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microRNA perspective on cardiomyocyte development and cardiovascular diseases

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1. Abstract

Study of micro-RNA regulatory networks (known as miRNA's or miR's), during development and in known pathologies have been the basis of study over the past decades. Herein, we recapitulate these findings in order to highlight the best underlying mechanisms found to date. We also seek to elucidate how miRNA dysregulation can be associated with many cardiovascular diseases. Furthermore, we discuss miR regulation mechanism during in early development in vivo and invitro. Since many of the miR's are precursors to transcriptional regulation, we relate back to their molecular control as we can then look together at the fundamental disease they might be exacerbating by this dysregulation.

2. miRNA's

Initial studies by Ruvkon et al., and Ambros et al., in the last decade of the 20th century, demonstrated that a group of small non-coding RNA influenced the development of *C. elegans* by regulating translation by a process of base pairing (inhibiting translation) to the 3'UTR and in a few cases 5'UTR of mRNA [1,2]. These small RNA were over time shown to be conserved in many cellular eukaryotic species including human cells [2–5].

MicroRNAs (miRNAs), commonly designated as mir for the precursor product or miR for the mature product, are a set of 18-24 nucleotides, which when processed (a process of complementary base pairing to mRNA) can silence or downregulate expression; either by destabilizing and/or cleaving the mRNA or by reducing the efficiency for which it can be processed [6,7]. Unlike other small RNA's, miR's have a predictable hairpin loop structure from

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their precursor transcript [8]. The small region required for miRNA regulation is only about eight nucleotides This small area possibly supports the idea that one miR could regulate as much as 60% of available mRNAs, although this has been found not to be the case [9]. Suggesting, that miRNA translation regulation is a tightly regulated mechanism that is controlled in a spatial-temporal manner, including how a handful of miR's can be secreted in specific organs and are uptaken far from their origin [5,6,10].

2.1 miRNA Biogenesis

Briefly, mir's are initially transcribed from the genomic region by pol II (up to 3kb) as a combined primary-precursor mir's (pri-mir). Further, processing by Drosha/DGCR8 permits the cleavage into a ~70-100bp stem-loop hairpin or precursor-mir (pre-mir). The pre-mir can now be exported to the Cytoplasm via complex to Exportin-5/Ran-GTPase. Once exported, it is at this stage where further enzymatic cleavage by Dicer/TRBP complex produces the commonly known miR duplexes (22 bp). These duplexes now serve as the guides that come into pair with mRNA; this is achieved initially by a dissociation of the duplexes followed by the assimilation of one of the miR strands with Argonaute (AGO), thus forming the RNA-induced Silencing Complex (RiSC), which consequently binds to the complementary sequence of the mRNA. Typically, complementation is on the 3' UTR of the mRNA promoting posttranslational degradation or downregulate expression [1,11–14], Fig. 1.

Given this brief overview, it's no surprise that miR's are such important regulatory molecules. In many instances self-regulating by co-expression with target genes, therefore many labs have dedicated a considerable amount of time and resources to study and profile miR's to development, as well as, to underlying diseases which they can incur into by mis-expression, errors in transcription, promoter defects such as hypo- or hyper- methylation and other noticeable conditions [5,8,15–18].

3. miRNA's in Cardiomyocytes

Fundamentally the adult heart has little potential to regenerate when given an insult by injury or disease. Normally cardiomyocytes are lost leading to heart failure and in more complicated cases to death [19–21]. Hence, understanding cardiomyocyte development has been key in trying to understand repair.

Studies of individual miR's using developmental models of the heart, have led to demonstrate that miR-1/miR-133 are fundamental in the control of proliferation and the muscle transcriptional network; SRF, MEF2c, MyoD, Hand2 and Myocardin. Interestingly, SRF has been found to be a requirement for miR-1 expression during development. This double mechanism is particularly evident as SRF/miR-1 also come together to regulate the sodium calcium exchanger NCX1 promoter [22]. Additionally miR-1 overexpression blocks ventricle myocyte expansion [23]. Meanwhile, miR-1 was linked to NOTCH1 receptor. Dlk-1, a critical factor involved in specification thought asymmetric division [24]. Mouse studies by Wu et al., found additional evidence of miR-34a is a repressive regulator to NOTCH1, while upregulating Jagged1, Hey2 and Hes [25]. A variant of miR-1(-1) is miR-1-2, has been found to have ~50% of embryonic lethality, while ~20% of survivors have major cardiac defects. Deletion miR-1-2 has been reported to repress Kcnd2, crucial factor in the repolarization of the heart [26–28]. In adult rats experiments miR-1 has been also revealed to target KCNQ1 and KCNE1 [18]

The miR-17-92 cluster is has also been implicated in cardiac proliferation by negatively regulating PTEN tumor suppressor gene [9,29,30]. Additional reports suggest the implication of the miR-17-92 cluster overexpression may cause Tsc1 repression. Thereby, causing mTOR mediated hypertrophy, proposing that the conditional downregulation relation of Tsc1 is a negative regulator of mTOR and downstream target of PTEN [31]. Meanwhile the miR-15 family, balances proliferation by repressing cell cycle regulators, in particular decreasing HSP-20, targeting Bcl2, and repressing TGFβ activity through [30].

Finally, a distinguishing trait of cardiomyocytes is their contraction. This is provided in part by a delicate balance of α/β– MHC chiefly controlled by miR-208. Principally, miR-208a was found to regulate GATA4 and CX40, thereby partially regulating the conduction system. Additional to this, Callis et al., states that both isoforms of miR-208 (a/b) target TRAP1 and myostatin, whom are important negative regulators of muscle growth and hypertrophy [32,33]. (A synopsize of the miR regulation thought development, Table 1)

3.1 miRNA's in Stem Cells to Cardiomyocyte Differentiation

In order to best bypass limitations for cardiomyocyte regeneration, direct differentiation of somatic cells into, induced pluripotent stem (IPS) cells and embryonic stem (ES) cells has been

the focus for many groups during the past 2 decades and continues to be a great field of study within organ repair [33,34].

Direct differentiation of fibroblasts to cardiomyocytes was initially achieved using transcription factors: Hand2, GATA4, MEF2c and TBX5 [35], and later enhanced by the addition of MYOCD, SRF, SMARCD3 and MESP1, never addressed the role of miR's [33,36,37]. Nonetheless, Dazu's group began to show the potential of miR-1, by demonstrating its overexpression was sufficient to convert cells [38].

In ES cells miR-1/-133, collectively induce mesoderm formation, during this stage both miR-199, and miR-483 are induced [33]. In addition to this, Srivastava's group, demonstrated at E4 (EB) suppression of both endoderm and neuroectoderm resulting in the repression of Dlk-1, consistent with the expression and possible dependency of *twitst* [39]. Moreover, by expression of miR-1/-133 on SRF null ES cells; known to block muscle differentiation, cardiac differentiation was achieved (null sarcomeres) [20,39,40].

Particularly, expression of miR-1/-499 in ES cell-derived cardiomyocytes predominantly controls the electrical/conduction system. Their expression upregulated Kir2.1, Kv1.4, HERG, and DHPR while downregulating HCN4, resulting in increased I_{to}, I_{ks}, and I_{kr}, and decreased I_f [41,42]. A result consistent with the expression of RYR2 L-type channel [43]. miR-499 has been implicated in expression of MHC6, MHC7, MLC2, and TNNT2.

Poon et al., recently described the importance of miR-200c using ES cells, denoting GATA4, TBX5, and SRF to be its targets. They also noted that knockouts in ES cells altered Ca^+, Na^+, and K^+ ion channels (CACNA1C, KCNJ2 and SCN5A) increasing contractility. Additionally, conduction seems to also be altered if deleting miR-1-2, by dysregulating the expression of Irx 4, 5 and Kcnd2. Meanwhile, transcription factor MESP1, once described as the key regulator to heart by Bondue and Blanpain, 2010, regulates Nkx2.5, Tbx5, Hand2, FoxH1, Isl1 and others, while at the same time induces mesoderm lineage by blocking Bry, Fgf6, FoxA2, Sox17, and Gsc. Thereby sitting atop of the hierarchy of cardiomyocyte formation and regulation [46]. At a step earlier, we can find that transactivation is succeeded by miR-322/503, this by targeting RNA binding factor Celf1, which would else induce a neural fate [47]. Regulation via the most important miR's during differentiation can be viewed in Fig. 2.

4. miRNA's in Cardiovascular Diseases (CVD's)

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CVD's, the leading cause of morbidity and mortality amid developed countries. Over the past couple of decades much effort has been put into finding both the physiological fundamentals and molecular mechanisms of control. Research done over miR's in CVD's has pointed out the specificity of certain miR's and clusters to certain conditions. Here we put emphasis on most important and clinically relevant miR's for conditions such as congenital heart disease (CHD), atherosclerosis, myocardial infarction (MI), severe coronary artery disease (CAD), and heart failure (HF) [4,9,15,33,48]. Fig. 3 and Supplementary Tables 1 and 2.

CHD's are the leading cause of prenatal deaths (~ 40%), while at the same time comprising the majority of all congenital malformations [49]. Reports form the Euro Heart Survey suggests, around 20% of patients with CHD undergo surgery or a catheter-based intervention, resulting in major economic burden for the patient [15,33,50,51].

Accounting for 30 ~ 40% of CHD's is the ventricular septal defect (VSD), and in a smaller proportion is the atrial septal defect (ASD). VSD (ASD) can be viewed as a discontinuation in the septal wall dividing the left and right ventricles (or atria) of the heart. VSD can produce left ventricle overload resulting in pulmonary hypertension [52,53]. miR-1/181c regulates the expression of BMPR2 [54,55]. During VSD conditions, elevated levels of GJA1 and SOX9 overlap with reduced expression of miR-1-1, and elevate miR-181c [15]. Additional data form Li et al., showed that let-7e-5p, miR-222-3p and miR-433 maybe the underlying cause for abnormalities since they target NOTCH1, HAND1, GATA3 , and ZFPM2 resulting in altered morphogenesis and VSD[27].

DiGeroge syndrome is a direct result of deletion in region 8 of chr:22, thereby producing loss-of-function mutation on TBX1 culminating in haploinsufficiency. TBX1 is has a role in differentiation of the neural crest cells, where mutated TBX1 hinders correct formation of the outflow track [15]. In addition, this condition leads to DGCR8 downregulation and promotes an accumulation of both pri-miR's and pre-miR's [56].

Recently, studies in Down syndrome (CHD expectancy 50~60%) have confirmed 5 miR's to be directly correlated miR-99a, let-7c, miR-125b-2, miR-155 and miR-802, all linked to over expression in the heart [57]. miR-99a has been associated with repression of cardiogenesis when expressed at early stage by regulating Smarca5, let-7c was found to induce it, but only if expressed during mesoderm formation, thereby repressing the activity of Ezh2 [58]. Additional studies in cancer biology have concluded that the loss of the let-7 family contributes to the upregulation of

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Ezh2, while miR-99a found typically in prostate cancer can contribute to general cell proliferation [58]. Moreover, miR-155 overexpression, (Down syndrome) can inhibit necrosis. However studies conducted suggest a mechanism by repressing receptor interacting protein 1 (RIP1), which is independent of both the Wnt/β-catenin and Akt survival pathways [15,59,60]. miR-155 is involved in many know aspects of regulatory biology: promoting cell proliferation by PTEN signaling pathway [29], promoting tumor growth by ARID2 repression [61], regulating cell proliferation in glioma by targeting FOXO3a [62].

According to the AHA website (http://www.heart.org/) "Atherosclerosis is a big word for a big problem". They define, atherosclerosis as buildup of fat deposits turning into plaques in the arteries. A multidimensional problem, not only dependent on the amount of circulating fat, but also on factors such as endothelial cell (EC) dysfunction, vascular smooth muscle cell (VSMC) differentiation and inflammation. These buildups can lead to partial or full blockage, and thus restricting blood flow, nutrition, and/or oxygen. Consequently, being the initiator of many diseases such as CHD, angina, carotid artery disease amongst others [63]. A major component of atherosclerosis, are the EC dysfunction as response to sheer stress. Schober et al., deciphered that mir-126 directly affected vascular integrity, leading to the notion that miR-126-5p was mainly responsible for EC repair by inhibiting NOTCH1 and Dlk1, [64]. Additionally, a second isoform miR-126-3p, is responsible for reducing inflammation signaling by promoting VCAM1. By blocking these 2 mechanisms atherosclerosis protection at the EC level is reduced [65,66]. Romano's group led to the mechanics of understating the cascade activation of miR-126-5p by Lipoxin A4. A response via pro-inflammatory endothelial microvesicles packed with miR-126-5p. An antagonizing effect to TNFα, leading to the upregulation of VCAM1 and the downregulation of SPRED1 [64]. Another component of atherosclerosis mentioned is VSMC differentiation. miR-145 deficiency has shown to reduce the medial layer in arteries. In addition, differentiation genes myocardin, KLF4, KLF5, calmodulin kinase, cholesterol transporter ABCA1 were found to be a direct target of miR-145 [67].

miR-33a and miR-33b have been shown to be in regulation of ABCA1 and ABCG1as they control the sterol regulatory element-binding proteins, hence their control can be a useful tool in potential therapies for dyslipidemia and atherosclerosis [68,69]. Hypertension or high blood pressure can lead to atherosclerosis due to the added force at the artery walls/ miR-145/-143 seems to play an important role in high blood pressure, mainly, having as target the angiotensin

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converting enzyme [4].. In addition, inhibition of miR-145 might improve diabetic resistance via nitric oxide [70]. We should reference that, mouse studies have enlighten the role of miR-21, demonstrating reduce blood pressure in inverse correlation with miR-130a and miR-195, whom have been positively shown to be upregulated in serum [71].

MI described as severe CAD or a myocardial cell death due to sustained ischemia. Patients with MI have shown to have a heavy upregulation of miR-1, miR-133, miR-208 and miR-499 [72]. The dysregulation of all 4 miR's has been linked to MI [18,33,72,73]. miR-208 by itself has been shown to be sufficient to induce heart hypertrophy as a response to overload, while inducing β-MHC expression [74]. Mentioned in Sun et al., levels of expression of miR-1, miR-16, miR-21, miR-92a, miR-195, miR-208, miR-375, miR-494, miR-103, miR-107, miR-325, and miR-874 are appreciably upregulated in heart tissue MI, while the levels of tissue miR-133a/b, miR-214, miR-873, miR-2861, miR-30b, miR-188-3p, and miR-145 are decreased, this panel of miR's contribute to the notion of specific spatial-time regulation, since many (if not all) of these miR's are involve in other conditions. In addition, protective signaling to reduce damage in the heart can be achieved by expression of miR-873 and miR-2861[75]. The finesse required to precisely achieve the protection in the mist of so much disruption, can tell us a bit more about the recurrent self-protective and pro-survival mechanisms present in the heart. As it was mentioned MI constitutes severe cell death, and cell death itself comes in 3 "flavors"; Necrosis (Necroptosis), Autophagy , and Apoptosis [75–77]. Each flavor comes with a set of miR regulators acting on specific targets to both promote and inhibit each process.

Necrosis is known a form of death due to exacerbation in cellular or pathogenic damage. In cardiomyocytes, necrotic death induced by $^{-}O_2$, elevates levels of miR-103 and miR-107, whom act on the Fas-associated protein with death domain [78]. Meanwhile miR-874 expression can lead to necrosis by activating FOXO3a and Caspase-8 [79]. Recently, research revealed that there exists a form of necrosis via program death: Necroptosis, which itself is initiated by TNF-α with directed interactions to RIP (1 and 3) proteins. Concisely, deficiency in interferon-β and MLK, as a result of TNFα/RIP induce pyruvate dehydrogenase to induce, what is now referred to as the necrosome (independent of caspase-8). Nevertheless, this pathway can be mediate by activation of miR-874 [80,81]. Additionally miR-155 can block the RIP1 interaction, thereby inhibiting the necrosome [29,59].

Autophagy is a highly conserved process of delivering intracellular components, including

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mitochondria and long-lived macromolecules via a double- membrane structure, to the lysosomes for degradation [76]. When activated miR-188-3p, miR-290 and miR-375 acts as mediators reducing autophagitic activity by activating ATG7. ATG7 acts thought a thiol-ester bond on the E1 activator to free Ubiquitin molecules beginning degradation [82]. At a molecular level, master regulators for autophagy are mTOR and AMPK, both regulatable by miR-155 and miR-17-92 complex. Previously, it was mentioned that miR-155 could repress the activation of the RIP complex by inhibition with PTEN, as well as interfering with the Wnt/β-catenin and the Akt-pro survival pathway [29,60]. Additionally miR 17-92 complex results in mTOR negative regulation [31].FOXO3a a pro-autophagitic factor, is negatively regulated by miR-212/132. Over expression of miR-212/132 significantly disturbs autophagy and results in drastic cardiac hypertrophy and heart failure [62,83]. Additionally, energy sensing pathway of AMPK can be blocked in particular by the disruption given to the α1 subunit (AMPKα1), activation of miR-148b directly inhibits its expression [84], therefore blocking the full AMPK assembly.

Apoptosis or programed death is a process driven by cell death receptors. Cascading signals mediated by many pro- and anti- apoptotic signals; caspases, Bcl-2 family and p53 [76,85]. Hypoxia inducible apoptosis, a condition where low oxygen concentration induces the over expression of HIF-1; initializing apoptotic conditions by inducing high concentrations of BNIP3 and causing stabilization of p53 [86–88]. miR-138 can exhibit protective effect against hypoxia-induced apoptosis via MLK3/JNK/c-jun pathway [89]. Down-regulating JNK, p38, Bax and Caspase-3 levels, and upregulating Bcl-2 we can find an apoptotic target to miR-320 IGF-1. Note that in inhibition of miR-320 up-regulates the level of IGF1 mRNA (Sun et al., 2017). Also the anti-apoptotic signaling is the downregulation of miR-200c, as it increases levels of Bcl2 [90].

An important study by the American Heart Association / American Stroke Association have been on Cavernous malformations, which are best defined as circumscribed vascular lesions with thin-walled sinusoidal spaces lined with endothelial tissue and containing intravascular or intervascular calcifications [91]. Developmentally, these malformations control cardiac development via endothelial signaling of MEKK and KLF [92]. PDCD10 a major role-playing factor in this condition, is heavily involved in cardiomyocyte autophagy. Has been shown to be susceptible to regulation by miR-613 by acting over LXRα [93], while PDCD4 is a direct target miR-155[94].

Yan et al., describe HF as a terminal stage of most types of cardiovascular diseases, which always leads to a negative prognosis. Their study on the clinical relevance of using circulating levels of miR-423-5p as a potential biomarker, they note that the standard marker is B type natriuretic peptide (BNP) [95], similarly BNP in MI miR-208b and miR-499are not the optimal but are under great scrutiny [75]. Meanwhile, research is conducted to use miR-1and miR-30a as they play crucial roles in cardiac hypertrophy and apoptosis they target key molecules in the signaling pathways that govern cardiac fibrosis, hypertrophy and apoptosis [96]. Nonetheless no defined biomarkers for HF stage.

4. Future directions

The clinical potential underlying miR's can be seen as a great area of opportunity both for their targeting and as biomarkers. miR's themselves are clearly expressed in a tightly regulated fashion throughout development and during organ maintenance. At the same time miR's can show peculiarities in expression during pathological altered states. For the clinic, knowing and understanding these in-balances gives us a step up in the game.

Important elements should be summarized for the usage or target of miR's, as certain elements can be contemplated as strengths or weaknesses. Let's consider the size for the mature miR, this can vary around 22bp and can be feasibly seen as a target by an antagonizing sequence (antagomiR or anti-miR); analogous to the mRNA outcompeting and thereby not inhibiting transcription [24,60,68]. The remarkable feature here, is that a single upstream target can determine the fate of a whole signaling pathway. Instead of targeting individual factors by knockdowns or having to obliterate by fully knocking out a gene; a not always viable solution. Counterintuitively, pairing a 22bp fragment is to an extent easy, yet, we need to consider the dynamics as targeting of miR to their mRNA. This beings with the "seed", a 5bp region at the 5' end + up to 2 more adjacent bp, using the seed alone in not a great method for pairing, so it is common for non-canonical pairing to occur [1]. Hence having the potential for silencing off targets exist so sequences need to be fully vetted by bioinformatics systems [23]. Another aspect to consider is these microRNA's have a short half-life, hence modifications or high quantities are considered as a possibility when intended for therapeutics, this might led to toxicity issues and alterations of biological properties [97]. Numerous miRNA inhibitors have been designed with different adjustments in particular at the 2′position of the. Moreover, 2′-MOE (2′-O-methoxyethyl)

and 2′-fluoro are the most commonly used modification. While an alternative LNA (locked nucleic acid) a miR mimetic, uses O, 4′-C methylene bridge to lock the furanose ring backbone [98].

Remarkably in the blood and body fluids miRNAs are stable (28h – 5d) [98], in part due to the fact they are garrisoned by their association to lipoproteins in extra cellular vesicles, form RNA-degrading molecules [4]. An interesting limitation for detection stands form the use of oligonucleotides to do PCR, if no under the precise conditions for small nucleic acids, this can easily led to large amount of artifacts [99]. Nevertheless, due to their high sensibility and specificity using circulating miR's is almost a given advantage. miR's as serum biomarkers have been rigorously reviewed [67,72,95,99,100]. Well known markers such as those mentioned by Sun et al., for MI serve as a perfect example of well-defined and circulating in the plasma. They denote miR-1, miR-16, miR-21,miR-92a, miR-195, miR-208, miR-375, miR-494, miR-103, miR-107, miR-325, miR-499, and miR-874 to be upregulated during an before an MI, while at the same time the make mention that miR-133a/b, miR-214, miR-873, miR-2861, miR-30b, miR-188-3p, and miR-145 are downregulated [75]. Moreover, we have previously mentioned that both atherosclerosis and HF also have clearly denied biomarkers such as: miR-21, miR-130a, miR-195, miR-92 (atherosclerosis) and miR-423-5p, mir-208a, miR-499, miR-16, miR-27a, miR-101, miR-150 (HF) [71] (Supplementary Table 2).

Attempts to try and direct mimetics and antagomiRs to a specific organ poses a fascinating challenge, a direct attempt to inject the antagomiR to the organ is usually the best shot and delivery system range from the use of liposome vesicles, polymers, and other viral particles, yet for the patient this could lead to potential high costs [101]. The first set of experiments using antagomiR was deemed to observe the systematic reduction of miR's. Intravenous administrations was done against miR-16, miR-122, miR-192 and miR-194 resulting in a striking decline of the resultant miRNA levels in liver, lung, kidney, heart, intestine, fat, skin, bone marrow, muscle, ovaries and adrenals [102]. Further studies have revealed the value of miR's to downregulate miRNAs in primates, using LNA modification [103], the first set of clinical trials (~20) are currently underway using diverse delivery systems [17] .

Previously it was mentioned that miR's can have many potential targets, taking this notion let's consider the well documented case of miR-29. miR-29 can regulate fibrosis in the heart by targeting a whole set of different components such as elastins, fibrillins and collagens; all components of the ECM. Therefore by using a well design antagomiR to miR-29, the group was

able to protect cardiomyocytes [98]. Furthermore, during MI experiments it was found that antagomiRs for miR-92a and miR-320 could reduce infarct size by contributing to recovery of blood vessels and reduction of apoptotic signals [48]. Meanwhile, Huang et al., considered the use of mesenchymal stem cells expressing miR-1. Elsewhere mentioned miR-1 is sufficient to lead conversion of stem cell to cardiomyocytes. This experiment overall improved both cardiac function and reduce overall infarcted size [104]. Another set of experiments looked at miR-21. Dong et al., upregulated the expression of miR-21 by ischemic preconditioning, before MI, demonstrating considerable reduction in infarcted area. Moreover, the effect was even greater when injecting alongside of miR-1 and miR-24 [48]. Experiments in Atherosclerosis using AntagomiRs (miR-33) showed a substantial elevation in HDL and reduction in VDL. Additionally, LNA-AntagomiR (miR-122) also showed promise in cholesterol reduction while 2'-O-methoxyethyl phosphorothioate (miR-122) enhanced liver steatosis [99]. Promoting a reduction of plaque size and enhanced vasculature, AntagomiR (miR-145) was used rodents. Results showed a marked reduction in plaque in aortic sinuses, necrotic core, increase collagen promoting contractile VSMC [67,99]. These studies demonstrated how using the antagomiR or the mir-mimic, it's possible to achieve a preferred outcome.

Since their initial discovery, small non-coding RNA's, have played an instrumental role in deciphering the nature and mechanics of human biology and its conditions. As we have seen in this overview of CVD's they are instrumental both in the way they orchestrate through positive and negative feedbacks, and direct control. Ultimately, we see that they work in groups, many of these belonging to a family or super family of miR's, which ultimately in the right balance are responsible for the correct cardiac environment. We can look forward into the next couple of years, where technological advances, will take us further in understanding and uncovering more insights into miR's, mimics and antagomiRs, it's very likely that their local use will be a way of personalized treatment in many illnesses we face. In addition, we expect an even faster uptake of the clinics in using screening methods for identifying miR level in patients as a way to easily access disease information.

5. References

1. Hydbring, P.; Badalian-Very, G. Clinical applications of microRNAs. *F1000* **2014**, *2*, 1–

16, doi:10.12688/f1000research.2-136.v1.

2. Wightman, B.; Ha, I.; Ruvkun, G. Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell* **1993**, *75*, 855–862, doi:10.1016/0092-8674(93)90530-4.

3. Zhang, L.; Hao, C.; Li, J.; Qu, Y.; Bao, L.; Li, Y.; Yue, Z.; Zhang, M.; Yu, X.; Chen, H.; Zhang, J.; Wang, D.; Yao, W. Bioinformatics methods for identifying differentially expressed genes and signaling pathways in nano-silica stimulated macrophages. *Tumour Biol.* **2017**, *6*, 1010428317709284, doi:10.1177/1010428317709284.

4. Romaine, S. P. R.; Tomaszewski, M.; Condorelli, G.; Samani, N. MicroRNAs in cardiovascular disease : an introduction for clinicians. *Heart* **2015**, *101*, 921–8, doi:10.1136/heartjnl-2013-305402.

5. Li, Y.; Kowdley, K. V. MicroRNAs in Common Human Diseases. *Genomics, Proteomics Bioinforma.* **2012**, *10*, 246–253, doi:10.1016/j.gpb.2012.07.005.

6. Sohel, M. H. Extracellular/Circulating MicroRNAs: Release Mechanisms, Functions and Challenges. *Achiev. Life Sci.* **2016**, *10*, 175–186, doi:10.1016/j.als.2016.11.007.

7. Bartel, D. P. MicroRNA Target Recognition and Regulatory Functions. *Cell* **2009**, *136*, 215–233, doi:10.1016/j.cell.2009.01.002.MicroRNA.

8. Gulyaeva, L. F.; Kushlinskiy, N. E. Regulatory mechanisms of microRNA expression. *J. Transl. Med.* **2016**, *14*, 1–10, doi:10.1186/s12967-016-0893-x.

9. Small, E. M.; Frost, R. J. A.; Olson, E. N. MicroRNAs add a new dimension to cardiovascular disease. *Circulation* **2010**, *121*, 1022–1032, doi:10.1161/CIRCULATIONAHA.109.889048.

10. Hunter, M. P.; Ismail, N.; Zhang, X.; Aguda, B. D.; Lee, E. J.; Yu, L.; Xiao, T.; Schafer, J.; Lee, M. L. T.; Schmittgen, T. D.; Nana-Sinkam, S. P.; Jarjoura, D.; Marsh, C. B. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One* **2008**, *3*, e3694, doi:10.1371/journal.pone.0003694.

11. Lee, Y.; Jeon, K.; Lee, J.-T.; Kim, S.; Kim, V. N. MicroRNA maturation: stepwisee processing and subcellular localization. *EMBO J.* **2002**, *21*, 4663–4670, doi:10.1093/emboj/cdf476.

12. Garzon, R.; Marcucci, G.; Croce, C. M. Targeting MicroRNAs in Cancer: Rationale, Strategies and Challenges. *Nat Rev Drug Discov.* **2013**, *9*, 775–789,

doi:10.1109/TMI.2012.2196707.Separate.

13. Demongeot, J.; Glade, N.; Moreira, A.; Vial, L. RNA relics and origin of life. *Int. J. Mol. Sci.* **2009**, *10*, 3420–3441, doi:10.3390/ijms10083420.
14. Chendrimada, T. P.; Gregory, R. I.; Kumaraswamy, E.; Cooch, N.; Nishikura, K.; Shiekhattar, R. TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature* **2005**, *436*, 740–744, doi:10.1038/nature03868.TRBP.
15. Smith, T.; Rajakaruma, C.; Caputo, M.; Emanueli, C. MicroRNAs in congenital heart disease. *Ann Transl Med* **2015**, *3*, 1–10, doi:10.3978/j.issn.2305-5839.2015.12.25.
16. Alhendi, A. M. N.; Haider, S.; Jagannathan, S.; Anaissie, E.; Driscoll, J. J. MicroRNA theragnostics for the clinical management of multiple myeloma. *Leukemia* **2014**, *28*, 732–738, doi:10.1038/leu.2013.262.
17. Chakraborty, C.; Sharma, A. R.; Sharma, G.; Doss, C. G. P.; Lee, S. S. Therapeutic miRNA and siRNA: Moving from Bench to Clinic as Next Generation Medicine. *Mol. Ther. - Nucleic Acids* **2017**, *8*, 132–143, doi:10.1016/j.omtn.2017.06.005.
18. Fu, J.-D.; Rushing, S. N.; Lieu, D. K.; Chan, C. W.; Kong, C.; Wilson, K. D.; Chiamvimonvat, N.; Boheler, K. R.; Wu, J. C.; Hajjar, R. J.; Li, R. A. Chiamvimonvat N, Li RA. Na+/Ca2+ exchanger is a determinant of excitation-contraction coupling in human embryonic stem cell-derived ventricular cardiomyocytes. *Stem Cell Dev* **2010**, *19*, 773–782.
19. Iyer, D.; Belaguli, N.; Flu, M.; Rowan, B. G.; Wei, L.; Weigel, N. L.; Booth, F. W.; Epstein, H. F.; Schwartz, R. J.; Balasubramanyam, A. Novel Phosphorylation Target in the Serum Response Factor MADS Box Regulates. **2003**, 7477–7486.
20. Zheng, G.; Tao, Y.; Yu, W.; Schwartz, R. J. Brief report: Srf-dependent MiR-210 silences the sonic hedgehog signaling during cardiopoesis. *Stem Cells* **2013**, *31*, 2279–2285, doi:10.1002/stem.1464.
21. Liu, Y.; Schwartz, R. J. A driver of cardiac cell fate determination © 2013 Landes Bioscience . Do not distribute. *Transcription* **2013**, *4*, 92–96.
22. Tritsch, E.; Mallat, Y.; Lefebvre, F.; Diguet, N.; Escoubet, B.; Blanc, J.; De Windt, L. J.; Catalucci, D.; Vandecasteele, G.; Li, Z.; Mericskay, M. An SRF/miR-1 axis regulates NCX1 and Annexin A5 protein levels in the normal and failing heart. *Cardiovasc. Res.* **2013**, *98*, 372–380, doi:10.1093/cvr/cvt042.

23. Tian, J.; An, X.; Niu, L. Role of microRNAs in cardiac development and disease (Review). *Exp. Ther. Med.* **2017**, *13*, 3–8, doi:10.3892/etm.2016.3932.
24. Chaitra, K. L.; Ulaganathan, K.; James, A.; Ananthapur, V.; Nallari, P. miRNA regulation during cardiac development and remodeling in cardiomyopathy. *EXCLI J.* **2013**, *12*, 980–992.
25. Wu, K. H.; Xiao, Q. R.; Yang, Y.; Xu, J. L.; Zhang, F.; Liu, C. M.; Zhang, Z. M.; Lu, Y. Q.; Huang, N. P. MicroRNA-34a modulates the Notch signaling pathway in mice with congenital heart disease and its role in heart development. *J. Mol. Cell. Cardiol.* **2018**, *114*, 300–308, doi:10.1016/j.yjmcc.2017.11.015.
26. Mishima, Y.; Stahlhut, C.; Giraldez, A. J. miR-1-2 Gets to the Heart of the Matter. *Cell* 2007, *129*, 247–249.
27. Li, J.; Dong, X.; Wang, Z.; Wu, J. MicroRNA-1 in cardiac diseases and cancers. *Korean J. Physiol. Pharmacol.* **2014**, *18*, 359–363, doi:10.4196/kjpp.2014.18.5.359.
28. Zhao, Y.; Ransom, J. F.; Li, A.; Vedantham, V.; von Drehle, M.; Muth, A. N.; Tsuchihashi, T.; McManus, M. T.; Schwartz, R. J.; Srivastava, D. Dysregulation of Cardiogenesis, Cardiac Conduction, and Cell Cycle in Mice Lacking miRNA-1-2. *Cell* **2007**, *129*, 303–317, doi:10.1016/j.cell.2007.03.030.
29. Xu, L.; Len, H.; Shi, X.; Ji, J.; Fu, J.; Len, H. MiR-155 promotes cell proliferation and inhibits apoptosis by PTEN signaling pathway in the psoriasis. *Biomed. Pharmacother.* **2017**, *90*, 524–530, doi:10.1016/j.biopha.2017.03.105.
30. Yan, S.; Jiao, K. Functions of miRNAs during mammalian heart development. *Int. J. Mol. Sci.* **2016**, *17*, 1–8, doi:10.3390/ijms17050789.
31. Danielson, L.; Park, D.; Rotllan, N Chamorro-Jorganes, A.; Guijarro, MV Fernandez-Hernando, C.; Fishman, GI Phoon, C.; Hernando, E. Cardiovascular dysregulation of miR-17-92 causes a lethal hypertrophic cardiomyopathy and arrhythmogenesis. *FASEB J.* **2013**, 1460–7, doi:10.1096/fj.12-221994.
32. Callis, T. E.; Pandya, K.; Hee, Y. S.; Tang, R. H.; Tatsuguchi, M.; Huang, Z. P.; Chen, J. F.; Deng, Z.; Gunn, B.; Shumate, J.; Willis, M. S.; Selzman, C. H.; Wang, D. Z. MicroRNA-208a is a regulator of cardiac hypertrophy and conduction in mice. *J. Clin. Invest.* **2009**, *119*, 2772–2786, doi:10.1172/JCI36154.
33. Xin, M.; Olson, E. N.; Bassel-duby, R. Mending broken hearts: cardiac development as a

basis for adult heart regeneration and repair. *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 529–541, doi:10.1038/nrm3619.Mending.

34. Guo, X.-M. Creation of Engineered Cardiac Tissue In Vitro From Mouse Embryonic Stem Cells. *Circulation* **2006**, *113*, 2229–2237, doi:10.1161/CIRCULATIONAHA.105.583039.
35. Srivastava, D.; Ieda, M. Critical Factors for Cardiac Reprogramming. *Circ. Res.* **2013**, *111*, 5–8, doi:10.1161/CIRCRESAHA.112.271452.Critical.
36. Christoforou, N.; Chellappan, M.; Adler, A. F.; Kirkton, R. D.; Wu, T.; Addis, R. C.; Bursac, N.; Leong, K. W. Transcription Factors MYOCD, SRF, Mesp1 and SMARCD3 Enhance the Cardio-Inducing Effect of GATA4, TBX5, and MEF2C during Direct Cellular Reprogramming. *PLoS One* **2013**, *8*, doi:10.1371/journal.pone.0063577.
37. Belian, E.; Noseda, M.; Abreu Paiva, M. S.; Leja, T.; Sampson, R.; Schneider, M. D. Forward Programming of Cardiac Stem Cells by Homogeneous Transduction with MYOCD plus TBX5. *PLoS One* **2015**, *10*, e0125384, doi:10.1371/journal.pone.0125384.
38. Jayawardena, T.; Egemnazarov, B.; Finch, E.; Zhan, L.; Payne, A.; Pandya, K.; Zhang, Z.; Rosenberg, P.; Mirotsou, M.; Dazu, V. MicroRNA-mediated in vitro and in vivo Direct Reprogramming of Cardiac Fibroblasts to Cardiomyocytes. *Circ. Res.* **2013**, *110*, 1465–1473, doi:10.1161/CIRCRESAHA.112.269035.MicroRNA-mediated.
39. Ivey, K. N.; Muth, A.; Arnold, J.; King, F. W.; Yeh, R.; Jason, E.; Hsiao, E. C.; Schwartz, R. J.; Conklin, B. R.; Harold, S.; Srivastava, D. MicroRNA Regulation of Cell Lineages in Mouse and Human Embryonic Stem Cells. *Cell Stem Cell.* **2009**, *2*, 219–229.
40. Zhang, S. X.; Garcia-Gras, E.; Wycuff, D. R.; Marriot, S. J.; Kadeer, N.; Yu, W.; Olson, E. N.; Garry, D. J.; Parmacek, M. S.; Schwartz, R. J. Identification of direct serum-response factor gene targets during Me2SO-induced P19 cardiac cell differentiation. *J. Biol. Chem.* **2005**, *280*, 19115–26, doi:10.1074/jbc.M413793200.
41. Fu, J.; Jiang, P.; Rushing, S.; Liu, J.; Chiamvimonvat, N.; Li, R. A. Na+/Ca2+ exchanger is a determinant of excitation-contraction coupling in human embryonic stem cell-derived ventricular cardiomyocytes. *Stem Cells Dev.* **2010**, *19*, 773–782, doi:10.1089/scd.2009.0184.
42. Lieu, D. K.; Fu, J.; Chiamvimonvat, N.; Tung, K. W. C.; McNerney, G. P.; Huser, T.; Keller, G.; Kong, C.-W.; Li, R. A. Mechanism-Based Facilitated Maturation of Human Pluripotent Stem Cell-Derived Cardiomyocytes. *Circ. Arrhythmia Electrophysiol.* **2013**, *6*,

463 CIRCEP.112.973420--201, doi:10.1161/CIRCEP.111.973420.
464 43. Terentyev, D.; Belevych, A. E.; Terentyeva, R.; Martin, M. M.; Malana, G. E.; Kuhn, D.
465 E.; Abdellatif, M.; Feldman, D. S.; Terry, S.; Gyorke, S. Mir-1 overexpression enhances
466 Ca(2+) release and promotes cardiac arrhythmogenesis by targeting pp2a regulatory
467 subunit b56alpha and causing camkii-dependent hyperphosphorylation of ryr2. *Circ. Res.*
468 **2015**, *104*, 514–521, doi:10.1161/CIRCRESAHA.108.181651.MiR-1.
469 44. Poon, E.; Hao, B.; Guan, D.; Li, M.; Lu, J.; Yang, Y.; Wu, B.; Wu, S.; Webb, S.; Liang,
470 Y.; Miller, A.; Yao, X.; Wang, J.; Yan, B.; Boheler, K. Integrated transcriptomic and
471 regulatory network analyses identify microRNA-200c as a novel repressor of human
472 pluripotent stem cell-derived cardiomyocyte differentiation and maturation. *Cardiovasc.*
473 *Res.* **2018**, *114*, [Epub ahead of print], doi:10.1093/cvr/cvy019.
474 45. Bondue, A.; Blanpain, C. MESP1. A Key Regulator of Cardiovascular Lineage
475 Commitment. *Circ. Res.* **2010**, 575–578, doi:10.1161/CIRCRESAHA.110.227058.
476 46. Islas, J. F.; Liu, Y.; Weng, K.-C.; Robertson, M. J.; Zhang, S.; Prejusa, A.; Harger, J.;
477 Tikhomirova, D.; Chopra, M.; Iyer, D.; Mercola, M.; Oshima, R. G.; Willerson, J. T.;
478 Potaman, V. N.; Schwartz, R. J. Transcription factors ETS2 and MESP1 transdifferentiate
479 human dermal fibroblasts into cardiac progenitors. *Proc. Natl. Acad. Sci.* 2012, *109*,
480 13016–13021.
481 47. Shen, X.; Soibam, B.; Benham, A.; Xu, X.; Chopra, M.; Peng, X.; Yu, W.; Bao, W.;
482 Liang, R.; Azares, A.; Liu, P.; Gunaratne, P. H.; Mercola, M.; Cooney, A. J.; Schwartz, R.
483 J.; Liu, Y. miR-322/-503 cluster is expressed in the earliest cardiac progenitor cells and
484 drives cardiomyocyte specification. *Proc. Natl. Acad. Sci.* **2016**, *113*, 9551–9556,
485 doi:10.1073/pnas.1608256113.
486 48. Schulte, C.; Zeller, T. microRNA-based diagnostics and therapy in cardiovascular disease-
487 Summing up the facts. *Cardiovasc. Diagn. Ther.* **2015**, *5*, 17–36, doi:10.3978/j.issn.2223-
488 3652.2014.12.03.
489 49. Bensemlali, M.; Bajolle, F.; Ladouceur, M.; Fermont, L.; Lévy, M.; Le Bidois, J.;
490 Salomon, L. J.; Bonnet, D. Associated genetic syndromes and extracardiac malformations
491 strongly influence outcomes of fetuses with congenital heart diseases. *Arch. Cardiovasc.*
492 *Dis.* **2016**, *109*, 330–336, doi:10.1016/j.acvd.2016.01.006.
493 50. Mozaffarian, D.; Benjamin, E. J.; Go, A. S.; Arnett, D. K.; Blaha, M. J.; Cushman, M.;

Das, S. R.; Ferranti, S. De; Després, J. P.; Fullerton, H. J.; Howard, V. J.; Huffman, M. D.; Isasi, C. R.; Jiménez, M. C.; Judd, S. E.; Kissela, B. M.; Lichtman, J. H.; Lisabeth, L. D.; Liu, S.; MacKey, R. H.; Magid, D. J.; McGuire, D. K.; Mohler, E. R.; Moy, C. S.; Muntner, P.; Mussolino, M. E.; Nasir, K.; Neumar, R. W.; Nichol, G.; Palaniappan, L.; Pandey, D. K.; Reeves, M. J.; Rodriguez, C. J.; Rosamond, W.; Sorlie, P. D.; Stein, J.; Towfighi, A.; Turan, T. N.; Virani, S. S.; Woo, D.; Yeh, R. W.; Turner, M. B. Heart disease and stroke statistics-2016 update a report from the American Heart Association. *Circulation* **2016**, *133*, e38–e48, doi:10.1161/CIR.0000000000000350.

51. Mercola, M.; Ruiz-lozano, P.; Schneider, M. D. Cardiac muscle regeneration : lessons from development Cardiac muscle regeneration : lessons from development. *Genes Dev.* **2011**, 299–309, doi:10.1101/gad.2018411.

52. Bigdelian, H.; Sedighi, M. The role of preoperative sildenafil therapy in controlling of postoperative pulmonary hypertension in children with ventricular septal defects. *J. Cardiovasc. Thorac. Res.* **2017**, *9*, 179–182, doi:10.15171/jcvtr.2017.31.

53. Lucchese, G.; Rossetti, L.; Faggian, G.; Luciani, G. B. Long-Term Follow-Up Study of Temporary Tricuspid Valve Detachment as Approach to VSD Repair without Consequent Tricuspid Dysfunction. *Texas Hear. Inst. J.* **2016**, *43*, 392–396, doi:10.14503/THIJ-14-4797.

54. Li, J.; Cao, Y.; Ma, X.; Wang, H.; Zhang, J.; Luo, X.; Chen, W.; Wu, Y.; Meng, Y.; Zhang, J.; Yuan, Y.; Ma, D. Roles of miR-1-1 and miR-181c in ventricular septal defects.le. *Int. J. Cardiol.* **2013**, *168*, 1441–1446.

55. Das, S.; Bedja, D.; Campbell, N.; Dunkerly, B.; Chenna, V.; Maitra, A.; Steenbergen, C. miR-181c Regulates the Mitochondrial Genome, Bioenergetics, and Propensity for Heart Failure In Vivo. *PLoS One* **2014**, *9*, e96820, doi:10.1371.

56. Landthaler, M.; Abdullah, Y.; Tuschi, T. The Human DiGeorge Syndrome Critical Region Gene 8 and Its D. melanogaster Homolog Are Required for miRNA Biogenesis. *Curr. Biol.* **2004**, *14*, 2162–2167, doi:10.1016/j.

57. Zhao, Y.; Jaber, V.; Percy, M. E.; Lukiw, W. J. A microRNA cluster (let-7c, miRNA-99a, miRNA-125b, miRNA-155 and miRNA-802) encoded at chr21q21.1-chr21q21.3 and the phenotypic diversity of Down's syndrome (DS; trisomy 21). *J Nat Sci* **2017**, *3*, 1–11, doi:10.1007/s00210-015-1172-8.The.

525 58. Coppola, A.; Romito, A.; Borel, C.; Gehrig, C.; Gagnebin, M.; Falconnet, E.; Izzo, A.;
526 Altucci, L.; Banfi, S.; Antonarakis, S. E.; Minchiotti, G.; Cobellis, G. Cardiomyogenesis
527 is controlled by the miR-99a/let-7c cluster and epigenetic modifications. *Stem Cell Res.*
528 **2014**, *12*, 323–337, doi:10.1016/j.scr.2013.11.008.
529 59. Jablonska, E.; Gorniak, P.; Prusisz, W.; Kiliszek, P.; Szydlowski, M.; Sewastianik, T.;
530 Bialopiotrowicz, E.; Polak, A.; Prochorec-Sobieszek, M.; Szumera-Cieckiewicz, A.;
531 Warzocha, K.; Juszczynski, P. MiR-155 Amplifies AKT and NFkB Signaling By
532 Targeting Multiple Regulators of BCR Signal in DLBCL. *Blood* **2015**, *126*, 2455–2455.
533 60. Yan, Q.; Chen, J.; Li, W.; Bao, C.; Fu, Q. Targeting miR-155 to Treat Experimental
534 Scleroderma. *Sci. Rep*. **2016**, *6*, 1–11, doi:10.1038/srep20314.
535 61. Zhang, L.; Wang, W.; Li, X.; He, S.; Yao, J.; Wang, X.; Zhang, D.; Sun; X. MicroRNA-
536 155 promotes tumor growth of human hepatocellular carcinoma by targeting ARID2. *Int J*
537 *Oncol.* **2018**, *48*, 2425–34., doi:10.3892/ijo.2016.3465.
538 62. Ling, N.; Gu, J.; Lei, Z.; Li, M.; Zhao, J.; Zhang, H.; Li, X. microRNA-155 regulates cell
539 proliferation and invasion by targeting FOXO3a in glioma. *Oncol Rep* **2013**, *30*, 2111–8,
540 doi:10.3892/or.2013.2685.
541 63. Andrews, J. P. M.; Fayad, Z. A.; Dweck, M. R. New methods to image unstable
542 atherosclerotic plaques. *Atherosclerosis* **2018**, *272*, 118–128,
543 doi:10.1016/j.atherosclerosis.2018.03.021.
544 64. Codagnone, M.; Recchiuti, A.; Lanuti, P.; Pierdomenico, A. M.; Cianci, E.; Patruno, S.;
545 Mari, V. C.; Simiele, F.; Di Tomo, P.; Pandolfi, A.; Romano, M. Lipoxin A4stimulates
546 endothelial miR-126-5p expression and its transfer via microvesicles. *FASEB J.* **2017**, *31*,
547 1856–1866, doi:10.1096/fj.201600952R.
548 65. Voora, D. The Last Line of Defense Against Atherosclerosis. *Sci. Transl. Med*. **2014**, *6*,
549 228ec51, doi:10.1126/scitranslmed.3008868.
550 66. Boon, R. A.; Dimmeler, S. MicroRNA-126 in Atherosclerosis. *Arter. Thromb Vasc Biol*
551 **2014**, *34*, :e15-e16, doi:10.1161/ATVBAHA.114.303572.
552 67. Faccini, J.; Ruidavets, J. B.; Cordelier, P.; Martins, F.; Maoret, J. J.; Bongard, V.;
553 Ferrières, J.; Roncalli, J.; Elbaz, M.; Vindis, C. Circulating MIR-155, MIR-145 and let-7c
554 as diagnostic biomarkers of the coronary artery disease. *Sci. Rep*. **2017**, *7*, 1–10,
555 doi:10.1038/srep42916.

68. Rayner, K. J.; Sheedy, F. J.; Esau, C. C.; Hussain, F. N.; Temel, R. E.; Parathath, S.; Van Gils, J. M.; Rayner, A. J.; Chang, A. N.; Suarez, Y.; Fernandez-Hernando, C.; Fisher, E. A.; Moore, K. J. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. *J. Clin. Invest.* **2011**, *121*, 2921–2931, doi:10.1172/JCI57275.

69. Rayner, K. J.; Esau, C. C.; Hussain, F. N.; Mcdaniel, A. L.; Marshall, M.; Gils, J. M. Van; Ray, T. D.; Sheedy, F. J.; Goedeke, L.; Liu, X.; Khatsenko, O. G.; Kaimal, V.; Lees, C. J.; Fernandez-, C.; Fisher, E. A.; Temel, R. E.; Moore, K. J. Inhibition of miR-33a/b in non-human primates raises plasma HDL and reduces VLDL triglycerides Katey. *Nature* **2012**, *478*, 404–407, doi:10.1038/nature10486.Inhibition.

70. Shiuchi, T.; Cui, T.-X.; Wu, L.; Nakagami, H.; Takeda-Matsubara, Y.; Iwai, M.; Horiuchi, M. ACE Inhibitor Improves Insulin Resistance in Diabetic Mouse Via Bradykinin and NO. *Hypertension* **2002**, *40*, 329–334, doi:10.1161/01.HYP.0000028979.98877.0C.

71. Yong, W.; Jin, L. miRNA 145 is associated with spontaneous hypertension by targeting SLC7A1. *Exp. Ther. Med.* **2017**, *15*, 48–552, doi:10.3892/etm.2017.5371.

72. Wang, G.-K.; Zhu, J.-Q.; Zhang, J.-T.; Li, Q.; Li, Y.; He, J.; Qin, Y.-W.; Jing, Q. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. *Eur. Heart J.* **2010**, *31*, 659–666, doi:10.1093/eurheartj/ehq013.

73. Corsten, M. F.; Dennert, R.; Jochems, S.; Kuznetsova, T.; Devaux, Y.; Hofstra, L.; Wagner, D. R.; Staessen, J. A.; Heymans, S.; Schroen, B. Circulating MicroRNA-208b and MicroRNA-499 reflect myocardial damage in cardiovascular disease. *Circ. Cardiovasc. Genet.* **2010**, *3*, 499–506, doi:10.1161/CIRCGENETICS.110.957415.

74. Kumarswamy, R.; Thum, T. Non-coding RNAs in cardiac remodeling and heart failure. *Circ. Res.* **2013**, *113*, 676–689, doi:10.1161/CIRCRESAHA.113.300226.

75. Sun, T.; Dong, Y.-H.; Du, W.; Shi, C.-Y.; Wang, K.; Tariq, M.-A.; Wang, J.-X.; Li, P.-F. The Role of MicroRNAs in Myocardial Infarction: From Molecular Mechanism to Clinical Application. *Int. J. Mol. Sci.* **2017**, *18*, 745, doi:10.3390/ijms18040745.

76. Chiong, M.; Wang, Z. V; Pedrozo, Z.; Cao, D. J.; Troncoso, R.; Ibacache, M.; Criollo, A.; Nemchenko, A.; Hill, J. a; Lavandero, S. Cardiomyocyte death: mechanisms and translational implications. *Cell Death Dis.* **2011**, *2*, e244, doi:10.1038/cddis.2011.130.

77. Graham, R. M.; Frazier, D. P.; Thompson, J. W.; Haliko, S.; Li, H.; Wasserlauf, B. J.; Spiga, M. G.; Bishopric, N. H.; Webster, K. A. A unique pathway of cardiac myocyte death caused by hypoxia-acidosis. *J Exp Biol* **2004**, *207*, 3189–3200, doi:10.1242/jeb.01109.

78. Wang, J.; Zhang, X.; Li, Q.; Wang, K.; Wang, Y.; Jiao, J.; Feng, C.; Zhou, L.; Gong, Y.; Zhou, Z.; Liu, J.; Wang, J.; Li, P. MicroRNA-103 / 107 Regulate Programmed Necrosis and Myocardial Ischemia / Reperfusion Injury Through Targeting FADD. *Circ. Res.* **2015**, *117*, 352–63, doi:10.1161/CIRCRESAHA.117.305781.

79. Wang, K.; Liu, F.; Liu, C.; An, T.; Zhang, J.; Zhou, L.; Wang, M.; Dong, Y.; Li, N.; Gao, J.; Zhao, Y.; Li, P. The long noncoding RNA NRF regulates programmed necrosis and myocardial injury during ischemia and reperfusion by targeting miR-873. *Cell Death Differ.* **2016**, *23*, 1394–1405, doi:10.1038/cdd.2016.28.

80. Yang, Z.; Wang, Y.; Zhang, Y.; He, X.; Zhong, C.-Q.; Ni, H.; Chen, X.; Liang, Y.; Wu, J.; Zhao, S.; Zhou, D.; Han, J. RIP3 targets pyruvate dehydrogenase complex to increase aerobic respiration in TNF-induced necroptosis. *Nat. Cell Biol.* **2018**, *20*, 186–197, doi:10.1038/s41556-017-0022-y.

81. Weber, K.; Roelandt, R.; Bruggeman, I.; Estornes, Y.; Vandenabeele, P. Nuclear RIPK3 and MLKL contribute to cytosolic necrosome formation and necroptosis. *Commun. Biol.* **2018**, *1*, 6, doi:10.1038/s42003-017-0007-1.

82. Yamaguchi, M.; Satoo, K.; Suzuki, H.; Fujioka, Y.; Ohsumi, O.; Inagaki, F.; Noda, N. N. Atg7 Activates an Autophagy-Essential Ubiquitin-like Protein Atg8 through Multi-Step Recognition. *J. Mol. Biol.* **2018**, *430*, 249–257.

83. Sermersheim, M. A.; Park, K. H.; Gumpper, K.; Adesanya, T. M. A.; Song, K.; Tan, T.; Ren, X.; Yang, J.; Zhu, H.; Heart, D.; State, T. P.; Hershey, M. S. MicroRNA regulation of autophagy in cardiovascular disease. *Biosci (Landmark Ed)* **2017**, *22*, 48–65.

84. He, X.; Li, C.; Ke, R.; Luo, L.; Huang, D. Down-regulation of adenosine monophosphate – activated protein kinase activity : A driver of cancer. *Tumor Biol.* **2017**, doi:10.1177/1010428317697576.

85. Samarel, A. M. IGF-1 overexpression rescues the failing heart. *Circ. Res.* **2002**, *90*, 631–633, doi:10.1161/01.RES.0000015425.11187.19.

86. Greijer, A. E.; Wall, E. Van Der The role of hypoxia inducible factor 1 (HIF-1) in hypoxia

induced apoptosis. *J. Clin. Pathol.* **2004**, *57*, 1009–1014, doi:10.1136/jcp.2003.015032.

87. Chu, W.; Wan, L.; Zhao, D.; Qu, X.; Cai, F.; Huo, R.; Wang, N.; Zhu, J.; Zhang, C.; Zheng, F.; Cai, R.; Dong, D.; Lu, Y.; Yang, B. Mild hypoxia-induced cardiomyocyte hypertrophy via up-regulation of HIF-1α-mediated TRPC signalling. *J. Cell. Mol. Med.* **2012**, *16*, 2022–2034, doi:10.1111/j.1582-4934.2011.01497.x.

88. Ng, K. M.; Lee, Y. K.; Chan, Y. C.; Lai, W. H.; Fung, M. L.; Li, R. A.; Siu, C. W.; Tse, H. F. Exogenous expression of HIF-1?? promotes cardiac differentiation of embryonic stem cells. *J. Mol. Cell. Cardiol.* **2010**, *48*, 1129–1137, doi:10.1016/j.yjmcc.2010.01.015.

89. Zhu, H.; Xue, H.; Jin, Q.-H.; Guo, J.; Chen, Y.-D. MiR-138 protects cardiac cells against hypoxia through modulation of glucose metabolism by targetting pyruvate dehydrogenase kinase 1. *Biosci. Rep.* **2017**, *37*, 1–9, doi:10.1042/BSR20170296.

90. Chen, Z.; Zhang, S.; Guo, C.; Li, J.; Sang, W. Downregulation of miR-200c protects cardiomyocytes from hypoxia-induced apoptosis by targeting GATA-4. *Int. J. Mol. Med.* **2017**, *39*, 1589–1596, doi:10.3892/ijmm.2017.2959.

91. Roach, E. S.; Golomb, M. R.; Adams, R.; Biller, J.; Daniels, S.; Ferriero, D.; Jones, B. V; Kirkham, F. J.; Scott, R. M.; Smith, E. R. Management of Stroke in Infants and Children A Scientific Statement From a Special Writing Group of the American Heart Association Stroke Council and the Council on Cardiovascular Disease in the Young. **2008**, doi:10.1161/STROKEAHA.108.189696.

92. Zhou, Z.; Rawnsley, D. R.; Goddard, L. M.; Pan, W.; Cao, X. J.; Jakus, Z.; Zheng, H.; Yang, J.; Arthur, J. S. C.; Whitehead, K. J.; Li, D.; Zhou, B.; Garcia, B. A.; Zheng, X.; Kahn, M. L. The Cerebral Cavernous Malformation Pathway Controls Cardiac Development via Regulation of Endocardial MEKK3 Signaling and KLF Expression. *Dev. Cell* **2015**, *32*, 168–180, doi:10.1016/j.devcel.2014.12.009.

93. Ou, Z.; Wada, T.; Gramignoli, R.; Li, S.; Strom, S. C.; Huang, M.; Xie, W. MicroRNA hsa-miR-613 Targets the Human LXRα Gene and Mediates a Feedback Loop of LXRα Autoregulation. *Mol. Endocrinol.* **2011**, *25*, 584–596, doi:10.1210/me.2010-0360.

94. Liu, F.; Song, D.; Wu, Y.; Liu, X.; Zhu, J.; Tang, Y. MiR-155 inhibits proliferation and invasion by directly targeting PDCD4 in non-small cell lung cancer. *Thorac. Cancer* **2017**, *8*, 613–619, doi:10.1111/1759-7714.12492.

95. Yan, H.; Zhang, Y.; Wang, C.; Qiu, D.; Zhou, K.; Hua, Y.; Li, Y. miRNAs as biomarkers

649 for diagnosis of heart failure. *Med. (Baltimore).* **2017**, *22*, e6825.,
650 doi:10.1097/MD.0000000000006825.
651 96. Wong, L. L.; Wang, J.; Liew, O. W.; Richards, A. M.; Chen, Y. MicroRNA and Heart
652 Failure. *Int. J. Mol. Sci.* **2016**, *17*, 1–31, doi:10.3390/ijms17040502.
653 97. Joladarashi, D.; Thandavarayan, R. A.; Babu, S. S. Small Engine , Big Power :
654 MicroRNAs as Regulators of Cardiac Diseases and Regeneration. *Int. J. Mol. Sci.* **2014**,
655 15891–15911, doi:10.3390/ijms150915891.
656 98. Kwekkeboom, R. F. J.; Lei, Z.; Doevendans, P. A.; Musters, R. J. P.; Sluijter, J. P. G.
657 Targeted delivery of miRNA therapeutics for cardiovascular diseases: opportunities and
658 challenges. *Clin. Sci.* **2014**, *127*, 351–365, doi:10.1042/CS20140005.
659 99. Condorelli, G.; Latronico, M. V. G.; Cavarretta, E. microRNAs in Cardiovascular
660 Diseases Current Knowledge and the Road Ahead. *J. Am. Coll. Cardiol.* **2014**, *63*, :2177–
661 87.
662 100. Gomes da Silva, A. M.; Silbiger, V. N. miRNAs as biomarkers of atrial fibrillation.
663 *Biomarkers* **2014**, *19*, 631–636, doi:10.3109/1354750X.2014.954001.
664 101. Thomson, D. W.; Bracken, C. P.; Szubert, J. M.; Goodall, G. . On measuring miRNAs
665 after transient transfection of mimics or antisense inhibitors. *PLoS One* **2013**, *8*, e55214.
666 102. Krützfeldt, J.; Rajewsky, N.; Braich, R.; Rajeev, K.; Tuschl, T.; Manoharan, M.; M., S.
667 Silencing of microRNAs in vivo with “antagomirs”. *Nature* **2005**, *438*, 685–9.
668 103. Elmen, J.; Lindow, M.; Schutz, S.; Lawrence, M.; Petri, A.; Obad, S.; Lindholm, M.;
669 Hedtjarn, M.; Hansen, H.; Berger, U.; Gullans, S.; Kearney, P.; Sarnow, P.; Straarup, E.;
670 Kauppinen, S. L. LNA-mediated microRNA silencing in non-human primates. *Nature*
671 **2008**, *452*, 896 – 899.
672 104. Huang, F; Li, ML; Fang, ZF; Hu, XQ; Liu, QM; Liu, ZJ; Tang, L; Zhao, YS; Zhou, S.
673 Overexpression of MicroRNA-1 improves the efficacy of mesenchymal stem cell
674 transplantation after myocardial infarction. *Cardiology* **2013**, *125*, 18–30.
675
676

6. Figures

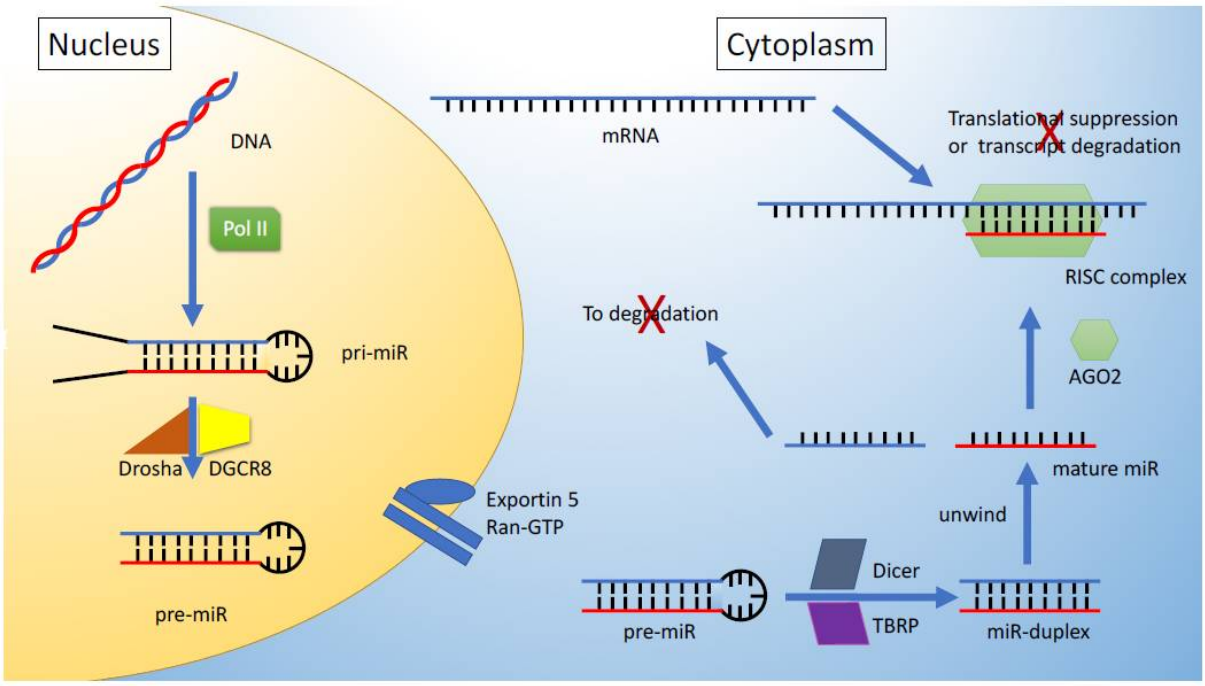

Figure 1. mRNA biogenesis. Transcription of pri-miR by pol II, followed by cleavage (Drosha/DGCR8 complex) to pre-miR. pre-miR is later exported to the cytoplasm via exportin-5/Ran-GTP, where now it can be further cleaved by Dicer/TRBP complex and unwind into a mature form. This mature form is further packed by AGO2 into the RISC complex (with mRNA). The consequence of such loading is either a transcriptional suppression or transcript degradation.

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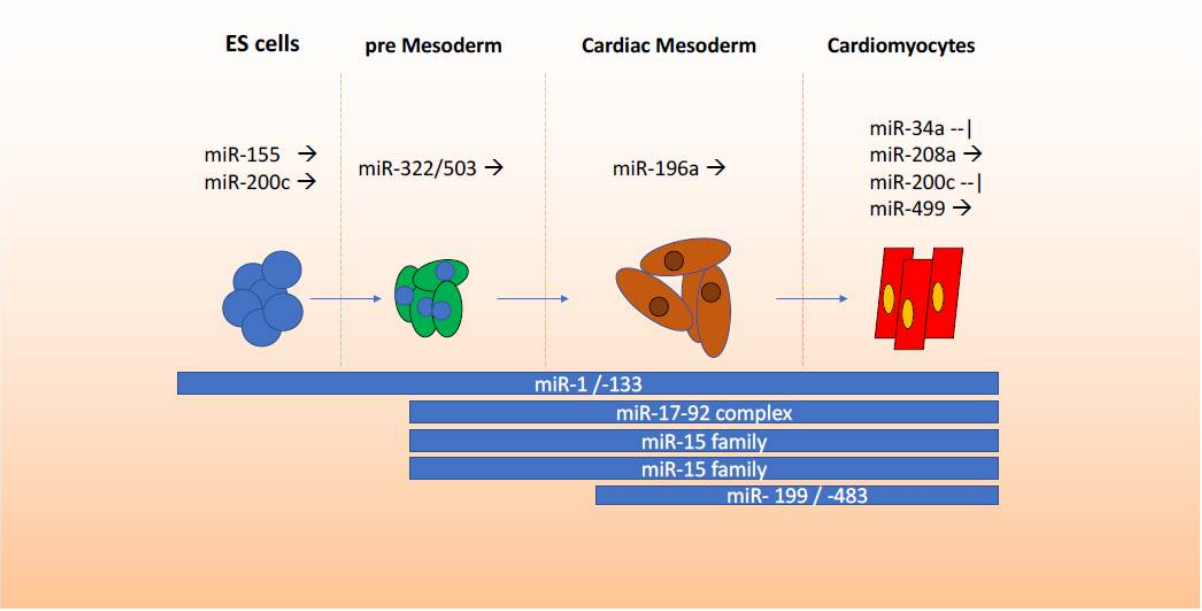

Figure 2. ES cells to cardiomyocytes. Left to Right, demonstrates the tight regulation of miRNA's during ES cell differentiation to cardiomyocytes including pre- and mesoderm stages. Top half show important activators and repressors at specific stages. Bottom show continuous miR activation during prolonged stages.

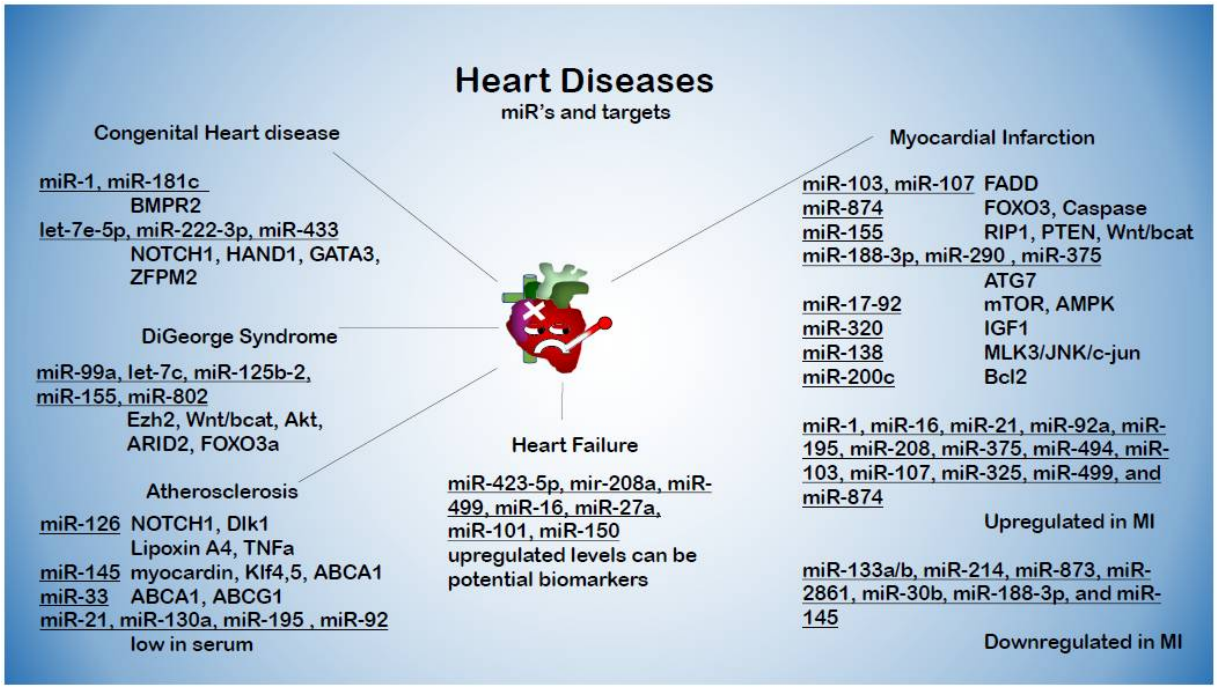

Figure 3. Major mRNA's and targets in Heart Diseases (Congenital Heart diseases, DiGeorge Syndrome, Atherosclerosis, Heart Failure, Myocardial Infarction, Biomarkers).

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694 7. **Tables**

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696 Table 1. Principal miRNA during Heart development and ES differentiation

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698 Supplemental Table 1. Principal miRNA dysregulation during Cardiovascular Diseases

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700 Supplemental Table 2. Principal miRNA dysregulation during Myocardial Infraction

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Table 1 Principal miRNA during Heart development and ES differentiation

MicroRNA	Regulation	Targets	References
miR -1/-133	Proliferation and muscle growth Signal mesoderm formation	SRF, MEF2c, MyoD, Hand2, Myocardin Twist	Tiang et al, 2017 Ivey et al., 2009
miR -1	Conduction Signaling	NCX1 Repression of HDAC4 Activation of MEF2	Tritsch et al., 2013 Ivey et al., 2009; Zhang et al., 2005; Zheng et al., 2013
miR-1-2	Repolarization	Kcnd2	Li et al., 2014; Mishima et al., 2007; Zhao et al., 2007
miR-15 family	Cell Cycle	Represion of HSP-20	Yan and Jiao, 2016
miR-17-92 complex	Second heart field Signaling Signaling	BMP signaling, SMAD repression Isl1, Tbx1 Represion of PTEN mTOR	Yan and Jiao, 2016 Small et al., 2010; Xu et al., 2017; Yan and Jiao, 2016) Danielson et al., 2013
miR-34a	Specification	NOTCH1, Dlk1, Jagged, Hey, Hes	Chaitra et al., 2013; Wu et al 2017
miR-155-3p	Regulates	MEF2c, KRAS (activate contractile factors)	Ivey et al., 2009; Zhang et al., 2005; Zheng et al., 2013
miR-196a	SHH patway during spetation	HOXB8	Hornstein et al., 2005; Mansfield et al., 2004
miR-208	Hypertrophy and muscle growth Myosin Heavy chain Conduction	a (208a), b (208b) GATA4, CX40	Callis et al., 2009; Xin et al., 2013
miR-199/-483	Signal mesoderm formation	Represion of Dlk-1	Ivey et al., 2009
miR-200c	Cardiac TF Conduction system	GATA4, TBX5, SRF CACNA1C, KCNJ2, SCN5A	Poon et al., 2018
miR322/503	Mesoderm formation	Celf1	Shen et al., 2016
miR-1/-499	Electrical/conduction	Upregulates Kir2.1, Kv1.4, HERG, and DHPR Downregulates HCN4	Fu et al., 2010; Lieu et al., 2013
miR-1/-133/-208/-499	Enahnced ES conversion		Christoforou et al., 2013; Shen et al., 2016; Xin et al., 2013; Jayawardena et al., 2013

Supplemental Table 1. Principal miRNA dysregulation during Cardiovascular Diseases

MicroRNA	Disease	Regulation	Targets	References
miR-1/181c	Congenital Heart Disease	Proliferation	BMPR2, SOX9, GJA1	Das et al., 2014; Li et al., 2013)
let-7e-5p, miR-222-3p, miR-433	Congenital Heart Disease	Morphogenesis	NOTCH1, HAND1, GATA3, ZFPM2	Li et al., 2014
miR-99a, let-7c, miR-125b-2, miR-155, miR-802	DiGeorge Syndrome			Zhao et al., 2017
miR-99a	DiGeorge Syndrome	Cardiogenic represion	Smarc5	Coppola et al., 2014
let-7c	DiGeorge Syndrome	Cardiogenic induction	Ezh2	Coppola et al., 2014
miR-155	DiGeorge Syndrome	Necrotic represion Tumor growth signaling Glioma targeting Proliferation	RIP1 ARID2 FOXO3a PTEN	Jablonska et al., 2015; Smith et al., 2015; Yan et al., 2016 Zhang et al., 2018 Ling et al., 2013 Xu et al., 2017
miR-126	Artherosclerosis	Proinflamatory response	Lipoxin A4, TNFa, NOTCH1, Dlk1	Codagnone et al., 2017
miR-155, let7c	Artherosclerosis / CAD	Proinflamatory response serves as Biomarker		Faccini et al., 2017; Jablonska et al., 2015; Ling et al., 2013; Vogelstein et al., 2013; Yan et al., 2016; Zhao et al., 2017
miR-145	Artherosclerosis	Targeting ACE NO Regulation Arthery volumn reduction	ACE enzyme Nitric Oxide myocardin, Klf4,5, ABCA1	Romaine et al., 2015 Faccini et al., 2017
miR-33	Artherosclerosis / dislipidemia	Control	ABCA1, ABCG1	Rayner et al., 2011, 2012
miR-21, miR-130a, miR-195 , miR-92	Artherosclerosis	Biomarkers	low in Serum	Yong and Jin, 2017
miR-423-5p, mir-208a, miR-499, miR-16, miR-27a, miR-101, miR-150	Heart Failure	Biomarkers	high in Serum	Yong and Jin, 2017

Supplemental Table 2. Prinicipal miRNA disregulation during Myocardial Infaction

MicroRNA	Regulation	Targets	References
miR-1, miR-133, miR-208, miR-499	Heavily upregulated		Corsten et al., 2010; Fu et al., 2011; Wang et al., 2010; Xin et al., 2013
miR-103, miR-107	Necrosis	FADD	Wang et al., 2016
miR-874	Necrosis	FOXO3a, Caspase	Wang et al., 2016
miR-155, miR-874	Necrosis	RIP1, PTEN, Wnt/bcat	Weber et al., 2018; Yang et al., 2018
miR-188-3p, miR-290, miR-375	Autophagy	ATG7	Weber et al., 2018; Yang et al., 2018
miR-17-92 complex	Autophagy	mTOR	Weber et al., 2018; Yang et al., 2018; Danielson et al., 2013
miR-212/132	Autophagy	AMPK sensing	Ling et al., 2013; Sermersheim et al., 2017); He et al., 2017
miR-320	Apoptosis	IGF1	Zhu et al., 2017
miR-138	Apoptosis	MLK3/JNK/c-jun	Zhu et al., 2017
miR-1, miR-16, miR-21,miR-92a, miR-195, miR-208, miR-375, miR-494, miR-103, miR-107, miR-325, miR-499, and miR-874	Biomarker upregulated		Sun et al., 2017
miR-133a/b, miR-214, miR-873, miR-2861, miR-30b, miR-188-3p, and miR-145	Biomarker downregulated		Sun et al., 2017