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Review Article

MicroRNAs in ischemia-reperfusion injury

Jakob BW Weiss[1,2], Steffen U Eisenhardt[2], G Björn Stark[2], Christoph Bode[1], Martin Moser[1], Sebastian Grundmann[1]

[1]Department of Internal Medicine III, [2]Department of Plastic and Hand Surgery, University Hospital Freiburg, Germany

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Abstract: Ischemia-reperfusion injury (IRI) is a major causal factor of tissue injury in various clinical settings including myocardial infarction, stroke, and free microsurgical tissue transfer. MicroRNAs (miRNAs, miRs) are short, non-coding RNA molecules involved in post-transcriptional regulation of gene expression. During the last years they have emerged as regulators of IRI as well as ischemic preconditioning and ischemic postconditioning. Here we give an overview of the current literature and describe the potential use of miRNA-based therapeutics for the treatment and prevention of ischemia-reperfusion injury in the future.

Keywords: MicroRNA, ischemia-reperfusion injury, inflammation, acute myocardial infarction, revascularization

Introduction

MicroRNAs

During the last decade, miRNAs have emerged as important regulators of gene expression in different biological processes and were found in over 160 organisms. MiRNAs are short, non-coding RNA molecules of 21-23 nucleotides length, that are currently under intensive investigation as a novel regulatory mechanism of gene expression in a wide range of species. At present, 1527 human and 741 murine miRNAs have been described [74], many of them being highly conserved in either exonic or intronic regions of the genome. After transcription as long pri-miRs by RNA polymerase 2, miRNAs undergo several processing steps towards the mature miRNA. Following cleavage by the endonuclease Drosha and cofactor DGCR8 to pre-miRs in the nucleus [1, 2], Exportin-5 transports pre-miRNAs across the nuclear membrane [3]. The final step of maturation is carried out by the nuclease Dicer in the cytoplasm, which processes the pre-miR to mature miRNA. To work effectively, mature miRNAs have to be introduced into the RNA -induced silencing complex (RISC), where they are able to bind to a specific target sequence often located in the 3'UTR of messengerRNAs (mRNAs) in a partly complementary way. This leads to either translational repression or degradation of the mRNA, resulting in inhibition of protein expression [4-6]. Most miRNAs are eventually found in the cytoplasm, but some are shuttled back into the nucleus, e.g. miR-29b. This transport from the cytoplasm to the nucleus is mediated by a hexanucleotide terminal motif in these miRNAs. Additionally, miRNAs are able to leave the cell within exosomes and can even be exchanged between cells this way [7] (See **Figure 1**).

Most interestingly, one miRNA is able to regulate several different mRNAs and at the same time one mRNA can be regulated by several miRNAs [8]. Thereby miRNAs represent a complex mechanism of genetic regulation that can modulate biological processes at different levels.

It is estimated that more than 30% of all genes are regulated by miRNAs [9-10]. In summary, the discovery of miRNAs exposed an entirely new aspect of the regulation of protein expression [11] and they can be considered a cellular mechanism of short or long acting fine tuning of

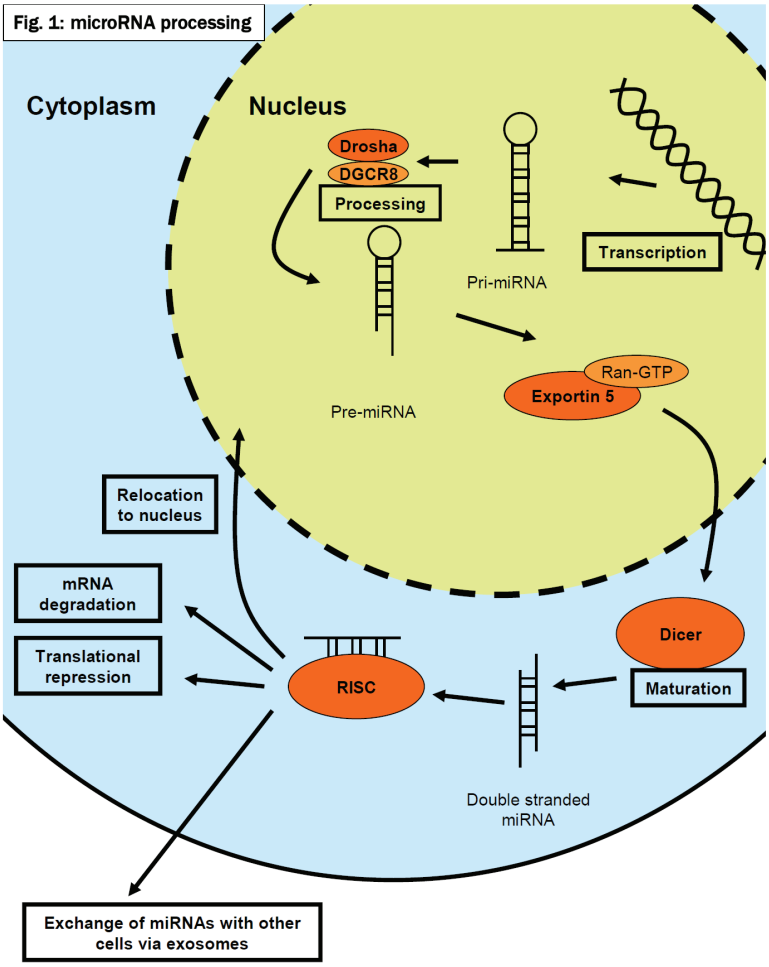

Figure 1. microRNA processing.

post-transcriptional gene regulation.

Ischemia reperfusion injury

Ischemia reperfusion injury (IRI) is of importance in every clinical setting in which blood flow is restored after a critical time of ischemia because it is a relevant cause of postischemic tissue damage. In the past decades, interventional or pharmaceutical revascularization in stroke or myocardial infarction has become a routine procedure in many hospitals and the prognosis of these patients has greatly improved. However, 6% of the patients admitted to the hospital with acute coronary syndrome do not survive [12]. Also stroke remains one of the top causes of mortality and disability in the world [13]. Hence, therapy of diseases related to acute ischemia should not only focus on restoration of blood flow, but also on the inflammatory sequelae of reperfusion.

Ischemia is followed by hypoxia which leads to accumulation of metabolites, increased permeability of the cell membrane [14-16], vascular leakage [17], subsequent interstitial edema [18] and endothelial dysfunction [19].

The following reperfusion is associated with an inflammatory response. Circulating leukocytes, coming to the post-ischemic tissue with the restored blood flow get activated by interaction with the damaged endothelium [17] and by generation of inflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin 1 (IL-1), interleukin 6 (IL-6) and interferon γ (IFNγ) [20, 21] as well as complement factor C5a [22, 23]. Rolling and adhesion of leukocytes in the postcapillary venules inhibit the re-established blood flow [18].

Reactive oxygen species (ROS) produced in the "respiratory burst "of activated neutrophile granulocytes [20] are regarded as the main cause of tissue damage in IRI. H_2O_2, O_2^- and OH^- are the most common radicals. They cause damage to cell membranes, protein structures and nucleic acids due to their aggressive chemical features. Hence, reperfusion initially aggravates the tissue and vascular damage and interstitial edema formation caused by ischemia. This eventually can lead to a "no-reflow" phenomenon, as the microcirculation can be impaired by the interstitial edema.

MicroRNAs in Ischemia Reperfusion Injury

Cardiac IRI

Acute myocardial infarction (MI) still is one of the most common causes of death throughout the industrialized nations. In the United States, coronary heart disease, mostly myocardial infarction, is responsible for approximately one

Table 1. Apoptotic effects of miRNAs in IRI

Pro-apoptotic miRs	reference	Anti-apoptotic miRs	reference
miR-1 (heart)*	[28]	miR-1 (heart)*	[31]
miR-29 (heart)	[39]	miR-21 (heart, kidney)	[36, 62]
miR-145 (brain)	[65]	miR-133 (heart)	[31]
miR-320 (heart)	[45-46]	miR-144 (heart)	[41]
		miR-451 (heart)	[41]
		miR-494 (heart)	[47]

*Contradictions may be due to different study protocols

Table 2. Regulation of miRNAs in IRI

Down-regulated	reference	Up-regulated	reference
HEART		*HEART*	
miR-1**	[31]	miR-1**	[28, 30]
miR-133	[31]	miR-494***	[47]
miR-320	[45]	miR-21****	[36-37]
miR-494***	[47]		
miR-21****	[36]		
KIDNEY		*KIDNEY*	
miR-21	[62]	miR-805	[62]
miR-20a	[62]	miR-194	[62]
miR-146a	[62]	miR-192*****	[62]
miR-199a-3p	[62]	miR-187	[62]
miR-214	[62]		
miR-192*****	[62]		
miR-187	[62]		
BRAIN		*BRAIN*	
miR-290	[65]	miR137	[65]
miR-145	[65]	miR27a	[65]
miR-26	[65]	miR18	[65]
SKELETAL MUSCLE			
miR-21	[57]		
miR-200c	[57]		
miR-205	[57]		

** Contradiction may be a result of different study protocols; *** Up- or down-regulation of miR-494 depends on the time point of measurement; **** Up- or down-regulation depends on the location in the infarct area; ***** MiR-192 and miR-186 are up-regulated initially, then down-regulated.

out of three deaths [24]. IRI is of critical importance in this patient population because even patients that are undergoing percutaneous coronary intervention (PCI) or intravenous thrombolytic therapy might not profit adequately from this therapy due to the "no-reflow" phenomenon caused by extensive IRI [25-27]. Hence, it is of great interest to further understand the pathomechanism involved.

The heart was one of the first organs in which miRNAs were investigated in the context of IRI. Also, several studies have shown the involvement of miRNAs in ischemic preconditioning (IPC), and in ischemic postconditioning (IPost), as well as in heat shock preconditioning. For an overview of the differential regulations and effects of miRNAs in IRI, see **Tables 1** and **2**.

miR-1

One of the first miRNAs investigated in the context of cardiac IRI was miR-1. It was found to be up-regulated in rat hearts undergoing 60 minutes of ischemia and 24 hours of reperfusion [28]. Similar results were obtained in pig hearts

undergoing IRI, with a peak of expression at 120 minutes of reperfusion [29], as well as in STEMI patients undergoing primary percutaneous coronary intervention [30]. On the contrary, another group found this miRNA to be down-regulated by IRI in rat hearts after 30 minutes of coronary artery occlusion and 180 minutes of reperfusion [31]. These controversial results might be due to different study protocols and time points of measurement. MiR-1 was also found to be up-regulated in IPC [32], IPost [31] and heat shock preconditioning [33]. In IPost, the restored blood flow after ischemia is repeatedly disrupted by short periods of ischemia. It is able to protect the rat heart against the sequelae of IRI by a decrease of the apoptosis rate in cardiomyocytes *in vivo* and thereby reducing infarct size [1, 31].

Following these expressional profiling studies, Tang *et al.* showed that over-expression of miR-1 can decrease and antimiRNA-1 oligonucleotides (AMO-1) can increase cell viability in cultured rat cardiomyocytes exposed to H_2O_2 [28]. In contrast, the apoptosis rate of rat cardiomyocytes treated with oligonucleotides representing the base sequence of miR-1 (miR mimics) before simulated IRI was lower compared to the control group, whereas it was higher after treatment with AMO-1 in the studies of He *et al.* [31]. This could possibly be due to a direct regulation of the anti-apoptotic factor Bcl-2 by miR-1 in cardiomyocytes [28]. In summary, these data suggest miR-1 as an anti-apoptotic factor in cardiac IRI.

miR-21

One of the miRNAs that is well described in the context of IRI is miR- 21. According to Roy *et al.* miR-21 is up-regulated in the area of myocardial infarction in mice undergoing an IRI protocol. Knockdown of miR-21 in cultured cardiac fibroblasts leads to increased expression levels of phosphatase and tensin homologue (PTEN), whereas application of miR-21 mimicking oligos leads to down-regulation of PTEN. This phosphatase seems to play a role in cardiac IRI, IPC and cardiac fibrosis [34, 35]. MiR-21 directly targets the 3'UTR of PTEN [36] and inhibition of this phosphatase results in up-regulation of fibroblast metalloprotease-2 (MMP-2) [37], which contributes to cardiac dysfunction after IRI [38]. Although these data suggest a deleterious effect of miR-21 in cardiac IRI, this does not seem to be the case *in vivo*. MiR-21 over-expressing transgenic mice show a significantly smaller infarct area than WT mice when subjected to cardiac IRI. Interestingly, miR-21 is down-regulated in core ischemic region of the mouse hearts. At the same time, the miR-21 level is increased in the border zone [36]. The up-regulation of miR-21 in IRI seems to be a consequence of reperfusion or reparation and not of ischemia.

miR-29a and miR-29c

Rats treated with antimiR-29a and antimiR-29c show a decrease in infarct size and less apoptosis in the myocardium of mice undergoing cardiac IRI. Additionally, the IRI-induced increase of the pro-apoptotic protein Bax is attenuated by these antimiRs. In cultured H9c2 cardiac muscle cells undergoing simulated IRI, these antimiRs increase the expression of Mcl-1, which is a member of the anti-apoptotic Bcl-2 family. These data suggest miR-29a and miR-29c to be pro-apoptotic by suppressing anti-apoptotic gene products and the expression of Bax.

Interestingly, application of the PPAR-γ agonist pioglitazone decreases the expression levels of miR-29a and miR-29c in the rat heart [39]. Modulation of miRNA expression and in particular down-regulation of these pro-apoptotic miRNAs seem to be an additional mode of action of these pharmaceutical drugs, which are widely used in the treatment of type 2 diabetes [40].

miR-133a

Another miRNA with anti-apoptotic properties is miR-133a, which is down-regulated in IRI, but up-regulated by ischemic post-conditioning. PCR analysis shows that the pro-apoptotic factors Bax and Caspase9 are up-regulated by IRI. On the other hand, the anti-apoptotic Bcl-2 is down-regulated and IPost inverses these effects.

In vivo, pre-treatment of rat hearts with antimiR-133a increases the apoptosis rate induced by IRI along with an increased activity of caspase-9, both of which are decreased by application of miR-133a mimics. Similar effects are found *in vitro,* where treatment of cardiomyocytes with antimiR-133a increases the apoptotic rate and a mimic of miR-133a has a cytoprotective effect

in simulated IRI [31].

miR-144/451

MiR-144 and miR-451 are two clustered miRNAs that are transcribed from the same gene locus on chromosome 17. Over-expression of either of them decreases cardiomyocyte cell death in simulated IRI *in vitro*, whereas knock-down results in the opposite effect. Both miRNAs can act synergistically and target CUG triplet repeat-binding protein 2 (CUGBP2), which represses COX-2 protein expression. Hence, over- expression of either and both of the miRNAs increases the expression level of COX-2 [41], which is protective in cardiac IRI [42] and mediates the beneficial effects of ischemic pre- and postconditioning [43, 44].

miR-320

MiRNA-320 is down-regulated in murine hearts after IRI *in vivo* as well as *ex vivo* and knock-down of miR-320 in cultured rat cardiomyocytes decreases cell death and apoptosis in simulated IRI, whereas over-expression has the opposite effect. These functional effects could be confirmed *in vivo*, where transgenic mice with cardiospecific over-expression of miR-320 show increased infarct size and apoptosis rate and *ex vivo,* where functional recovery is impaired in perfused isolated transgenic mouse hearts. Again, systemic application of antimiR-320 leads to an opposing effect, showing less apoptosis and a smaller infarct size. MiR-320 was shown to target heat shock protein 20 (Hsp20) [45], which could mediate the observed effects by its known cardioprotective properties [46].

miR-494

This anti-apoptotic miRNA shows a complex temporal expression pattern in the postischemic mouse heart (45 minutes of ischemia), where its expression increases at one hour of reperfusion, returns to baseline after 4 hours of reperfusion, then followed by a down-regulation until 24 hours of reperfusion. Its over-expression reduces caspase-3 activity and cell death in simulated IRI. Wang *et al.* created a transgenic mouse model in which miR-494 is specifically over-expressed in the heart. *Ex vivo,* functional recovery in transgenic hearts is better than in WT hearts, assessed in the Langendorff system. Transgenic hearts, subjected to IRI *in vivo* show smaller infarction areas than WT hearts. MiR-494 suppresses three anti-apoptotic factors (ROCK1, PTEN, CAMKIIδ) and two pro-apoptotic factors (FGFR2, LIF) by binding to the 3'UTR of their mRNAs. In summary, the cardioprotective properties seem to be dominant *in vivo*, where miR-494 has a strong beneficial impact in IRI [47].

MicroRNAs in cardiac IPC and heat shock preconditioning

Ischemic preconditioning denominates a process, in which repeated brief episodes of ischemia are applied to the heart. It can protect the heart from future myocardial infarction [48]. Adenosine, endothelial nitric oxide synthase (eNOS), inducible nictric oxide synthase (iNOS), cyclooxygenase-2, heat shock protein 70 and other endogenous mediators have been proposed to protect the heart from myocardial infarction in the context of IPC [49, 50]. Interestingly, miRNAs seem to have a special importance for these processes.

The cardioprotective effect of IPC can be partly abolished with antimiR-21 applied to the heart before IPC. In cultured cardiac myoyctes, antimiR-21 treatment increases the apoptosis rate in a hypoxia/reoxygenation model. Programmed cell death 4 (PDCD4) is up-regulated in these cells. PDCD4 is a target of miR-21. Treatment with PDCD4 increases the apoptosis rate. This suggests that miR-21 exerts its cardioprotective effect by inhibiting PDCD4 [51].

MiRNA-199a is down-regulated in ischemic preconditioning in swine hearts. It targets hypoxia inducible factor 1α, which in turn stabilizes p53 which has pro-apoptotic properties. Assumingly, down-regulation of miR-199a gives way to the induction of pro-apoptotic factors [52].

MiR-378* and miR-711 expression levels were shown to be down-regulated in IPC-treated mouse hearts. In $NF\kappa B^{-/-}$ mice this effect can only be reproduced for miR-378* but not for miR-711, suggesting that miR-711 suppression depends on NFκB. Both miRNAs target Hsp70.3 by binding to its 3'UTR. Hsp70.3 shows a robust increase in IPC-treated WT mouse hearts. This increase is attenuated in $NF\kappa B^{-/-}$ mice, which is consistent with the above mentioned results [53]. These results deserve attention, as our current knowledge on how miRNAs are regu-

lated is still restricted.

MiRNAs isolated from the plasma of mice in the late phase of IPC and injected into the heart of normal mice can reduce the infarct size after IRI. An up-regulation of eNOS, HSF-1 and Hsp-70 can be observed [32]. eNOS and Hsp-70 are able to protect the heart against IRI [50, 54] which is consistent with the cardioprotective effect of IPC induced miRNAs.

Exposing hearts to mild heat shock (heat shock preconditioning) makes them more resistant to subsequent myocardial infarction [55]. MiRNAs are differentially regulated in mouse hearts subjected to cytoprotective mild heat shock. Several miRNAs are up-regulated, including miR-1, miR-21 and miR-24. A cocktail of isolated miRNAs that is injected in the peritoneum of mice is able to reduce infarct size in these animals, when undergoing IRI. Several pro-apoptotic factors were found to be decreased in the cardiac tissue of the treated mice compared to non-treated mice. On the contrary, several pro-apoptotic miRNAs are up-regulated [33].

Both publications of Yin *et al.* show consistently that miRNAs induced by stress (heat shock or IPC) can protect the heart against further ischemia reperfusion injury.

MicroRNAs as biomarkers of myocardial infarction

Besides the therapeutic modulation of IRI, miRNAs could potentially serve as biomarkers for the detection of cardiac injury and risk stratification. So far, more than 256 miRNAs were detected in the human plasma. In patients with STEMI undergoing percutaneous coronary intervention (PCI), many miRNAs are regulated differentially. MiR-1, miR-133a, miR-133b and miR-208b are strongly up-regulated in these patients within 12 hours after the onset of symptoms [29, 30, 56] and their values peak close to the cardiac biomarker troponin I [30] indicating that they could also serve as biomarkers. All of them are detectable in the urine as well. Interestingly, there is a negative correlation between the ejection fraction and the plasma level of miR-208b [29], suggesting the potential use of miR-208b in the prognosis assessment after acute coronary syndrome. However, contradictions exist between the reports about miR-208a: Wang *et al.* reported it to be up-regulated in STEMI patients undergoing PCI with 100% sensitivity and 100% specificity [56], whereas D'Alessandra *et al.* found it to be up-regulated only in some of their STEMI patients undergoing PCI. Possibly these differences can be explained by differences in the study protocols, for example the time points of measurements.

After coronary artery ligation in mice, miR-1, miR-133a, miR-133b and miR-499-5p are up-regulated in the plasma but down-regulated in the myocardial infarct area, indicating that the increase of miRNAs in plasma could be due to release from the cytoplasm of cardiac cells [30].

These data show that changes in the level of miRNAs in the plasma could possibly serve as highly sensitive and predictive biomarkers in acute myocardial infarction.

IRI in striated muscle

Besides cardiac muscle, first experimental studies also describe a differential regulation of microRNAs in IRI of striated muscle groups: Hsieh *et al.* found that miR-21, miR-200c and miR-205 are up-regulated in the postischemic gracilis muscle of the rat [57]. However, besides these first profiling experiments, little is known about the involvement of miRNAs in IRI of striated muscle, as it occurs e.g. during reconstructive surgery. Free muscle flap transfer still is associated with a risk of failure necessitating revision in 10-25% [15] and thus this field leaves ample room for further studies.

Renal IRI

IRI in kidney transplantation has a fundamental impact on the short- and longterm clinical outcome [58, 59]. It is also a crucial factor in acute kidney injury (AKI), which can arise from various conditions such as dehydration, sepsis, renal artery stenosis, coronary artery bypass grafting or other major surgery. The main consequence of IRI in the kidney is acute tubular necrosis, resulting in a decrease in the glomerular filtration rate [60, 61]. Several miRNAs have been identified to be differentially regulated in renal IRI or to have a functional impact on this process.

miR-21

Nine miRNAs are differentially expressed in

mouse kidneys following renal IRI. MiR-21, miR-20a, miR-146a, miR-199a-3p and miR-214 are up-regulated. MiR-192 and -187 are initially up-regulated, but after five days their expression levels are lower than in the control group. MiR-805 and -194 are consistently down-regulated. R^0/γ^0 mice which lack Nk cells, NKT cells, B and T cells expose the same expression profiles, indicating that the deregulation is independent of the invasion of circulating immune cells [62]. Similar changes occur in immunodeficient RAG-1 deficient mice (Rag-12/2 mice) and RAG-2/common c-chain cytokine receptor double knockout mice (Rag-2/cc2/2 mice) compared with wild type mice.

In vitro, cultured tubular epithelial mice show up-regulation of miR-21 after exposure to ischemia and knockdown of this miRNA increases cell death. Interestingly, TGF-β also induces pri-miR-21, whereas ischemia decreases the pri-miR-21 level, suggesting that induction of miR-21 in ischemia is due to an increased processing of pri-miR-21. The up-regulation of miR-21 in renal IRI *in vivo* however seems to be independent of TGF-β as the expression of the latter is not altered by ischemia in this setting [62].

One consequence of renal IRI is an increase of blood urea nitrogen (BUN) and creatinine in the plasma. Specific deletion of the miRNA processing enzyme Dicer and thus inhibition of all miRNAs in the proximal tubules attenuates the increase of blood urea nitrogen and creatinine. It also results in less tubular injury indicated by tubular dilation and brush border loss and decrease in caspase-3 activity, resulting in a lower apoptosis rate. Finally, the survival of Dicer-knockout mice after renal IRI is significantly better than in WT mice [63], leading to the assumption that miRNAs at least partly contribute to renal damage after IRI.

Cerebral IRI

Stroke is the third leading cause of death in the industrialized nations and many of the surviving stroke patients suffer from severe disability for the rest of their lives. There are different therapeutic approaches to cure acute ischemic insult including intravenous or intra-arterial thrombolysis [13]. Again, as soon as the obstruction is removed and blood flow re-established, the ischemic tissue is threatened by reperfusion injury.

In response to oxygen and glucose deprivation (OGD), miRNAs are dysregulated in cultured neurons and astrocytes *in vitro*. However, the expression profiles of the two cell types differ significantly. In neurons, miR-21 and miR-29b are up-regulated, whereas miR-30b, miR-107 and miR-137 remain unaffected. In astrocytes, miR-21 and miR-29b are up-regulated as in neurons, but miR-30b, miR-107 and miR-137 are also up-regulated. MiR-210, the expression levels of which show high variations in neurons, is significantly up-regulated in astrocytes [64].

In vivo, the miRNA expression profile in the rat brain is altered after transient focal ischemia. After 1 hour of ischemia by occlusion of the middle cerebral artery, 8 miRNAs are increased and 12 are decreased at 4 out of 5 time points from 3 hours to 3 days of reperfusion. After 24 hours of reperfusion, miR-290, miR-145 and miR-26 are up-regulated and miR137, miR27a and miR18 are down-regulated. The expression levels of the miRNA processing proteins Dicer, Drosha, cofactor Pasha and pre-miRNA transporter exportin-5 do not change in cerebral IRI [65]. Intracerebral pre-treatment with antimiR-145 increases the expression of super oxide dismutase-2 in the peri-infarct area and decreases the infarct area. Bioinformatic analysis of miRNA and mRNA expression profiles reveals a positive correlation between the expression of miR-145 and several markers of inflammation, suggesting a modulation of the IRI-induced inflammatory response as the underlying mechanism for this beneficial effect [65].

Clinical implications and problems

With the growing evidence for the involvement and the regulatory function of miRNAs in many pathophysiological processes, these small regulatory RNAs are evolving as promising therapeutic targets. However, therapeutic modulation of miRNAs in cardiovascular disease remains experimental so far and several obstacles need to be overcome before miRNA-based therapeutics will progress towards clinical practice.

MicroRNA-inhibition

Current strategies targeting miRNAs include classical “antagomiRs” and several new oligonucleotide technologies, with locked nucleic acids (LNA) being the most prominent. AntagomiRs are single-stranded RNA molecules,

modified by 2'-O-methyl and phosphorothioate substitution for stability. They bind to the targeted miRNA by complementary base pairing. To enable cellular uptake, they are conjugated with cholesterol. These compounds can achieve significant miRNA knockdown [66] and have been used successfully to treat experimentally induced diseases in different organs and tissues [67-69]. LNAs use a principle similar to "antagomiRs" but in these molecules the oligonucleotide backbone is modified by an accessory bridge between the 2' oxygen and the 4' carbon. This modification causes increased resistance to degradation and affinity to the target miRNA and allows the use of shorter sequences resulting in an enhanced cellular uptake. Clinical trials have successfully tested LNA-based drugs for the treatment of hepatitis C [70, 71] and several other LNA-based therapeutics are under development [75].

MicroRNA-replacement therapy

While miRNA-antagonists directly block their target miRNA in the cytoplasm, miRNA-mimicking oligonucleotides (miR-mimics) function differently: After passage of the cellular membrane, they need to be integrated in the RNA induced silencing complex [62] and induce translational inhibition of degradation of their mRNA targets. Hence, the possibilities for chemical modifications that increase the resistance of these oligonucleotide chemistries towards degradation or facilitate cellular uptake are limited. Although over-expression of miRNAs by pre-miR-oligonucleutides or miR-mimics is a well-established method for characterization of miRNA-function *in vitro*, only a few successful applications for the *in vivo* treatment of mammals exist so far. For example, intranasal application of the tumor suppressor miRNA let-7 in mice can attenuate tumor progression and cell proliferation in an experimental lung cancer model [72] and the anti-apoptotic properties of miR-133a can reduce the apoptotic rate of cardiomyocytes in mouse hearts undergoing IRI, when it is directly injected into the myocardium [31].

Challenges and advantages

Despite the rapid advances in experimental miRNA research and the theoretical advantages of miRNA-based therapies in comparison to conventional pharmaceuticals, several obstacles need to be overcome before these promising new compounds can progress towards clinical practice.

One of the advantages of antagomir-based miRNA-inhibitors is their prolonged effect: A single dose can suppress the target miRNA for several weeks [73]. The duration of this inhibition can be modulated by chemical modification, making a compound more or less prone to degradation. As the potency of an anti-miR drug is limited by the targets' availability, miRNAs with a highly specific expression pattern could theoretically be targeted in selected tissues.

In contrast to these miRNA-inhibitors, the delivery of miR-mimics *in vivo* is more challenging, as mentioned above. Furthermore, artificial overexpression of administered miRNA might affect the natural miRNA-network, e.g. by overloading the RISC or by off target effects.

Conclusion

The differential regulation of miRNAs in IRI is now well described and it is very likely that further miRNAs influencing IRI will be discovered. However, still little is known about the actual mechanisms by which miRNAs influence the individual components of IRI e.g. endothelial damage, leukocyte activation, ROS production and complement activation. Hence, further research is required to understand the functional impact of miRNAs on IRI in the heart and other tissues. Theoretically, the treatment of IRI in myocardial infarction or stroke would be a very convenient field for miRNA-based therapy, because a single-dose application before revascularization could be sufficient for a sustained treatment effect.

Address correspondence to: Dr. Sebastian Grundmann, Department of Internal Medicine III (Cardiology and Angiology), University Hospital Freiburg, Hugstetter Straße 55, 79106 Freiburg Germany Tel: +49 761 270 70460; Fax: +49 761 270 70450; E-mail: sebastian. grundmann@ universitaets-herzzentrum.de

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