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TOPICAL REVIEW

# MicroRNAs in cardiovascular ageing

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**Abstract** MicroRNAs (miRs) have emerged as potent regulators of pathways in physiological and disease contexts. This review focuses on the role of miRs in ageing of the cardiovascular system. Several miRs have been described to be regulated during ageing and some of these miRs are involved in the regulation of ageing-related processes. We discuss the roles of miR-34, miR-217 and miR-29, which are induced during ageing in the vasculature. The roles of miR-34, miR-29 (age-induced) and miR-18/19, which are decreased during ageing in the heart, are discussed as well. Furthermore, numerous miRs that play a role in diseases associated with ageing, like diabetes, atherosclerosis, hypertension, cardiac hypertrophy and atrial fibrillation, are also briefly discussed. miRs also serve as circulating biomarkers for cardiovascular ageing or ageing-associated diseases. Finally, pharmacological modulation of ageing-related miRs might become a promising strategy to combat cardiovascular ageing in a clinical setting.

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## Introduction

MicroRNAs (miRs) belong to the rapidly growing family of non-coding RNAs with crucial and substantial regulatory functions in almost all cellular biological mechanisms. They act via RNA-mediated gene silencing through RNA interference-like pathways. The first microRNAs were described in the early 1990s regulating and timing larval development in the nematode *Caenorhabditis elegans* (Lee *et al.* 1993; Reinhart *et al.* 2000; Lee & Ambros, 2001). Since then, detailed insights have been gained into the biogenesis and regulation of microRNAs as well as specific roles in physiological regulatory mechanisms and dysregulation in pathophysiological conditions. To date, over 2500 miRs have been catalogued in humans in the latest release of the microRNA database miRBase, the majority of them by deep sequencing approaches and for many of these annotations, the functional importance has not yet been evaluated (Kozomara & Griffiths-Jones, 2014).

MicroRNAs are small, ~22 nucleotides in length, single stranded RNA molecules. By binding to their target mRNAs, miRs induce translational repression, mRNA deadenylation and mRNA decay (Huntzinger & Izaurralde, 2011). Predominantly, miR-binding sites are located in the 3′ untranslated region (UTR) of their

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target mRNAs (Bartel, 2009). Target recognition is mainly driven by the miR 'seed sequence', a domain at the 5′ end reaching from nucleotide 2 to nucleotide 7 of the mature miR, and miRs with identical seed sequences belong to the same 'miR family' (Bartel, 2009). In the majority of target–miR interactions, the seed region binds perfectly complementarily, while the downstream nucleotides also contribute to base pairing but with varying numbers of mismatches (Fig. 1*A*). The 3′ UTR of mRNAs can contain binding sites for different miRs and furthermore, due to the variations in binding of the nucleotides downstream of the seed region, one miR can target several hundred mRNAs (Fig. 1*A*). Through these highly diversified binding interactions miRs can 'fine tune' entire signalling cascades and gene networks (Bartel, 2009).

## MicroRNA biology

MicroRNAs encoded in the genomic DNA are mainly intergenic being located in the non-coding regions between genes and transcribed by often unidentified promoters. However, specific miRs have been found to be transcribed from intronic regions (Lin *et al.* 2006; van Rooij *et al.* 2007). miRs are transcribed by RNA polymerase II (Pol II) and controlled by RNA Pol II-associated transcription factors and epigenetic regulators as a long (>1 kb) primary RNA transcript (pri-miR) (Lee *et al.* 2004). The pri-miR forms a characteristic stem–loop structure and has the miR embedded in its stem (Fig. 1*B*) (Ha & Kim, 2014). Each precursor miR contains two mature miRs, one in its 5′ and one in its 3′ strand (for

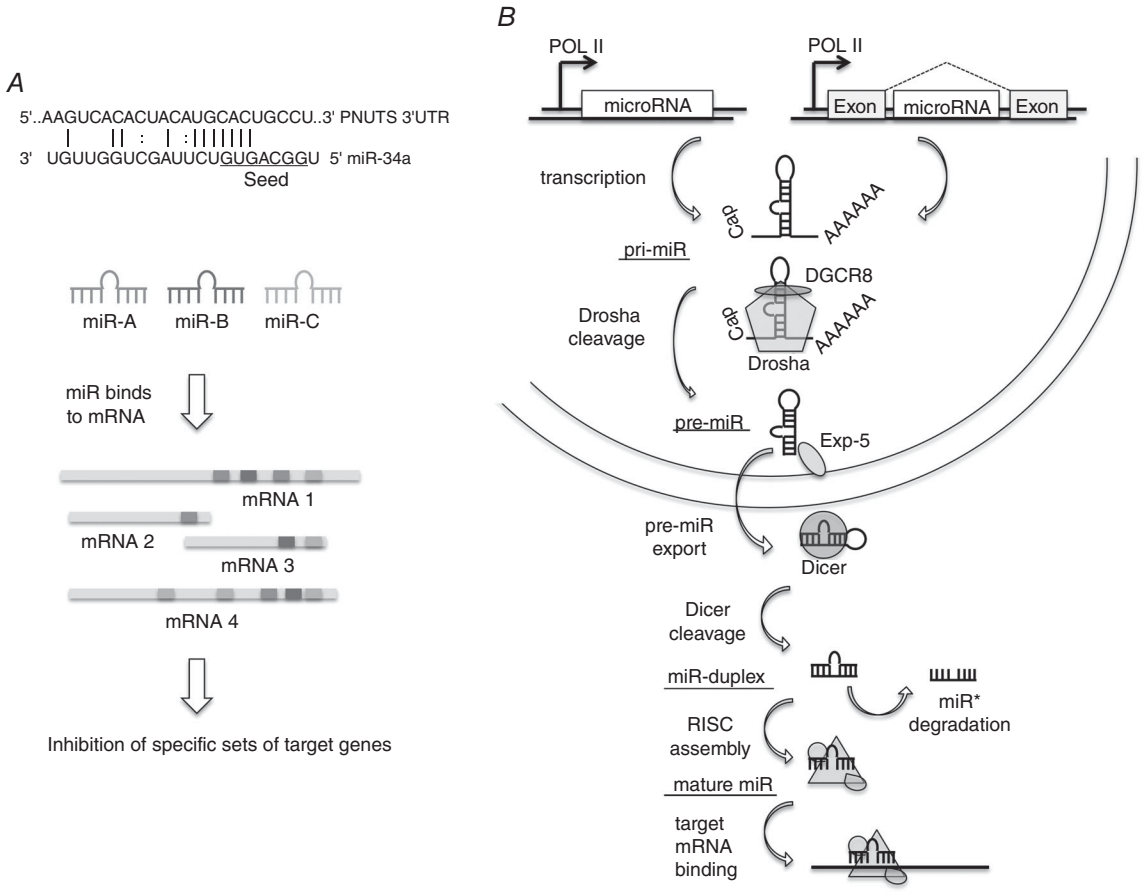


**Figure 1. MicroRNA binding and biogenesis**
*A*, microRNAs (miRs) bind to target mRNAs mainly in a partially complementary fashion. Nucleotides 2–7 of the miR are referred to as a seed sequence and these nucleotides bind fully complementarily to the target sequence, whereas the other nucleotides of the miR only show interspersed complementarity as shown for miR-34a and its target PNUTS (Boon *et al.* 2013). Different miRs (depicted by various grey tones) bind to a specific set of mRNA targets and these sets may be overlapping. One mRNA can be bound by multiple different miRs or even multiple times by the same miR. Each miR targets tens to hundreds of mRNAs, thereby coordinating complex regulatory networks. *B*, miRs are transcribed from the genome either from intergenic regions or within introns of genes. The primary miR transcript (pri-miR) is processed by Drosha in association with DGCR8 (DiGeorge critical region 8), yielding a precursor miR (pre-miR) that is exported by exportin 5 (Exp-5). Pre-miRs are cleaved by Dicer in the cytoplasm and one of the strands from the resulting miR duplex is incorporated in the RNA-induced silencing complex (RISC) that binds to target mRNAs, thereby repressing expression levels.

example miR-34a-3p and miR-34-5p). In most cases, one is usually biologically more prevalent and is called the guide strand. The other is called the passenger strand or miR* and is mostly degraded. However, many examples also exist where the passenger strand is stable and functions as a miR as well.

In the nucleus, the pri-miR is processed by the nuclear RNase III Drosha and its essential co-factor DGCR8 (named by its implication in a genetic disorder called DiGeorge syndrome) together forming the microprocessor complex (Lee *et al.* 2003; Denli *et al.* 2004; Gregory *et al.* 2004). Recently, it has been shown that methyltransferase-like 3 (METTL3) methylates pri-miRNAs, marking them for recognition and processing by DGCR8 (Alarcón *et al.* 2015). After binding of the microprocessor to the pri-miR, the single-stranded 5′ and 3′ arms are cut releasing the pre-miR, a short hairpin-like RNA with around 65 nucleotides in length. Deficiency of Drosha in the germline is embryonically lethal (Chong *et al.* 2010). Further, cardiac specific deletion of DGCR8 in mice leads to heart failure and dilated cardiomyopathy (DCM; Rao *et al.* 2009), highlighting the importance of miRs in cardiovascular homeostasis.

After nuclear processing by Drosha, the pre-miR is exported from the nucleus for further maturation in the cytoplasm. In a complex with the protein exportin-5 and GTP-binding nuclear protein RanGTP the pre-miR passes the nuclear core complex and is released into the cytoplasm (Bohnsack *et al.* 2004; Lund *et al.* 2004). After export, the pre-miR is cleaved by Dicer, an RNase III-type endonuclease, close to the terminal loop and a small RNA duplex containing miR/miR* is released (Bernstein *et al.* 2001; Hutvágner *et al.* 2001). Deletion of Dicer in the mouse germline is embryonically lethal (Bernstein *et al.* 2003). The cardiomyocyte specific deletion of Dicer also rapidly leads to DCM and heart failure (Chen *et al.* 2008).

Together with Argonaute proteins (human AGO1–4) the miR duplex forms the RNA-induced silencing complex (RISC) which mediates all RNA-silencing pathways (Liu *et al.* 2004). By removing the passenger strand the pre-RISC turns into the mature RISC. The determination of the biologically active guide strand and the passenger strand, which is degraded quickly after release, is mainly dependent on the thermostability of the two ends of the RNA duplex (Schwarz *et al.* 2003).

Recently it has been shown that miRs may also act in the nucleus, even though miR-loading into RISC only takes place in the cytoplasm (Gagnon *et al.* 2014). In this review we discuss the importance of miR-mediated regulation of processes that are relevant for cardiovascular ageing. For an overview of miRs in cardiovascular biology in general, we refer to Quiat & Olson (2013), Boon & Dimmeler (2014), Greco *et al.* (2015), Schober *et al.* (2015) and Wronska *et al.* (2015).

## Vascular ageing

Vascular ageing is characterized by detrimental effects in most cell types found in the vessel wall. These include changes in endothelial cells, smooth muscle cells and inflammatory cells. A few miRs have been described to regulate ageing-related processes in these cells. One of the most described ageing-induced miRs, miR-34a, has been shown to regulate ageing-related processes such as senescence in most of these cells (as well as in the heart, mentioned below). miR-34a was first discovered in the context of cancer (He *et al.* 2007; Tarasov *et al.* 2007), where it was found to be induced by p53 and to regulate apoptosis (Hermeking, 2012). That miR-34a is also important in vascular biology became clear with the study by Ito and colleagues (Ito *et al.* 2010) which showed that miR-34a regulates the histone deacetylase silent mating-type information regulation 2 homologue (SIRT1) in endothelial cells (see also Tabuchi *et al.* 2012). As SIRT1 acts as a longevity promoting factor (Haigis & Guarente, 2006), this mechanism contributes to the endothelial senescence-inducing effects of miR-34a. Ageing also induces miR-34a expression in smooth muscle cells (Badi *et al.* 2014), where a reduction in SIRT1 likewise results in an increase in senescence and secretion of inflammatory factors. Like all miRs, miR-34a also has multiple target genes and it is likely that additional target genes beyond SIRT1 are involved in inducing senescence and ageing in the vasculature (Fig. 2, Table 1).

Other miRs that are involved in senescence and ageing of endothelial and smooth muscle cells include miR-217 and miR-29. The former was also shown to regulate the expression of SIRT1, thereby promoting endothelial ageing (Menghini *et al.* 2009). miR-29 was found to be induced during ageing and play a role in aneurysm formation (Boon *et al.* 2011). Ageing is the major risk factor for the development of aneurysms, the pathological widening of large arteries, which greatly increases the risk of rupture of the artery with a very high mortality rate. miR-29 regulates the expression of extracellular matrix proteins and thereby reduces the structural integrity of the vessel wall allowing aneurysm formation to take place (Boon *et al.* 2011; Maegdefessel *et al.* 2012).

Several other miRs have been described to be involved in disease processes that are associated with ageing; however, a direct role in ageing has not been firmly established. Many miRs were shown to affect atherosclerosis formation, which is reviewed elsewhere (Kumar *et al.* 2014; Menghini *et al.* 2014; Schober *et al.* 2015). Apoptosis in vascular cells, which is induced during ageing, is also regulated by miRs (Quintavalle *et al.* 2011). Ageing is a strong risk factor for arterial hypertension, partly via the $\beta$-adrenergic system, which is regulated by miRs as well (reviewed in Bátkai & Thum, 2012; Ling *et al.* 2013*a*). Furthermore, single nucleotide polymorphisms

(SNPs) linked to arterial hypertension have been found in miR-binding sites of genes of the renin–angiotensin system (Nossent *et al.* 2011). Finally, ageing is associated with an increase in diabetes mellitus and metabolic syndrome. The role of miRs in these age-associated diseases is reviewed in Fernández-Hernando *et al.* (2013), Paneni *et al.* (2013) and Beltrami *et al.* (2014).

One of the cellular mechanisms that causes ageing is oxidative stress. miR-200 and miR-210 have been described to regulate mitochondrial function and oxidative stress in the vasculature (for review, see Magenta *et al.* 2013). The oxidative stress response of endothelial cells includes expression changes in miRs as well, i.e. the upregulation of miR-92a, which induces endothelial dysfunction (Chen *et al.* 2015). Interestingly, one can also exploit the cell-type enriched expression patterns of miRs to specifically target certain cell types in the vasculature by including miR binding sites in the overexpression construct for miRs that are highly expressed in the tissue one does not want to target (so-called detargeting). The endothelial-enriched expression of miR-126 was used to specifically detarget an adenoviral construct to overexpress p27 in vascular smooth muscle cells in the context of restenosis (Santulli *et al.* 2014*b*).

## Cardiac ageing

Ageing affects cardiac function in multiple manners. The most common age-induced cardiac disease is diastolic dysfunction, also termed heart failure with preserved ejection fraction (Loffredo *et al.* 2014). This is caused by increased stiffness and fibrosis of the myocardium and is associated with endothelial dysfunction (Paulus & Tschöpe, 2013). Ageing-induced changes in virtually all cell types in the heart contribute to these processes and several miRs were described to play a role in cardiac ageing.

In cardiomyocytes, the main ageing-regulated miR that has been described is miR-34. The miR-34 family consists of miR-34a, miR-34b and miR-34c. All these family members are induced during ageing (Boon *et al.* 2013). miR-34a is the most highly expressed miR-34 family member in cardiomyocytes and the increased miR-34a expression in the aged heart is probably due to an increase in p53 signalling, known to be induced in ageing. miR-34 family members induce apoptosis during ageing, but also after acute myocardial infarction (Bernardo *et al.* 2012; Boon *et al.* 2013). Next to SIRT1, miR-34a directly inhibits the expression of several other target genes, including *POFUT1*, *BCL6*, *SEMA4b* (Bernardo *et al.* 2012) and *PNUTS* (Boon *et al.* 2013), thereby affecting cardiomyocyte apoptosis and heart function. Interestingly, miR-34a is also induced in a genetic model for cardiac ageing (calstabin-2 null mice) (Yuan *et al.* 2014) and integrated network analysis also confirmed a central role for miR-34a in cardiac ageing (Dimitrakopoulou *et al.* 2015) (Fig. 2, Table 1).

MicroRNAs that are present in cardiac fibroblasts and are regulated during ageing appear to have a link to fibrosis, which is known to be induced during ageing. miR-18 and miR-19, which are expressed from the same primary cluster, miR-17-92, are reduced in aged mouse hearts (van Almen *et al.* 2011). Connective tissue growth factor (CTGF) and thrombospondin-1 (TSP-1) are the

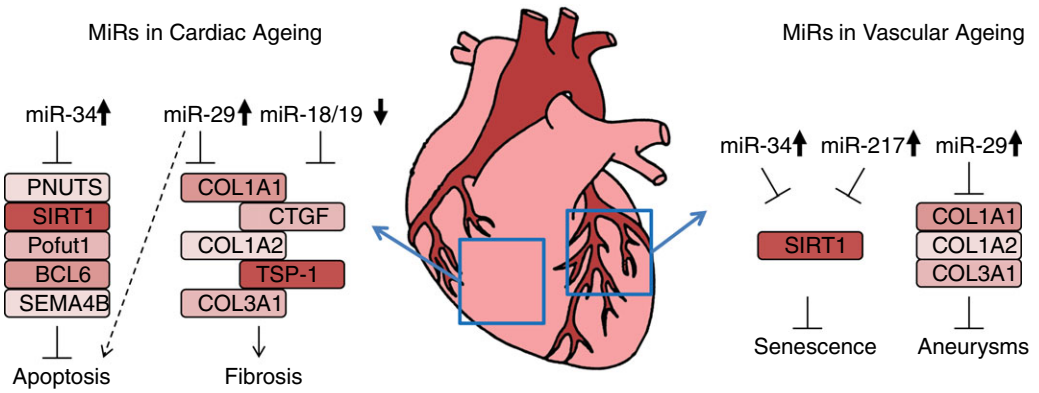


**Figure 2. Several miRs are involved in cardiac ageing and in vascular ageing**
miRs that are involved in cardiac ageing include miR-34, miR-29, miR-18 and miR-19. miR-34 is upregulated during ageing and induces apoptosis by inhibiting expression of anti-apoptotic genes. miR-29 is also upregulated and induces apoptosis, but counteracts fibrosis via suppression of extracellular matrix components. miR-18 and miR-19 are down-regulated during ageing and normally inhibit pro-fibrotic genes, thereby facilitating fibrosis. miR-34, miR-217 and miR-29 are involved in vascular ageing. miR-34 and miR-217 are upregulated in endothelium and cause senescence via repression of SIRT1. miR-29 is upregulated in smooth muscle cells, where it inhibits synthesis of extracellular matrix components, which contributes to aneurysm formation.

**Table 1. MicroRNAs that are regulated during ageing and/or ageing-associated diseases**

| MicroRNA | Associated disease | References |
|---|---|---|
| miR-1 | Atrial fibrillation, mitochondrial function | (Girmatsion *et al.* 2009; Zhang *et al.* 2014) |
| miR-133 | Cardiac hypertrophy | (Carè *et al.* 2007) |
| miR-144 | Mitochondrial function | (Csiszar *et al.* 2014) |
| miR-15 | Cardiac hypertrophy, atrial fibrillation | (Nishi *et al.* 2010; Tijsen *et al.* 2014) |
| miR-18 | Cardiac fibrosis | (van Almen *et al.* 2011) |
| miR-181 | Mitochondrial function, immunosenescence | (Das *et al.* 2012; Seeger *et al.* 2013) |
| miR-19 | Cardiac fibrosis | (van Almen *et al.* 2011) |
| miR-199 | Cardiac hypertrophy | (da Costa Martins *et al.* 2010) |
| miR-208 | Cardiac hypertrophy | (van Rooij *et al.* 2007) |
| miR-21 | Cardiac hypertrophy, cardiac fibrosis | (Thum *et al.* 2008; Patrick *et al.* 2010; Bang *et al.* 2014) |
| miR-214 | Mitochondrial function | (el Azzouzi *et al.* 2013) |
| miR-217 | Senescence | (Menghini *et al.* 2009) |
| miR-22 | Cardiac fibrosis, senescence, cardiac hypertrophy | (Huang *et al.* 2013; Jazbutyte *et al.* 2013) |
| miR-25 | Cardiac hypertrophy | (Dirkx *et al.* 2013) |
| miR-26 | Atrial fibrillation | (Luo *et al.* 2013) |
| miR-29 | Cardiac fibrosis, apoptosis, aortic aneurysms, atrial fibrillation | (van Rooij *et al.* 2008*b*; Ye *et al.* 2010; Boon *et al.* 2011; Maegdefessel *et al.* 2012; Dawson *et al.* 2013; Abonnenc *et al.* 2013) |
| miR-30 | Cardiac hypertrophy | (Wijnen *et al.* 2014) |
| miR-328 | Atrial fibrillation | (Lu *et al.* 2010) |
| miR-34 | Senescence, apoptosis, telomere attrition, cardiac hypertrophy | (Ito *et al.* 2010; Bernardo *et al.* 2012; Tabuchi *et al.* 2012; Boon *et al.* 2013; Yuan *et al.* 2014; Badi *et al.* 2014; Dimitrakopoulou *et al.* 2015) |
| miR-378 | Cardiac hypertrophy | (Ganesan *et al.* 2013) |
| miR-451 | Cardiac hypertrophy | (Kuwabara *et al.* 2015) |
| miR-499 | Atrial fibrillation, mitochondrial function | (Wang *et al.* 2011; Ling *et al.* 2013*b*) |
| miR-92 | Endothelial dysfunction | (Chen *et al.* 2015) |

main pro-fibrotic targets of these miRs and a reduction of miR-18 and miR-19 during ageing contributes to the increased expression of CTGF and TSP-1, resulting in increased fibrosis and a decline in heart function. Interestingly, the fibrosis-inhibiting miR-29 is also increased in the heart during ageing (Boon *et al.* 2013) and even though exogenous miR-29 reduces fibrosis (van Rooij *et al.* 2008*b*; Abonnenc *et al.* 2013), the endogenous induction does not seem to be able to prevent fibrosis during ageing. A possible mechanism could be the contribution of miR-29 to apoptosis of cardiomyocytes during ageing, since inhibition of miR-29 was described as preventing ischaemia-induced cardiomyocyte apoptosis (Ye *et al.* 2010). Finally, miR-22 has also been shown to be induced during ageing in the mouse heart, where it induces fibroblast migration and senescence that contribute to fibrosis in ageing (Jazbutyte *et al.* 2013).

Several other miRs have been described to play a role in processes related to cardiac ageing or ageing in general. We will briefly discuss these miRs here. miRs that are involved in heart failure in general are reviewed in Tritsch *et al.* (2013) and Zhuo *et al.* (2014). Further, the contribution of miRs to clinical management of heart failure was recently described (Sardu *et al.* 2014). Control of cardiac hypertrophy and fibrosis, which are also induced during ageing, by miRs was shown for miR-133 (Carè *et al.* 2007), miR-21 (Thum *et al.* 2008; Patrick *et al.* 2010; Bang *et al.* 2014), miR-208 (van Rooij *et al.* 2007), miR-15 (Tijsen *et al.* 2014), miR-25 (Dirkx *et al.* 2013), miR-199 (da Costa Martins *et al.* 2010), miR-22 (Huang *et al.* 2013), miR-451 (Kuwabara *et al.* 2015), miR-378 (Ganesan *et al.* 2013) and miR-30 (Wijnen *et al.* 2014). Ageing also induces the prevalence of atrial fibrillation and several miRs have been described in the context of atrial fibrillation: miR-1 (Girmatsion *et al.* 2009), miR-26 (Luo *et al.* 2013), miR-29 (Dawson *et al.* 2013), miR-328 (Lu *et al.* 2010) and miR-499 (Ling *et al.* 2013*b*), reviewed in Santulli *et al.* (2014*a*). Increased oxidative stress in the heart and impaired mitochondrial functional are hallmarks of cardiac ageing. Several miRs have been identified to control mitochondrial function and oxidative stress in the heart that may contribute to cardiac ageing: miR-1 (Zhang *et al.* 2014), miR-144 (Csiszar *et al.* 2014), miR-199 and miR-214 (el Azzouzi *et al.* 2013), miR-181 (Das *et al.* 2012), miR-499 (Wang *et al.* 2011) and miR-15 (Nishi *et al.* 2010).

## Clinical outlook

The development of miRNA therapeutics has gained considerable momentum over the past years. Inhibition of miRs with anti-miRs that sterically block the specific miR seems most promising and has been pioneered by anti-miRs against miR-122 to treat hepatitis C (Lanford *et al.* 2010). Phase II clinical trials using these anti-miRs (called miravirsen) are very promising (Janssen *et al.* 2013). There are no clinical trials reported to date with anti-miRs targeting cardiovascular disease, but inhibition of many miRs was shown to be therapeutically beneficial in mouse models (van Rooij *et al.* 2008*a*) or even in large animal models (Hinkel *et al.* 2013). Several of the ageing-related miRs discussed in this review may also prove promising therapeutic targets. For instance, inhibition of miR-34a in the myocardium may prevent or even ameliorate age-induced cardiac dysfunction. One should, however, be very careful in choosing delivery strategies for the anti-miRs, as many miRs are expressed in a variety of cell types and may have dissimilar roles in different cells. The pro-apoptotic miR-34a would be an interesting target to prevent cardiomyocyte apoptosis, but inhibition of miR-34a may simultaneously induce tumorigenesis (Hermeking, 2010). In fact, delivery of miR-34a mimics is being developed as treatment for liver cancer (clinical trial number NCT01829971).

Even though anti-miR chemistries appeared to be safe and well-tolerated in the clinical trials performed so far, a recent study showed that the phosphorothioate-modified RNA backbone used in anti-miRs can induce platelet aggregation via activation of glycoprotein VI on platelets (Flierl *et al.* 2015). However, most anti-miRs are only 16 nucleotides long and therefore too small to facilitate glycoprotein VI dimerization and subsequent platelet aggregation (Flierl *et al.* 2015).

Finally, miRs may serve as biomarkers of cardiovascular ageing. Many miRs have been proposed as biomarkers for cardiovascular disease (Fichtlscherer *et al.* 2011; Zampetaki *et al.* 2012; Watson *et al.* 2015), but only a few circulating miRs reflect cardiovascular ageing. Reduced expression of miR-181c in the peripheral blood was shown to be associated with ageing and chronic heart failure as well as immunosenescence (Seeger *et al.* 2013), circulating miR-34a was shown to correlate with age in mice (Li *et al.* 2011), and plasma levels of miR-21 are increased with ageing (Olivieri *et al.* 2012). Furthermore, several reports describe differential levels of circulating miRs in centenarians, compared to young control subjects (Noren Hooten *et al.* 2010; Gombar *et al.* 2012; Serna *et al.* 2012; Meder *et al.* 2014). As these miRs are not cardiovascular specific, the levels do not necessarily reflect cardiovascular disease but may help in risk stratification as well as in monitoring disease progression. For example, miRs found in blood plasma can function as biomarkers for diabetes mellitus (Guay & Regazzi, 2013). Circulating miRs that specifically reflect cardiovascular ageing or might emerge to be direct therapeutic targets are still elusive.

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## Additional information

### Competing interests

The authors have no conflicts of interest to declare.

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