia-reperfusion may be used to boost long-term cardiac output. Cardiomyocyte destruction, inflammatory cell invasion, and the production of inflammatory cytokines and chemokines may all be caused by a lack of oxygen ((139), (140), (141), (142)). Neutrophils are drawn to the infarct region by cell debris and inflammatory response signals within the first hours after ischemia begins. They contain a lot of reactive oxygen species and secrete granule components like myeloperoxidase and proteases, which exacerbates vascular and tissue damage (143).

A nonfunctional scar replaces the myocardium after a myocardial infarction, and is one of the leading causes of MI mortality. The synthetic cardiac scaffold has the ability to regain function and enhance myocardial remodeling. A poly-l-lactide scaffold was used to release the granulocyte colony-stimulating factor (GCSF) (PLLA). The development and release of granulocytes and stem cells from the bone marrow into the bloodstream is caused by this glycoprotein. In this research, PLLA-based scaffolds were successfully implanted into infarcted myocardium in rabbits without inducing a major inflammatory response. In comparison to the non-functionalized scaffold, the G CSF increased vessel density and extracellular matrix structure (144).

2.3.2 Patches made of polymeric fabrics for the heart

In contrast to scaffolds that become an indispensable part of the cardiac tissue, heart patches are intended to adhere to the surface of the injured area of the heart after a MI. Patches must be biocompatible and biodegradable, as well as have a mechanical feature that assists the beating heart's dynamics, despite not being rooted in tissue.

While cardiac patches display tremendous potential as a modern therapy for cardiovascular diseases ((145), (146), (147), (148), (149), the shortage of degradable polymeric patches on the market ensures that there is still space for improvement. Moreover, some cardiac patches (129,150,151) are fitted for epicardial delivery and must be implanted via open-chest surgery. They also increase the risk of pericardial adhesions, which can lead to heart failure and an increase in morbidity and mortality (152).

2.4. Osmotic pumps-based protein delivery systems

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A difference in osmotic pressure through a semipermeable membrane may be used as a driving force for regulated distribution systems to facilitate net water flow. With long-term release profiles, the osmotic pump is easy to build and operate (153). Hermans et al. (154) used pericardial catheters and a subcutaneously implanted osmotic minipump to see whether intrapericardial transmission is possible in rats. They found that by using this approach, therapeutic levels in the pericardial space could be sustained for longer periods of time. Landau et al. (155) injected bFGF into the intrapericardial space of rabbits with chronic ischemia. An osmotic pump supplied bFGF, which facilitated the construction of new epicardial vessels.

Systems for delivering DNA

3.1. Gene transmission via naked plasmid DNA

Gene transmission of VEGF through transient cardiac overexpression by adenovirus delivery or naked plasmid to promote angiogenesis has been shown to be successful in both animal models and patients with myocardial ischemia ((156), (157), (158), (159). Hao et al. measured the efficacy and side effects of the two therapies in a rat myocardial infarction model and discovered that both therapies improved left ventricular function to a similar degree. Adenoviral gene transfer, on the other hand, induced more apoptotic cells and caused more side effects than plasmid, with no discernible efficacy benefit over plasmid. These findings indicate that using plasmid-encoded VEGF rather than adenovirus to induce therapeutic angiogenesis in the heart could be a better option (69). This is most likely due to the high immunogenicity of adenovirus. It'll be fascinating to see whether cardiac-specific AAV vectors with low immunogenicity, including AAV8 and AAV9, outperform plasmids. In patients with severe ischemic heart disease, high-dose plasmid-mediated VEGF gene transfer was found to be safe. An intra-myocardial injection of 3.8 mg plasmid encoding VEGF into the ischemic region using an injection catheter over a 2-year period produced no major adverse effects associated with either VEGF expression or the injection procedure (160,161). Despite this, the study had no test subjects, was open-label, and the drug was only used for a brief period of time. As a result, in the Euroinject One phase II trial, 80 patients with acute stable ischemic heart disease were divided into two groups: 1. those who received 0.5 mg of plasmid-encoding VEGF, and 2. those who received a "clean" plasmid into the affected myocardial area. Although VEGF gene transfer did not improve myocardial perfusion as compared to placebo, it did significantly reduce local wall motion disturbances three months later. Despite the absence of a discernible clinical outcome in myocardial perfusion conditions, which may be due in part to the low dose, there is a movement toward beneficial therapeutic efficacy. These findings highlight the need for further studies to fully comprehend the potential value of plasmid VEGF gene therapy (162).

3.2. Nanoparticle-based DNA delivery systems

Nanoparticles could be more effective than naked plasmid delivery for gene transmission ((163), (164), (165), (166)). For example, nanoparticle synthesis and purification are typically faster and easier than viral distribution methods (167,168). Polymeric nanoparticles encapsulating plasmid DNA can also improve DNA uptake and protection, increasing target gene expression (169). The control of DNA release is also possible when DNA is encapsulated in biodegradable polymer particles (170).

Despite this, the lack of successful encapsulation due to the lack of cationic charge makes it challenging to implement this method. PEI (poly (ethylene imine)) is a widely used charged polymer with excellent transfection properties. It has a high charge density, which is useful for plasmid encapsulation, but it has poor cell specificity since it binds to every negatively charged cell membrane. To address this, researchers created a chitosan-graft-PEI-eprosartan copolymer with a lower charge density. PEI was grafted onto chitosan to create a polymer with a mild charge. Eprosartan, a compound that binds to the overexpressed angiotensin II type 1 receptor in cardiomyocytes, is also present in the polymer. Following local injection in the myocardium of a rat model of MI in which the polymer was prepared with plasmid VEGF and administered intramyocardial, the formulation improved heart function with a significant increase in ejection fraction. At the cellular level, reduced apoptosis and an increase in the number of capillaries in the infarct and peri-infarct area were also observed (171).

Several experiments in animal models have shown that local administration of such targeted cationic polymer structures with reduced charge density resulted in positive clinical outcomes. Many avenues have been pursued to enhance the applicability of these systems even more, including reducing the invasiveness of the process, enhancing voice, and performing the treatment ex vivo. Intramyocardial injection may be made less invasive by using an intravenously delivered delivery device. The phage-identified homing peptide CSTS MIKAC (173) can be used to target primary cardiomyocytes in the infarct area. Cystamine bisacrylamide-diamino hexane polymers containing the homing peptide and D-9-arginine will deliver to, accumulate in, and transfect the ischemic myocardium after intravenous administration (174).

The use of regulatory elements in the plasmid has been investigated in order to improve transfection performance. A hypoxia-inducible VEGF gene therapy system has been developed, which uses a combination of erythropoietin enhancer and water-soluble lipopolymer to improve VEGF expression and endothelial cell proliferation (175).

In a study focusing on cardiomyocyte apoptotic cell death, positive regulators of death receptor-mediated apoptotic signaling pathways were examined. Src homology region 2 domain-containing tyrosine phosphatase-1 (SHP1) is one of the most powerful apoptosis-regulating factors (176).

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3.2.1 Lipid-based DNA delivery pathways

In 1980, Fraley et al. (177) demonstrated the feasibility of using lipid-based nanoparticles to deliver plasmid DNA to monkey kidney cells, and Schaefer-Ridder et al. (178) demonstrated the effective delivery of genes using lipid-based nanoparticles. Currently, lipid-based gene delivery systems are being used in many clinical trials to express therapeutic proteins for the treatment of tumors via various administration routes. Xu et al. demonstrated that systemic p53 gene therapy for cancer had desirable gene transfer efficiency and anti-tumor efficacy in vivo using a novel cationic immune lipoplex procedure in 2001 (179).

In a randomized, placebo-controlled, double-blind phase II trial, patients with coronary heart disease received gene transfer using a perfusion-infusion catheter. VEGF adenovirus was given to 37 patients, VEGF plasmid lipidic carriers to 28 patients, and Ringer's lactate to 38 patients as a monitor. Although intracoronary gene transfer was shown to be safe after six months, there were no significant variations in minimum lumen diameter or proportion of diameter stenosis among all test groups (157). Since lipid-based gene delivery systems are widely used in other fields, it's possible that they'll make their way into the cardiac field as well, while tissue-specific modifications may be needed. One option is ex vivo cell transfection. Overexpressing growth factors in mesenchymal stem cells (MSCs) has been used as a carrier for cardiac regenerative potential ((180), (181), (182), (183)). Locatelli et al. used a combination of two lipid-based reagents to enhance plasmid-mediated transfection of ovine MSCs, resulting in high transfection performance and cell viability (184), meaning that this strategy could be used to introduce regenerative factors to the myocardium efficiently and transiently.

3.3 DNA distribution mechanisms based on the UTMD

Physical methods for temporarily disrupting cell membrane integrity, as well as designing chemical structures that can deliver plasmids, have all been investigated. The use of ultrasound-targeted microbubble disruption (UTMD) for nucleic acid delivery is currently being studied in many studies (185). The plasmid will be released to the targeted area following the administration of a plasmid-coated microbubble through ultrasound-controlled microbubble destruction. High-energy microbubble cavitation causes increased permeability of cell membranes and faster gene absorption into the targeted region, in addition to local release. The ultrasound-targeted microbubble disruption (UTMD) protocol has been used for gene and drug delivery in a variety of preclinical disease models, including cardiovascular diseases ((186), (187), (188), (189). Microbubbles have been used in the lab as an echocardiographic contrast agent, but they haven't been produced specifically for delivery. To improve plasmid transfection with UTMD, cationic microbubbles with a higher DNA-binding capability were tested. As an alternative to the distribution of growth factors that facilitate regeneration, inhibitors of negative remodeling have been proposed. The cardiac matrix metalloproteinase (MMP) plays an important role in the course of heart failure. Tissue antagonists of matrix metalloproteinases (TIMPs) have been shown to enhance cardiac remodeling and performance after ischemic injury (190). UTMD was used to successfully distribute cationic microbubbles containing plasmid-encoded TIMP to ischemic rat myocardium or mouse hind limb tumors (191). Finally, because of its non-invasiveness, low immunogenicity, and repeatability, multiple studies have shown that UTMD is a novel gene delivery system. The UTMD-mediated gene delivery approach has the ability to pave the way for multi-gene therapy in cardiovascular medicine and has a bright future. A disadvantage of this strategy is that blood opsonization and local shear stress of the blood flow will reduce the targeting ability of antibody/cationic surface and microbubbles (192).

3.4. Wrapping up the discussion

It has been possible to deliver growth factors or inflammation inhibitors through DNA to facilitate vascularization or to avoid adverse cardiac remodeling. This type of therapeutic action is made possible by the fact that the DNA molecules in both treatments are very identical. If the therapeutic protein is a growth factor or a cytokine, the plasmid DNA is the same molecule with the same physicochemical properties and stability. The main difference is the nucleotide sequence of the decoding nucleotides. As a result, DNA delivery processes seem to gravitate toward the same concepts. One of the most important obstacles to widespread usage is the inefficient entry of plasmid DNA into the nucleus, where transcription to mRNA would take place. mRNA delivery may be an appealing way to get around this.

V. SYSTEMS FOR RNA DELIVERY

4.1. Therapies based on RNA

Since they have a working protein that is transiently expressed in the target cells without the difficulties that DNA-based constructs have, messenger RNA (mRNA)-based therapies carry a lot of promise for treating a variety of diseases (193). The intracellular active site, the cytoplasm, has a significant advantage in that it eliminates the need to cross the nuclear membrane. More than three decades earlier, Wolff et al. (194) demonstrated that direct injection of mRNA could be translated into protein in vivo, ushering in a new period in the experimental translation of mRNA therapeutics. Many improvements in nucleotide chemistry, synthesis, scale-up, and dissemination were needed to get this approach closer to implementation.

The mRNA blueprint consists of five cis-acting structural components from the 5′ to the 3′ ends: (1) the optimized cap system, (2) the optimized 5′ untranslated region (UTR), (3) the codon-optimized coding sequence, (4) the optimized 3′ UTR, and (5) a stretch of repetitive adenine nucleotides (polyA tail). The cap structure and poly (A) tail are important regulatory determinants in deciding the mRNA's translational efficiency and stability against decay, while the UTR regulates the mRNA's half-life and translational efficiency (196). Orthodox mRNA-based treatment had little medicinal benefit due to its inconsistency and immunogenicity. For example, typical mRNA is easily

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degraded by extracellular or intracellular ribonucleases. Pattern recognition receptors (PRRs) that bind double-stranded and single-stranded RNA, such as Toll-like receptors (TLRs) 3 or 7/8, can also recognize mRNA. The innate immune system will be stimulated, resulting in the release of pro-inflammatory cytokines and type I interferons ((197), (198), (199), (200)). A number of improvements to the vectors used to produce mRNA, as well as to the synthetic mRNA itself, are needed to improve the translatability and stability of mRNA and reduce its immunostimulatory function in mammalian cells and mice (201).

Chemical manipulation of nucleotides in mRNA could be used to inhibit mRNA immunostimulatory activity. Adding naturally occurring nucleotides including pseudouridine 2-thiouridine (s2U), 5-methyluridine (m5U), N1-methylpseudouridine (m1), N6-methyladenosine (m6A), or 5-methylcytidine (m5C) to mRNA strengthens this (202). This is most likely due to changes in the secondary structure of the mRNA, which makes it difficult for TLRs and nucleases to detect it. Andries et al. (203) showed that using N1-methylpseudouridine (m1) in mRNA resulted in innate immune evasion and increased translational capacity in vitro and in vivo.

The 5′ and 3′ UTRs of mRNA are also involved in RNA-binding proteins and microRNA recruitment, as well as the regulation of mRNA half-life and translational capacity. The cap structure and poly A tail give mRNA essential stability and translatability by inhibiting RNA decapping and increasing resistance to enzymatic degradation (205). Both can be incorporated into RNA during transcription by including an anti-reverse cap analog (ARCA) and an extended polyadenylation region, but they can also be inserted enzymatically after in vitro translation. These modifications would significantly improve the properties of mRNA and expand its functional capabilities.

4.2. mRNA production that has been changed

In vitro transcription (IVT) of RNA with phage RNA polymerases is the most powerful method for producing long sequence-specific RNAs. Although there are a number of ways to make mRNA, all in vitro synthesis techniques use the same basic technique. A linear DNA template containing a bacteriophage promoter, optimized UTRs, codon-optimized sequence, and a mixture of different nucleoside triphosphates is used by phage RNA polymerase to generate RNA in vitro. As a result, the mRNA produced closely resembles fully processed mature mRNA present in eukaryotic cells' cytoplasm.

After mRNA synthesis, contaminants such as ions and residual polymerase must be removed. There are many purification methods, each with its own combination of advantages and disadvantages. Lithium chloride precipitation is a popular method for purifying RNA in the lab. Since RNA and water are both highly polar, they melt quickly. Monovalent cations in the form of salts can combine with negatively charged RNA through electrostatic interactions and precipitate via charge neutralization. Lithium chloride is desirable for mRNA purification since it efficiently precipitates larger RNA molecules after in vitro transcription but not DNA, tRNA, and other small RNA fragments, proteins, or unincorporated nucleotides (206). Another typical form of RNA purification in the lab is silica-based columns, which rely on RNA binding to the solid phase in a chaotropic buffer. Silica-based columns are commonly used because they allow easy extractions and high nucleic acid yields (207). Finally, size-exclusion chromatography (SEC) columns in fast performance liquid chromatography (FPLC) systems can rapidly purify homogeneous RNA samples. The method enables milligram quantities of pure RNA to be prepared quickly and easily in a single day (208). Several of the smaller toxins that can induce type I interferons (IFNs) and proinflammatory cytokines by PRRs are removed by these techniques (209,210). On the other hand, long RNA toxins aren't. These pollutants can be removed using high-performance liquid chromatography (HPLC), resulting in increased translation but no induction of IFNs or inflammatory cytokines, as well as no significant induction of genes linked to RNA sensor activation (211). In primary cells and in vivo, the most modern HPLC approach allows for the purification of mRNA, which can produce up to 1000-fold more protein than unpurified mRNA (212).

4.3 The Role of RNA Therapeutics in Cardiovascular Repair

Since mRNA is generated in the cytoplasm and does not require nuclear localization to mediate protein translation, it is less labor intensive than DNA. In conditions where the main approaches are growth factor distribution to promote vascularization and inflammation inhibition, mRNA expression is sporadic, reducing the risk of sustained protein expression and/or insertion mutagenesis. Ischemic injury causes the release of pro-inflammatory cytokines ((213), (214), (215), as well as a rapid inflammatory reaction that occurs 2–3 days after the injury. This window of potential for intervention (204) is proposed, in which desirable gene combinations could be delivered to rescue cardiomyocytes. Since mRNA-based approaches are still new, they will benefit from the most current research into disease-modifying therapies. A recent emerging therapeutic strategy (216) is introducing unique cytokines to facilitate cardiac myocyte regeneration to repair cardiac damage, which can be augmented by modulating miRNA-pathways, such as miRNA-15 (217, 218). 1. differentiation of resident stem progenitor cells to form new cardiomyocytes, 2. re-entry of pre-existing cardiomyocytes into the cell cycle to replace missing cells, and 3. reprogramming of fibroblasts to transdifferentiate into cardiomyocytes are both forms of endogenous cardiomyogenic pathways. The careful regulation of gene expression is needed for all three options for cardiomyocyte regeneration. In vivo, modified mRNA has been shown to articulate easily and can be detected for up to ten days (204). This applies to the amount of time that a psychiatric intervention should be used.

4.4. Cardiac healing issues with RNA therapeutics

To achieve their destination, RNA molecules must conquer many obstacles, including the fact that they function in the cytoplasm. Viral vectors can efficiently transmit RNA to cardiac muscle cells, but they can also cause strong immune responses and jeopardize genomic stability ((219),

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(220), (221), (222)). The topic of synthetic non-viral delivery vectors is discussed in this article.

4.6. Lipid-dependent RNA delivery pathways

Lipid nanoparticles are the most commonly used nano-systems for the delivery of RNA agents (223,224). To efficiently condense RNA, electrostatic activity may be used. The cationic charge can promote cellular uptake of RNA via endocytosis and/or endosomal escape (225). DOTMA (N-(1-(2,3-allyloxy)propyl)-N,N,N-trimethylammonium chloride) (226) was the first synthetic cationic lipid used to condense and inject mRNA into cells. For example, DOTAP (1,2-dioleoyl-3-trimethylammonium propane) is frequently used for RNA transmission, especially in vitro (227). To improve RNA transfection, neutral helper lipids like DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) can aid endosomal escape. These lipid systems are rapidly cleared by the mononuclear phagocytes system in the liver and spleen after intravenous infusion, reducing distribution efficiency. Furthermore, cationic lipids tend to accumulate with negatively charged blood components, resulting in negative effects through capillary blockage.

Electrostatic associations and recognition can be minimized using functional lipids such as polyethylene glycol (PEG)-phospholipid conjugates. Targeting ligands can also be applied to the particles' surface to improve interaction with target cells. Lipid carriers that target the heart display promise in the care of MI: For eg, conjugated-liposomes were able to specifically target the infarcted heart after intravenous injection in vivo using anti-P-selectin to target infarcted myocardium (65) or a ligand specific to angiotensin II type 1 receptor (AT1) (228). These results suggest that liposomes with complex modifications may aid in the more precise delivery of therapeutic agents to the infarcted heart. It's worth mentioning that RNA transfection in vivo has already been demonstrated using several readily available lipid formulations (229). They discovered that VEGF mRNA promotes vessel growth as a result of pulse-like VEGF overexpression. In the peri-infarct area, this is more successful than continuous protein expression in stimulating the endogenous epicardial progenitor pool.

The use of pH-dependent ionizable lipids to replace cationic lipids has been the subject of recent studies. They include charge restoration at low endosomal pH after intracellular absorption for endosomal escape, neutral charge after injection at physiological pH, and cationic charge during RNA complexation at low pH. The equilibrium between transfection and cytotoxicity is thus maintained as a result. In 2019, the FDA approved the first siRNA treatment for amyloid transthyretin, which contains the ionizable lipid (6Z,9Z,28Z,31Z)-hepta-triaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino) butanoate (MC3). Since then, several lipid nanoparticles (LNP) that were originally developed for siRNA delivery have been repurposed for mRNA delivery (230). This shows that different RNA lengths and underlining sequences can be delivered using the same delivery technology, and that these differences are much less important for RNA delivery than they are for protein and peptide delivery. Gilleron et al. created an analytical platform based on a combination of quantitative light and electron microscopy (EM) to dissect the mechanisms of LNP-mediated siRNA transmission with high precision (231). They discovered that endosomes released only a small percentage of siRNAs (1–2%), and that this release was restricted to a specific stage of endosomal-lysosomal maturation. Important changes can occur after intracellular arrival in the target cell.

By refining ionizable lipids for different RNA groups and target tissues, the delivery properties of LNP could be greatly improved. Fenton et al. developed a series of ionizable lipids based on alkenyl amino alcohols (232). For example, OF-2 was formulated into LNPs with cholesterol, DOPE, C14-PEG-2000, and mRNA coding for erythropoietin, and it outperformed other ionizable materials in terms of in vivo erythropoietin synthesis over a large dose-response window.

In animal models, the aggregation of non-biodegradable lipids has been shown to induce harmful effects or even death, so biodegradability is an important aspect of nanoparticles for in vivo applications. Ester groups are the most frequently used functional groups for enhancing biomaterial biodegradability (233). Lipid 5 (234), a biodegradable ionizable lipid with a key ester-containing lipid tail, was discovered by Sabnis et al. They discovered that the presence of this key ester resulted in quicker liver clearance than MC3 in nonhuman primates. The liver is usually in control of LNP aggregation (235,236).

In organs or tissues other than the liver, LNPs are more difficult to target. Cheng et al. (237,238) recently suggested a strategy called selective organ targeting (SORT), in which LNPs containing nucleic acid-based therapeutics are bioengineered to induce gene regulation specific to the liver, spleen, and lung. Unfortunately, no study into a heart-specific formulation has been completed, but there is a strategy for getting there. There have also been LNPs produced with ligands for cardiac tissue-specific receptors (239).

4.7. RNA distribution systems based on polymers

RNAs, including DNA, have been delivered using cationic polymers such as PEI, chitosan, PLGA, dendrimers, and mixtures of these. In recent years, polymers, including lipid-based delivery systems, have shifted from siRNA and DNA delivery to mRNA delivery. Polyethyleneimine (PEI) is one of the most potent and commonly used synthetic cationic polymers (240,241). Efficient and temporary protein expression was tested under hypoxia-induced apoptosis conditions, promoting cardiomyocyte survival and preventing cell apoptosis. Transient protein expression caused by mRNA during myocardial infarction has an important effect on IGF1's cardioprotective function. Zangi et al. (242) found that long-term activation of IGF-1 signaling pathways in the heart after MI has negative consequences. The involvement of IGF-1 activated the formation of epicardial adipose tissue after a heart attack (EAT). A fat compartment called epicardial fat exists between the

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myocardium and the visceral pericardium. Excessive epicardial fat deposition around the heart will lead to atherosclerotic plaques in the coronary arteries (243). As a result, transient mRNA expression can be preferred to long-term expression.(n=4.8) microbubbles RNA molecules can be accurately delivered to the cardiovascular system under the guidance of ultrasound, unlike the synthetic delivery vehicles discussed above. Tsunoda et al. successfully transmitted siRNA against GFP into the heart of GFP transgenic mice (244). In comparison, siRNA was only present in cardiac endothelial cells, not cardiomyocytes. Others have discovered that using UTMD (187), ultrasound control, ultrasound frequency, and ultrasound pulsing, marketed miRNAs can be directed to cardiomyocytes ((245), (246), (247)). UTMD-induced RNA transmission, on the other hand, is a promising technique for treating cardiovascular disease, especially myocardial disease, when both of these conditions are in balance (248).

VI. EXTRACELLULAR VESICLES PLAY A ROLE IN HEART REPAIR

Exosomes, also known as extracellular vesicles, are increasingly being recognized as vital biological information carriers for intercellular contact. EVs include proteins, small RNAs, mRNAs, and second messengers, some of which have been shown to play important roles in cardiac recovery. However, as previously mentioned, there are several risks to operating electric cars. EVs have high manufacturing and upscaling costs, and they lack efficient cargo loading at the moment. Several techniques for biofabrication of cell-derived EV-like vesicles with biological functions similar to EVs have recently been developed by us and others ((250), (251), (252)). These cell-derived nanovesicles are formed when the cellular membrane is physically disrupted. Despite the fact that these artificial vesicles look like EVs and activate the ERK pathway, a clear comparison with EVs is still important. The structural similarity of the lipid bilayer between liposomes and EVs has led researchers to explore bottom-up methods for creating fully artificial EV mimics. Using either traditional liposome formulation in the presence of exosomal membrane proteins (253) or synthetic lipids that mimic exosomal lipid composition (254), this bottom-up processing technique allows for large scale clinical grade vesicles. However, considering the biological structure of EVs, it's unknown how precisely they can imitate natural EVs.

VII. CONCLUSIONS AND PROSPECTS FOR THE FUTURE

It's now clear that widespread differences impair ncRNA function in cardiovascular diseases. Surprisingly, the majority of these SNPs are found in the 3'UTR of miRNA target genes, suggesting that selective selection may have succeeded to preserve ncRNA gene coding regions or genomic regions encoding transcriptional regulatory regions. Owing to selection bias in GWAS markers and a lack of knowledge of the relationship between ncRNAs and their regulatory elements or encoding genes, some modulating traditional variants may not have been linked to cardiovascular disorders. As a result, further research in this area is needed. Identifying disease-associated SNPs involving ncRNA would also help in assessing the genetic susceptibility for a particular disease, allowing for the early diagnosis of at-risk subjects who can be closely monitored. A much more detailed image of disease-associated SNPs affecting ncRNA activity is likely to be obtained in the not-too-distant future, thanks to the rapid development in next-generation sequencing technologies and the resulting decline in their cost. In the other hand, the experimental concept of ncRNA-regulated proteins and pathways is expected to provide not only new insights into the pathogenesis of human diseases, but also novel genetic diagnostic opportunities and the ability to assess the additional risk of disease associated with any of these variants, assisting in the development of selective and tailored therapeutic approaches.The most common arrhythmia in the world, especially among the elderly, is atrial fibrillation (AF), which has a strong genetic component (49). AF, which is also a risk factor for ischemic stroke and thromboembolism, may cause or exacerbate heart damage (50). MiRNAs have been related to increased atrial arrhythmogenicity and AF and have been shown to play a crucial role in regulating atrial excitability (51). In this regard, irregular miRNA expression in cardiac tissue and blood has been observed in animal models and AF patients on a daily basis (52). In atrial tissue samples from patients with AF, altered levels of miR-1 and miR-133 have been discovered in various studies (53), (54), (55). Genetic screening of the MIR1-1 and MIR 1-2 genomes, which encode miR-1-1 and miR-1-2, and the MIR133A1, MIR133A2, and MIR133B genes, which encode miR-133a-1, miR-133a-2, and miR-133b, was conducted in 120 probands with suspected inherited AF. Eight of the ten variants were well-known, with minor allele levels in the AF population that were equivalent to those found in public databases (56). The 79 T > C substitution in the MIR133A2 gene has been discovered to modify the way the miRNA duplex is processed, allowing the usually damaged miR-133a-5p strand to clump together. Since two affected family members are genotype-negative, the presence of the MIR133A2 mutation in the proband may have a modifying effect by altering gene expression profiles in the atrium, according to the authors (56).

The molecular mediators that cause MI, the lack of myocardial regeneration, and the development of heart disease are all being studied more thoroughly. It's intriguing to think about using biochemical pathways to control disease mediators. DNA, RNA, or proteins may both be included in this operation. However, direct delivery of these biomolecules is not always effective. Nanomedicine has an elegant endeavor for treating multiple gene polymorphisms and mutations in complex diseases (177-191) using gene therapy techniques (165-176). The stability of these biomolecules, as well as their controlled release and passage through barriers to the operating site, can be aided by delivery systems. Precision-tailored vector delivery has been shown to minimize toxicity and improve drug availability (255). Currently, a variety of distribution systems are being evaluated, with the frontrunners incorporating security and conclusions being selected. New biological mediators, as well as the complex interactions within them, as well as their pharmacokinetic and pharmacodynamic profiles, will be discovered in the future. This paves the way for a more advanced distribution system that can meet the biological requirements for maximum therapeutic efficacy.

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