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Emerging Roles for MicroRNAs in Perioperative Medicine

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ABSTRACT

MicroRNAs (miRNAs) are small, non–protein-coding, single-stranded RNAs. They function as posttranscriptional regulators of gene expression by interacting with target mRNAs. This process prevents translation of target mRNAs into a functional protein. miRNAs are considered to be functionally involved in virtually all physiologic processes, including differentiation and proliferation, metabolism, hemostasis, apoptosis, and inflammation. Many of these functions have important implications for anesthesiology and critical care medicine. Studies indicate that miRNA expression levels can be used to predict the risk for eminent organ injury or sepsis. Pharmacologic approaches targeting miRNAs for the treatment of human diseases are currently being tested in clinical trials. The present review highlights the important biological functions of miRNAs and their usefulness as perioperative biomarkers and discusses the pharmacologic approaches that modulate miRNA functions for disease treatment. In addition, the authors discuss the pharmacologic interactions of miRNAs with currently used anesthetics and their potential to impact anesthetic toxicity and side effects. **(Anesthesiology 2016; 124:489-506)**

MICRORNAS (miRNAs) are short, noncoding RNA molecules composed of a single-stranded sequence of 20 to 24 nucleotides. They predominantly act as negative regulators of gene expression.[1,2] Functionally, they regulate target genes at the posttranscriptional level *via* means of preventing the synthesis of the active protein. This can be achieved by binding of miRNAs to protein-coding transcripts, thereby preventing either translation of the mRNA to a functional protein or leading to mRNA degradation. Being involved in the regulation of essentially every aspect of cellular function, it is hardly surprising that miRNAs are thought of as critical regulators during various disease processes, such as sepsis, ischemia–reperfusion, or cancer.[3–7] miRNAs were first discovered in 1993 in studies reporting miRNA–mRNA interaction in *Caenorhabditis elegans*.[8] Similar to double-stranded RNA molecules manipulating gene expression by RNA interference as described by Nobel Prize winners Fire and Mello in nematodes,[9] miRNAs were initially thought to be only relevant in nonmammalian species. However, it was subsequently identified that miRNAs are expressed in mammalian systems and play important functional roles.[10,11] From that time onward, miRNA research focused on screening miRNA expressions in various human tissues and disease processes. Many of these studies revealed that miRNAs are expressed in tissue-specific patterns and play fundamental roles in tissue identity and in characteristic features of cell types and functions.[12,13] Initial analysis of miRNA expressions in diverse human pathologic states clearly revealed correlations between miRNA expression patterns and certain human diseases including inflammatory conditions and sepsis, ischemia–reperfusion, or cancer.[3,7,14] Subsequent studies focusing on miRNAs functional activities leveraged the importance of miRNAs as critical gene regulators and indicated the potential clinical relevance of miRNAs. Not only could miRNA expression patterns provide a new diagnostic tool, but also would aberrant miRNA functions present promising new therapeutic options by targeting miRNAs. Another conceivable application for miRNAs in a clinical setting is related to the emerging field of personalized medicine. Indeed, many studies point out the prognostic importance of miRNA expression. Important distinction between certain states of diseases (*e.g.*, subtypes of tumor entities) could lead to improved identification of the best suitable therapeutic treatment for a specific patient.

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For example, in the field of perioperative medicine, miRNA expression variations may serve as preoperative biomarkers to stratify the individual patient risks for specific organ injuries. The clinical availability of miRNAs could improve detecting preexisting risks for postoperative complications and thereby help preventing fulminant organ failure or severe inflammatory response following intervention. Some *in vitro* studies even indicate that miRNAs could play a functional role in neuroprotection from anesthetic toxicity.[15,16] The present review aims to provide the reader with an understanding of the function and disease implications of miRNAs. For this purpose, we attempted to include a discussion of exciting research studies from the field of miRNAs that could have an important impact on the perioperative medicine. Moreover, we are highlighting various scenarios of how miRNAs could enter daily anesthesia care of various patients and outline their potential impact on emergency, critical care, and perioperative medicine (fig. 1).

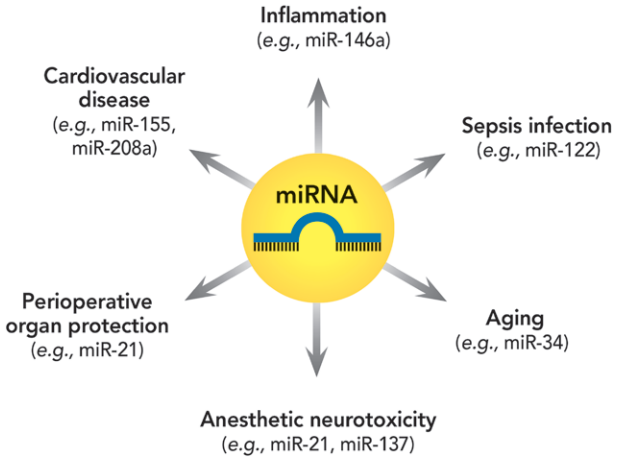

Fig. 1. MicroRNA (miRNA) functions in perioperative medicine. miRNAs represent targets for diagnostic or therapeutic approaches in various perioperative fields. A subset of miRNAs of which inhibition or overexpression has shown therapeutic promise are currently being pursued as clinical candidates for disease treatment or prevention. For example, in the setting of "cardiovascular disease," targeting miR-155 in patients with arteriosclerosis could reduce one of the main risk factors for myocardial infarction or stroke.[58] Detecting levels of miR-208a in the peripheral blood of patients with suspect of heart attacks could improve the early diagnosis and consequently accelerate therapeutic onset as its increase was shown to occur in the very first hours postinfarction.[104] As a target for "inflammation," detection of miR-146a could help modulating immune-mediated pathology because miR-146a was shown to regulate the suppressive activity of regulatory T cells.[85] In the setting of "sepsis or infection," patients suffering from chronic hepatitis C virus infection can be successfully treated with SPC 3649, a drug that targets miR-122 and thereby reduces hepatitis C virus replication.[103] Identified in studies of "aging" individuals, pharmacologic approaches inhibiting miR-34a (*e.g.*, anti-miR-34a) could improve cardiac function.[72] During anesthesia, specific miRNAs could represent promising candidates to protect from anesthesia-induced neurotoxicity. For example, experimental overexpression of miR-21 was found to attenuate the cell death of human embryonic stem cell–derived neurons induced by propofol.[15] Other miRNAs are implicated in mediating "perioperative organ protection," for example, miR-21 was shown to have a functional role during myocardial ischemia–reperfusion injury.[152]

Biological Functions

Maturation

To understand miRNA functions, it is important to be aware of the mechanism that regulates miRNA biogenesis. miRNA genes are located throughout the genome and can be found intergenic (in non–protein-coding regions) or in genomic regions that are within protein-coding genes and are therefore cotranscribed with the host gene.[17] miRNA biogenesis starts similar to the majority of protein-coding genes in the nucleus. In contrast, later maturation steps are different from other small RNAs (fig. 2). In the nucleus, RNA polymerase II (Pol II) generates long primary transcripts called pri-miRNAs,[18] which will then be further processed by two miRNA exclusive RNase III enzymes: Drosha and Dicer. The first one associates with a nuclear protein called DiGeorge Syndrome Critical Region 8 and produces a hairpin-structured shorter precursor miRNA named pre-miRNA, which subsequently is exported into the cytoplasm. After the nuclear export, the critical second nuclease Dicer further shortens the pre-miRNA, resulting in an unstable double-stranded short miRNA. One strand of this duplex structure becomes the functionally active miRNA, which gets incorporated into a nuclease complex called the RNA-induced silencing complex (RISC). The active single-stranded miRNA within the RISC subsequently interacts with its mRNA target and induces nuclease activity, thereby regulating protein expression.[2,19–22] According to an miRNA database entry (miRBase 21, a database of all published miRNA sequences, released in July 2014), a total of 1,881 human miRNA loci have been presently described.

Regulation of miRNA Expression

MicroRNA maturation is a tightly regulated process, including temporal and spatial coordination. Any dysregulation of miRNA biogenesis can alter the miRNA expression levels, which in turn can cause altered gene expression, thereby potentially contributing to disease. For example, this process has been implicated in the initiation of various cancers. Among many proteins involved in the proper maturation process, the most critical checkpoints are RNase III proteins and associated nuclear proteins DiGeorge Syndrome Critical Region 8, Drosha, and Dicer. Their proper function is essential for mammalian life, as it was shown in genetic models that mice with global genetic deletion of any of these "miRNA maturation enzymes" are not viable.[23,24]

In addition to regulatory elements of the miRNA maturation process, pathways that control tissue-specific miRNAs

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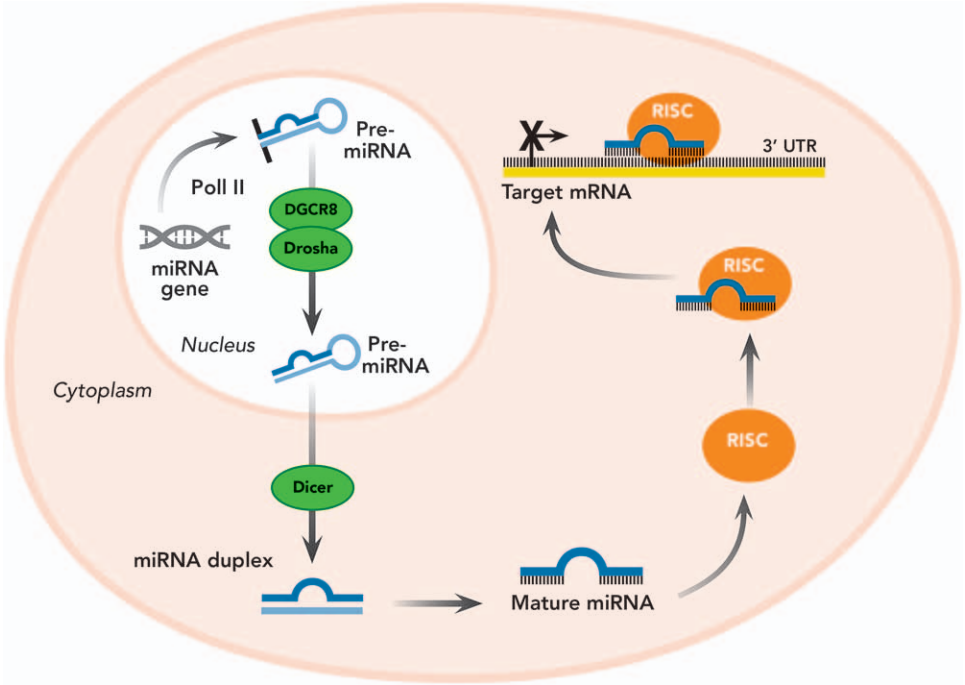

Fig. 2. MicroRNA (miRNA) biogenesis. miRNA biogenesis starts in the nucleus where RNA polymerase II (Pol II) generates large transcripts called primary miRNAs (pri-miRNA). Next, associated with DiGeorge Syndrome Critical Region 8 protein (DGCR 8), RNase nuclease Drosha synthesizes shorter hairpin-structured precursor miRNAs (pre-miRNA). The pre-miRNA is exported from the nucleus into the cytosol where RNase nuclease Dicer further shortens the hairpin-structured miRNA, resulting in double-stranded short duplex miRNAs. The mature strand gets incorporated into RNA-induced silencing complex (RISC) and guides this multiprotein complex to the target mRNA for gene repression. Binding of the miRNA to complementary sequences in the 3 prime untranslated region (3′UTR) of the mRNA of a target gene typically results in translational repression or mRNA degradation, thereby causing functional repression of the target gene.

expression have been described as well. For instance, some miRNAs are differentially processed after the pre-miRNA maturation. Depending on the tissue, ubiquitously expressed pre-miRNAs are selectively exported into the cytoplasm for further maturation.[25]

An additional level of miRNA expression control relates to miRNA decay. Rapid changes in miRNA expression allow for miRNAs to frequently react faster than other systems, thereby highlighting the high turnover rate of mature miRNAs. Even though the RISC-incorporated miRNA is considered to be relatively stable (*e.g.*, half-lives of several hours to days[26,27]), individual miRNAs have shown differences in miRNA stability.[27] These differences indicate that miRNA turnover is also involved in miRNA expression regulation. In particular in the neuronal system, active miRNA degradation was found to play a critical role,[28] and various brain-specific miRNAs revealed to have short half-lives compared with miRNAs in other systems.

Transcriptional regulation of miRNA expression can be controlled *via* classical transcription factors *via* binding of such transcription factors to the promoter region of genes encoding miRNAs. For example, during conditions of limited oxygen availability (hypoxia), the transcription factor hypoxia-inducible factor (HIF) is stabilized and was shown to regulate a panel of miRNAs. By direct binding to miRNA promoters, HIF can cause induction or repression of a gene. In addition, some of these miRNAs can target HIF (reviewed in the study by Shen *et al.*[29]). These interactions between miRNAs and HIF are feedback loops that are relevant to cellular processes such as proliferation, cell cycle progression, or angiogenesis, processes playing a role in tumorigenesis, but also in ischemia–reperfusion.

Mechanisms of Target Regulation

MicroRNAs predominantly act as negative regulators of gene expression.[19] In the majority of cases, miRNAs identify and bind their target gene in a specific mRNA region. Protein-coding mRNAs are organized in three main regions. Those regions are the five prime untranslated region (5′UTR), the three prime untranslated region (3′UTR), and the coding region or also called open reading frame, which is flanked by the two untranslated regions. The coding region ultimately contains the genetic information of the gene, which on a DNA level is composed of multiple regions, called introns and exons (fig. 3A). The final protein composition is defined by the sequence of bases within the exons, thereby making up the translated region of the mRNA. miRNAs preferentially bind their target mRNA in the 3′UTR of the mRNA. This is the mRNA region located downstream of the coding region. The 3′UTR is usually not translated into protein but

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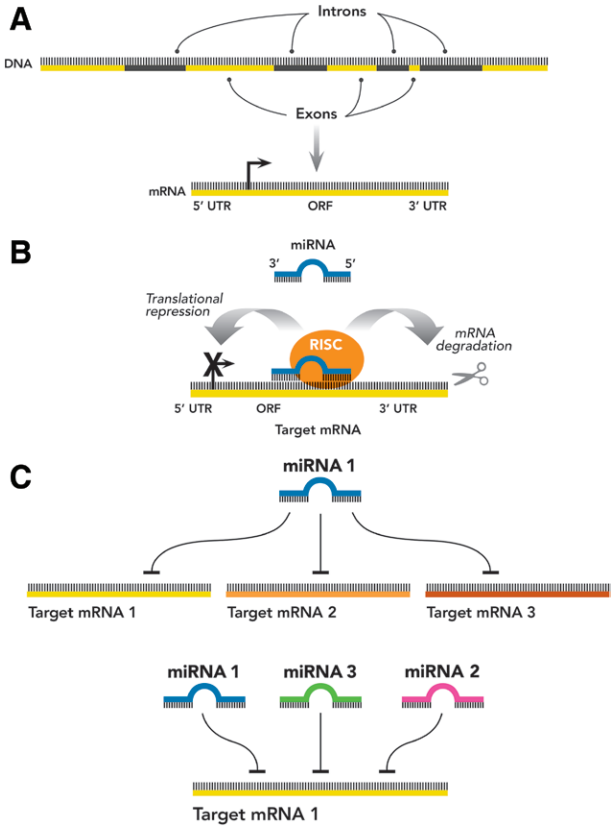

Fig. 3. MicroRNA (miRNA) functions. (*A*) Structure of mRNAs, transcribed from DNA. mRNAs are composed of three main regions: the 5 prime and 3 prime untranslated regions (5′UTR and 3′UTR) representing regions that are not translated into proteins. The untranslated regions flank the coding region or open reading frame (ORF). The ORF is composed of exons and introns, with only the exons being eventually translated into proteins. (*B*) Mature miRNAs are loaded into the RNA-induced silencing complex (RISC) and guide the RISC to the target mRNA. miRNA binding to its mRNA target sequence typically occurs within the 3′UTR of the mRNA. This process results in gene regulation, either by translational repression or mRNA degradation. (*C*) One single miRNA can bind to many different mRNA targets and therefore it regulatory can have a wide scope. However, one target mRNA can be repressed by different miRNAs, ensuring a profound miRNA-mediated regulatory effect.

serves as regulatory region that can affect posttranscriptional processes and subsequently gene expression. miRNA binding initiates the RISC-mediated gene regulation, resulting in mRNA degradation or translational repression (fig. 3B). In rare cases, miRNAs can also bind outside the 3′UTR, for example, in the 5′UTR or even in the coding region.[30–33] miRNA–mRNA base pairing in the 3′UTR prevents any further steps that could lead to the synthesis of a full protein encoded by the targeted mRNA. This regulatory function does not require perfect base pairing between miRNA and targeted mRNA. Therefore, a single miRNA can target many different mRNAs, leading to a wide scope of mRNA alterations (fig. 3C). However, one target mRNA can be repressed by multiple different miRNAs[34] (fig. 3C). Initially, it was assumed that miRNAs predominantly act as translational repressors.[35,36] Today, miRNA-mediated gene silencing is thought to affect both the levels of mRNA and the level of synthesized protein.[37–40] This complex network of miRNA–mRNA interactions explains the fact that the expression of more than 60% of all human genes are thought to be fine-tuned by miRNAs.[41]

Secreted miRNAs

Only recently discovered, miRNAs can also participate in intercellular communication, such as cross-talk between different cell types. As such, studies demonstrated that miRNAs can represent genetic information that is passed on from one cell type to another and thereby participating in the exchange of genetic information. Cell–cell communication based on miRNA exchange can take place within a tissue or among different cell types. The finding of extracellular miRNAs as a means of intercellular communication is intriguing from a clinical perspective. These findings highlight that miRNAs are not only not limited to intracellular functions. Instead, miRNAs can exist in the extracellular compartment and have the capability to participate in intercellular communication.[42,43] miRNAs were found in various human body fluids, including blood, urine, cerebrospinal fluid, bronchoalveolar lavage, or breast milk. Extracellular miRNAs are easily detectable in human body fluids and provide an additional noninvasive instrument to analyze patients' "health" state. Additional studies identified extracellular vesicles and lipids serving as carrier vehicles in miRNA-based cell–cell interactions.[42,44,45] Membrane-enclosed or lipid-bound secreted miRNAs are surprisingly stable[46] and can be taken up by recipient cells.[47,48] The exact mechanisms underlying this genetic exchange including miRNA selection and packaging and the process of transport and uptake are still not fully understood. However, it is becoming increasingly apparent that miRNAs can transfer signals between immune cells, thereby orchestrating acute immune response.[47,49] Other studies implicate miRNA transfer in chronic diseases such as atherosclerosis[50,51] or in promoting tumor growth and metastasis.[43,52] For example, miR-150 was found to be contained in extracellular microvesicles in the plasma of patients with atherosclerosis. Subsequent studies indicate that miR-150 mediates a cross-talk between blood cells and the vasculature. *In vitro* and *in vivo* experiments revealed that blood cell–secreted microvesicles containing miR-150 were delivered into blood vessel cells, where increased levels of miR-150 regulated cell migration (by targeting its target c-Myb).[44] C-Myb is a family member of the MYB transcription factors, a family of proteins including the conserved MYB DNA-binding domain. The MYB protooncogene was shown to play an essential role in tumorigenesis by regulating migration and tumor invasiveness, for example, in breast and colon cancer.[53] The existence of functional active extracellular miRNAs indicates a very auspicious approach

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for patient care. One could potentially take the advantage of modulating these endogenous regulators for diagnostics purposes or in order to optimize therapeutic strategies for instance during inflammatory disease conditions or during infections with pathogens.

miRNA Functions in Perioperative Medicine

Cardiovascular Disease

Shortly after discovering miRNAs in mammalians, miRNA profiling studies revealed correlations between miRNA expression patterns and human diseases.[3,6] Numerous studies in various human vascular and cardiac diseases affirmed that miRNAs play a critical role in cardiovascular disorders. Functional studies attribute significant roles for those miRNA with aberrant expressions in the development and maintenance of cardiovascular disorders, including myocardial infarction, heart failure, and fibrosis.[54–57] Here, in particular, we want to focus on miRNAs involved in the pathogenesis of arteriosclerosis (miR-155) and miRNAs that contribute to myocardial dysfunction and heart failure (miR-208a and miR-34a).

Studies focusing on arteriosclerosis analyzed miRNA expression in human carotid plaques, obtained during carotid endarterectomy. These studies revealed increased expression levels of miR-155 and miR-147b.[58] Subsequent studies in murine macrophages in an *in vivo* mouse model of arteriosclerosis confirmed that miR-155 promotes the proinflammatory activity of macrophages and that the lack of miR-155 *in vivo* leads to markedly reduced arteriosclerosis. MiR-155 was shown to directly repress the expression of B-cell lymphoma 6 (BCL6) protein, a transcription factor involved in the control of inflammation. BCL6 inhibits proinflammatory signaling, leading to attenuation of atherosclerosis.[59] This antiinflammatory effect of BCL6 is partially mediated by inhibition of nuclear factor κ-light-chain-enhancer of activated B cells signaling *via* different mechanisms. Together with the high levels of miR-155 found in human arteriosclerotic plaques,[58] these findings indicate that inhibition of miR-155 could be a novel therapeutic approach to the treatment of arteriosclerosis.

Myocardial infarction and heart failure are among the most frequent perioperative complication during noncardiac surgeries.[60] A study in hypertrophic or failing hearts from humans revealed 12 miRNAs that are modulated compared with healthy controls.[61] Subsequent studies identified miR-208a as important modulator of the cardiac stress response.[62] Interestingly, this miRNA is cardiac specific. The cardiomyocyte-specific expression pattern indicates a significant role of this miRNA in the regulation of myocardial function.[60] Mice carrying a genetic deletion of miR-208a showed reduced hypertrophy and fibrosis in response to cardiac stress.[62] Moreover, miR-208a knockout mice were unable to up-regulate pathologic cardiac markers suggesting that inhibition of miR-208a is beneficial in cardiac stress and, in a broader sense, in the context of heart disease. Pharmacologic inhibition of miR-208a in murine studies confirmed this potential advantage during cardiac stress and also ascribed promising pharmacologic features to the selected inhibition of this miR-208a using a so-called "locked nucleic acid" (LNA) specifically targeting this miRNA *in vivo*.[63] Myocardial contractility largely depends on the contractile protein Myosin Heavy Chain (MHC). Two MHC isoforms represent this major contractile protein of the cardiac muscle, α-MHC and β-MHC. Up-regulation of miR-208a lead to increased β-MHC expression and was associated with arrhythmia, fibrosis, and hypertrophic growth in mice.[64] In addition, miR-208a was revealed to be a strong predictor of cardiac death and heart failure.[65] However, it was shown that down-regulation or deletion of miR-208a was associated with decreased β-MHC expression in the adult heart[64,66] and attenuated pathologic cardiac remodeling.[63,64] Additional studies provided more insights into the positive effects of reduced miR-208a expression by revealing an unexpected resistance to obesity after miR-208a inhibition.[67] The authors found that miR-208a inhibition in mice lead to a resistance to high-fat diet–induced obesity. Inhibition of miR-208a caused increased levels of its target mediator complex subunit 13 (Med13), which is involved in the regulation of energy expenditure and regulation of numerous genes involved in energy balance in the heart. MED13 represents a transcriptional coactivator complex that is thought to be required for the expression of almost all genes. It serves as the molecular bridge between the general transcriptional machinery with specific transcription factors and the kinase submodules.[68] According to these findings, miR-208a qualifies as a potent target for therapeutic modulation of cardiac function during progression of heart disease. In addition, it could also be targeted for the treatment of metabolic syndrome.[63,67]

Interestingly, some studies provide evidence that miRNAs also play a functional role in the process of aging. Importantly, the mean age of today's patients is steadily increasing, rising the demand for special attention to the particular conditions of older patients. Aging itself represents a risk factor in the perioperative setting, especially with regard to cardiovascular disease.[69] Physiologic changes that come with age also include alterations in miRNAs expression and function. Consequently, recent studies indicate that miRNAs play important functional roles in the process of aging.[70,71] One of these age-associated miRNAs is miR-34a. A study analyzing the miRNA expression levels in human heart samples revealed that the level of expression of miR-34a significantly correlated with the patient's age when a cardiac biopsy was obtained. Subsequent mechanistic studies demonstrated that miR-34a contributes to an age-related cardiac function deterioration in the murine heart.[72] MiR-34a interaction with phosphatase nuclear targeting subunit (PNUTS), also known as protein phosphatase 1, regulatory subunit 10 (also known as PPP1R10) promotes telomere attrition and cell

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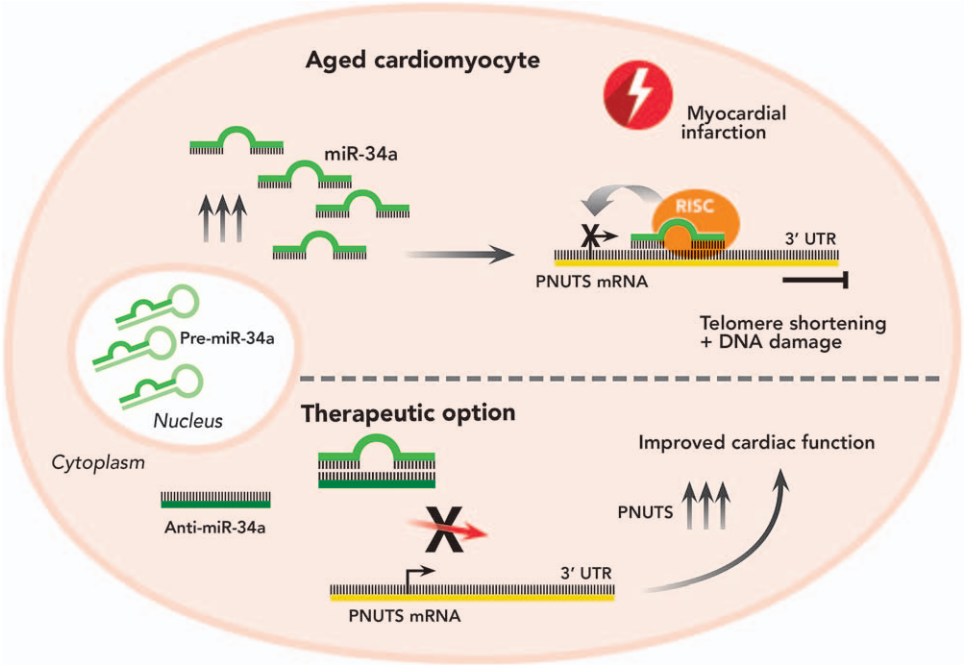

Fig. 4. MicroRNA miR-34a in the aged heart. In the aged heart, miR-34a levels are increased. MiR-34a interaction with its target phosphatase nuclear targeting subunit (PNUTS), also known as protein phosphatase 1, regulatory subunit 10 (PPR1R10) mRNA, prevents PNUTS translation. Lower levels of PNUTS promote telomere attrition and cell death, worsening the postinfarct cardiac function in the elderly patient. The inhibition of miR-34a by anti-miR-34a allows PNUTS protein synthesis. Higher levels of PNUTS prevent telomere shortening, reduce cardiomyocyte cell death, and improve cardiac contractility after myocardial infarction in the aged heart. Taken together, these findings suggest that pharmacologic approaches that would inhibit miR-34a (*e.g.*, anti-miR-34a) could improve cardiac function in the elderly patient suffering from myocardial infarction.[72] RISC = RNA-induced silencing complex.

death, thereby worsening the postinfarct cardiac function in the aged murine heart (fig. 4). PNUTS is predominantly localized to the interphase nucleus of mammalian cells. It can interact with the telomere-specific proteins, for example, telomere repeat factor 2,[73] and is thought to function in the DNA damage response in DNA repair.[74] *In vivo* inhibition of miR-34a prevented PNUTS down-regulation. As a result, higher levels of PNUTS prevented telomere shortening, reduced cardiomyocyte cell death, and improved cardiac contractility after myocardial infarction in older mice.[72] The findings indicate that preventing miR-34a from regulatory activity attenuates cardiac injury in elderly. Therefore, it is conceivable that when patients undergo interventions associated with a high risk for cardiac complications, inhibition of miR-34a could specifically improve perioperative outcomes in elderly patients.

Inflammation and Sepsis

Inflammation and sepsis occur frequently and represent major contributing factors to morbidity and mortality in perioperative medicine and during critical illness. In the following paragraph, we will highlight the roles of three miRNAs that were found to be important in assuring proper immune responses. As first, we will discuss miR-223 as a key regulator of innate immune responses. Second, we will describe findings on miR-146a that has been implicated in the function of regulatory T cells (Tregs) and their concomitant role in the resolution of inflammation. Finally, we will review studies of miR-27a as a biomarker and functional target miRNA during sepsis.

Effective responses to infectious or sterile immune stressors require tightly controlled and precisely regulated inflammatory cascades to be activated. At the same time, certain endogenous and exogenous signals have to be down-regulated in order to prevent collateral tissue damage or progression to chronicity. Representing a fast reacting group of fine-tuning regulators, it is not unexpected that miRNAs have been shown to play an important role in controlling inflammation and immune responses.[14,75,76] They are involved in the regulation of innate and adaptive immune responses, dynamically regulating proliferation, differentiation, and function of immune cells and thereby controlling a wide range of immune responses.[75–79] A key miRNA in this process is presented by miR-223, which has been shown to modulate innate immune responses on two distinct levels. On the one hand, it directly controls granulocyte cell differentiation and maturation.[80] In addition, it also regulates granulocyte function, thereby acting as an important regulatory break for granulocyte activity.[81] Mice with miR-223 deletion are prone to inflammation. When challenged with endotoxin, miR-223 knockout mice display exaggerated tissue injury and develop profound pulmonary

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inflammation. One of the critical targets regulated by miR-223 is the inflammasome NLR family, pyrin domain containing 3 (NLRP3), a multiprotein complex sensing cellular stress and mediating inflammatory responses.[82] NLRP3 is an important regulator of caspase-1, the enzyme regulating the level of active interleukin (IL)-1β protein and was shown to play a role in the regulation of inflammation and apoptosis. It is involved in the pathogenesis of hereditary cryopyrinopathies, a spectrum of autoinflammatory syndromes and was also associated with diseases such as gout, type 2 diabetes, and atherosclerosis. MiR-223 suppresses NLRP3 expression through its 3′UTR leading to reduced NLRP3 inflammasome activity and therefore limiting proinflammatory processes.

Depending on the regulated target gene, miRNAs can act as an amplifier of proinflammatory or antiinflammatory signals, meaning that depending on their target genes (proinflammatory or antiinflammatory targets), they have the capability to enhance or dampen an inflammatory immune response. One of the first miRNAs that was shown to be induced during inflammation is miR-146a. Indeed, it was subsequently demonstrated that miR-146a plays a central role in controlling innate and adoptive immune responses.[83–86] Deletion of miR-146a *in vivo* revealed a hyperresponsive and inflamed phenotype, as well as favored autoimmunity in mice. MiR-146a was shown to be one of the prevalent miRNAs expressed in Tregs, a subpopulation of T cells. Tregs are critical for self-tolerance and autoimmune disease and play a critical role in promoting the resolution of inflammation.[87] Lack of miR-146a is associated with increased levels of its direct target signal transducer and activator transcription 1 (Stat1). In response to stimulation by interferons, Stat1 induces genes involved in the activation of the immune system. It is known for its central role in the modulation of the interferon-mediated immune response. This immune response is mediated by Th1 helper cells, a subpopulation of T effector cells representing the host immunity effectors against intracellular bacteria and protozoa. For Treg-mediated control of these Th1 responses, an optimal level of Stat1 activation is important. Deregulated Stat1 levels in Tregs lead to loss of appropriate regulation of T effector cells and autoimmunity. These findings confirmed the studies that had earlier identified miR-146a as negative regulator of immune responses, acting as an autoregulatory brake in inflammatory feedback loops.[84]

Postoperative inflammation after surgery is a common and usually limited event. But, when paired with an infection might lead to sepsis—one of the leading causes of mortality on surgical intensive care units.[88] Although some patients are capable of mounting an adequate inflammatory response after surgery, some patients develop severe systemic reactions caused by inadequate immune response. miRNAs have been shown to be highly involved in the complex regulation of adequate immune responses.[75,76,89–91] Very recent findings from a functional study in a murine sepsis model revealed expressional changes of diverse miRNAs including the proinflammatory miR-27a during sepsis induction.[92] Previous studies revealed that the antiinflammatory gene peroxisome proliferator–activated receptor (PPAR) γ is one of the several genes miR-27a can target and reported that it is negatively regulated by miR-27a in adipocyte differentiation.[93] The three different PPARs, α, β/δ, and γ, are differentially expressed and represent nuclear receptor proteins that function as transcription factors. PPARs play essential roles in the regulation of various biological functions. In particular, PPARα and PPARγ have been implicated in the regulation of inflammatory responses in different cell types. Based on this, the authors examined the neutralization of miR-27a in a murine cecal ligation and puncture sepsis model.[92] In those septic mice, inhibition of miR-27a was accompanied by reduced expression levels of proinflammatory cytokines such as tumor necrosis factor-α and IL-6 and diminished pulmonary inflammation and revealed a benefit in survival of septic mice. This is also in accordance with a study that identified IL-10, a major antiinflammatory cytokine, as direct miR-27a target in human peripheral blood mononuclear cells.[94] Based on these findings, miR-27a is a top candidate target for miRNA-based sepsis therapeutics.

Infections with Pathogens

Besides, miRNAs controlling the host's defense mechanisms, miRNAs can also directly interact with invading pathogens, such as viruses.[95–97] In the following paragraph, we will highlight the involvement of miRNAs in controlling infections based on the studies of mice deficient in the miRNA-editing enzyme Dicer. Second, we will discuss the role of liver-specific miR-122 during hepatitis C. Indeed, these findings have led to one of the first successful clinical trials directly targeting an miRNA for disease treatment.

One of the key steps in the maturation process of miRNAs involves RNA processing by Dicer. As such, impaired miRNA maturation in models of dicer deficiency highlights the roles of miRNAs in controlling infections with pathogens. Initial studies in Dicer-deficient mice revealed impaired overall miRNA production. Because homozygous mice with targeted deletion of Dicer die during the early embryogenesis due to developmental defects, mice carrying a hypomorphic Dicer1 allele (Dicer [d/d]) can be used to bypass the embryonic lethality. These mice with partial dicer deficiency are vital and can be used to examine the functional role of dicer in a wide set of disease models.[98] Indeed, dicer deficiency was associated with an increased susceptibility to viral infections, thereby revealing a pivotal role for miRNAs in viral infections.[99] Dicer1 (d/d) mice experienced an increased susceptibility to vesicular stomatitis virus infection. At least in part, this phenotype was caused by a lack of miR-24 and miR-93, which are known to target viral proteins, and are, therefore, critical in attenuating viral replication. In summary, these findings indicate that the IFNβ-induced antiviral effects on hepatitis C virus (HCV) replication and infection are at least in part miRNA mediated.

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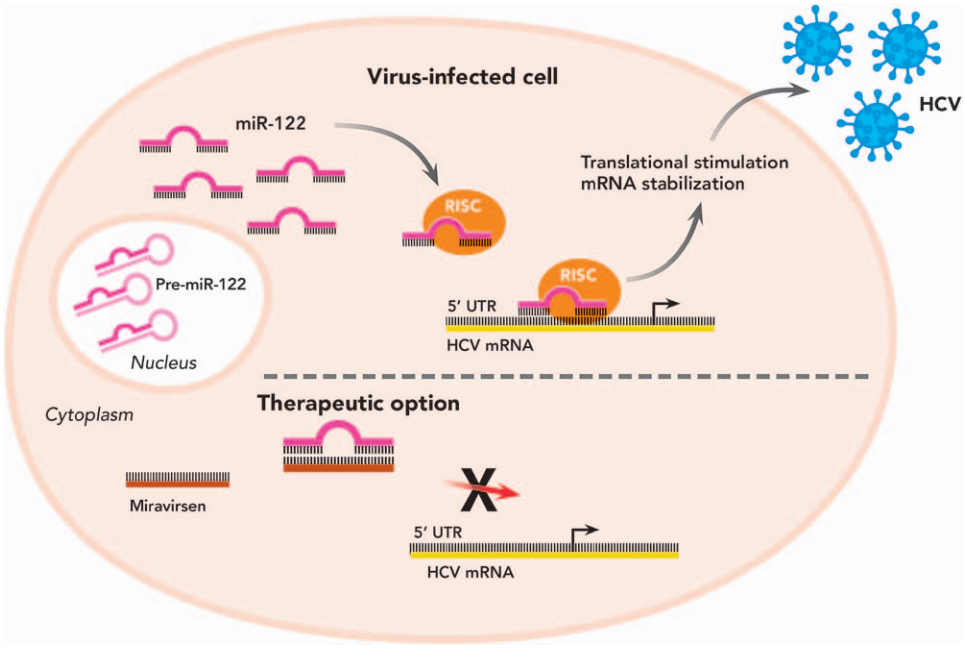

Fig. 5. MicroRNA miR-122 in hepatitis C virus (HCV) infection. After infection of a hepatocyte with the HCV, miR-122 promotes virus replication *via* different mechanisms. In contrast to the typical location of the microRNA binding in the 3 prime untranslated region (3′ UTR) of the target mRNA, miR-122 binds in the 5 prime untranslated region (5′UTR) of its target gene, the HCV mRNA. Opposite to target gene repression *via* binding to the 3′UTR, miR-122 binding in the 5′ UTR and recruiting RNA-induced silencing complex (RISC) stimulate viral protein translation and stabilize the viral mRNA thereby promoting the propagation of HCV. SPC 3649 (Miravirsen), a locked nucleic acid oligonucleotide that binds to and sequesters miR-122, results in functional miR-122 inhibition. A study with 36 patients with chronic HCV genotype 1 infection who received five weekly subcutaneous injections of SPC 3649 resulted in prolonged dose-dependent reductions in HCV RNA levels. The patients received doses of 3, 5, or 7 mg/kg of body weight or placebo subcutaneously over a 29-day period and had been followed until 18 weeks after randomization. The study revealed no evidence of viral resistance or dose-limiting side effects.[103]

A well-studied miRNA involved in the control of virus infection is the liver-specific miRNA miR-122. In contrast to most other miRNAs, this miRNA is known to preferentially bind to the 5′UTR of its target mRNA (or to viral RNA). Importantly, miR-122 binding to the 5′UTR of its target causes increased expression of its target. In other words, binding of miR-122 to its target promotes expression of its target gene. Indeed, miR-122 was shown to directly bind to the HCV RNA, and thereby promoting its replication by various mechanisms, including direct binding to the 5′UTR of the viral genome,[31] as well as by stimulation of viral protein translation[100] and delayed decay of the HCV RNA in infected cells[101] (fig. 5). Studies revealed that specific inhibition of this particular miRNA efficiently suppresses virus replication. This finding was first shown in simple cell culture experiments. The inhibitory compound, SPC 3649 (*miravirsen*), is an antisense oligonucleotide that is complementary to miR-122. It binds with a high affinity and specificity to miR-122. Sequestering of miR-122 prevents miR-122 from protecting HCV mRNA and from stimulating its translation (fig. 5). In subsequent studies, the antiviral effect of miR-122 inhibition was also successfully translated in nonhuman primates.[102] Inhibition of miR-122 in chronically infected chimpanzees lead to long-lasting suppression of HCV viremia and derepression of miR-122 target mRNAs. Although the treated animals showed no side effects during treatment, their liver biopsies revealed improved HCV-induced liver pathology. Following these studies, subsequent studies took these findings from the research laboratory into a clinical setting. Indeed, the antiviral effect of miR-122 inhibition was proven and confirmed in humans.[103] In a randomized study, 36 patients suffering from chronic HCV genotype 1 infection received five weekly injections of SPC 3649 at different doses (3, 5, or 7 mg/kg of body weight) or placebo. SPC 3649 was reconstituted to a concentration of 150 mg/ml and was administered subcutaneously over a 29-day period. All patients were followed for 18-week postrandomization. The SPC 3649 treatment resulted in a dose-dependent reduction in HCV RNA levels lasting beyond the 29-day period of therapy. In addition, in some patients who received higher doses of SPC 3649, HCV RNA was not detected in the 14 weeks of follow-up after treatment. Among all patients, there were no dose-limiting adverse events and also no escape mutations in the miR-122-binding sites of the HCV genome were discovered. Today, the specific miRNA-122 inhibitor SPC 3649 is the first miRNA therapeutic that successfully entered a clinical phase II.[103]

Biomarker

MicroRNAs are likely to "experience" their first successful integration into routine clinical practice based on their

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applicability as biomarkers of various disease processes. Due to their high abundance and stability and the ease in accessing them in body fluids, this could be particular relevant to the field of perioperative medicine. Expression levels of circulating miRNAs are significantly altered in various diseases indicating that they may serve in diagnosis and prognosis of disease, in monitoring treatment responses, and in evaluating perioperative risks. Changes in extracellular miRNA expression can occur due to damaged tissue where cells simply release their contained intracellular miRNAs. In other cases, extracellular miRNAs could be actively transcribed, secreted, and transferred to other cells as part of a cellular response and in order to interact with other cell types. In the cancer field, the potential diagnostic and prognostic value of circulating miRNAs is already well established. For example, a large prospective miRNA profiling study (phase III, ClinicalTrials.gov identifier: NCT02247453) used plasma from volunteers who are heavy smokers and assessed miRNA expression as a first-line screening test for lung cancer detection (table 1). Although the development of miRNAs in the cancer field is very advanced, other fields are catching up. Analysis of human plasma from patients with acute coronary occlusion revealed highest sensitivity and specificity for miR-208a for the early diagnosis of acute myocardial infarction.[104] Subsequent studies in a rat model of myocardial infarction revealed that miR-208a was undetected in plasma samples at 0 h but was significantly increased as early as 1 h postcoronary artery occlusion, presumably due to release from the injured myocardium. In patients with acute myocardial infarction, miR-208a appeared in less than 4 h after the onset of chest pain, with levels correlating with later peaking troponin and infarct size.

In patients suffering from sepsis, analysis of circulating miRNA levels revealed dynamic changes that correlate with different stages of disease, prognosis, and outcome.[92,105–107] Analysis of extracellular miRNAs could also help identifying the early onset of acute kidney injury (AKI). Kidney failure presents a frequent perioperative complication, and even minor increases of perioperative creatinine levels are associated with prolonged hospital length of stay and increased mortality.[108] Studies revealed that during kidney injury, miR-210 was up-regulated in plasma samples derived from AKI patients.[109] Moreover, circulating levels of miR-210 were shown to correlate with outcome of critically

Table 1. Examples of Ongoing Clinical Trials Involving miRNAs (Available at: https://www.clinicaltrials.gov)

Disease (Intervention)	Patient Population	Purpose of Study	ClinicalTrials.gov Identifier (Status)
Inflammatory bowel disease	Ulcerative colitis, Crohn's disease, healthy non-IBD colitis	Evaluate the diagnostic value of miRNAs for inflammatory bowel disease	NCT02020382 (recruiting)
Asthma (budesonide)	Asthmatics, healthy nonasthmatic controls	Evaluate the role of miRNAs in Th2-driven inflammation in asthma	NCT01484691 (recruiting)
PAH	Patients undergoing right heart catheterization for PAH diagnosis as part of their clinical care, controls: patients who present with symptoms of PAH and whose clinical right heart catheterization does not support this diagnosis	Analyze the expression and significance of miRNA profile and markers of inflammation in patients with PAH	NCT00806312 (recruiting)
Postoperative delirium in patients undergoing hip arthroplasty	Hypoactive and hyperactive delirious patients, patients not presenting any symptoms of hypoactive or hyperactive postoperative delirium	Identification of specific circulating miRNAs and microemboli formation (diagnosed by TC Doppler) in both delirious groups and nondelirious group	NCT02323984 (recruiting)
AKI in postcardiac surgery	Adult cardiac surgery patients (> 16 yr) undergoing coronary artery bypass grafting or valve surgery with moderately hypothermic blood cardioplegia at increased risk for AKI	Identify the role of microvesicles and microvesicle-derived miRNA in postcardiac surgery AKI	NCT02315183 (recruiting)
Acute lung inflammation (bronchial segmental endotoxin instillation, 4 ng/kg)	Healthy individuals (18–45 yr) with no significant medical problem, no medication, nonsmoker	Identification of cell-associated and secreted miRNAs with specific types of resident and inflammatory cells in the lung	NCT02392442 (recruiting)
Cardiac arrest	Patients with cerebral performance category score 1–2 *vs.* 3–5 judged half a year after cardiac arrest	Evaluate the plasma levels of miRNAs and circular RNAs after cardiac arrest and their ability to prognosticate neurologic outcome	NCT02297776 (recruiting)
Primary liver cancer/liver metastasis (miRNA miR-RX34 liposomal injection)	Patients with primary liver cancer or those with liver metastasis from other cancers	Evaluate the safety of MRX34 (multicenter phase I study)	NCT01829971 (recruiting)

AKI = acute kidney injury; IBD = inflammatory bowel disease; miRNA = microRNA; PAH = pulmonary arterial hypertension.

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ill patients suffering from AKI. This study suggested that miR-210 is an independent and powerful predictor of 28-day survival. This indicates that miR-210 could be used as novel biomarker of stratifying the prognosis of critically ill patients with AKI.[109] Some miRNAs (*e.g.*, miR-494) also showed altered expression in urine samples providing an additional source for detecting miRNAs during AKI.[110] Interestingly, it was shown that during AKI, many miRNA alterations in serum or urine can be detected even before serum creatinine starts increasing.[110] Consequently, these miRNAs have great potential as biomarkers, as they might allow an earlier diagnosis leading to an earlier onset of therapy resulting in benefit in patient outcome.

Currently, a major challenge for the field of miRNA biomarkers represents the technical difficulty for miRNA measurements using standardized technology. Still, more specific and sensitive miRNA detection methods are needed that help illuminating their role in diverse disease and critical health states. Working with reliable and reproducible miRNA measurement technologies will increase the opportunities to benefit from miRNAs in perioperative medicine.[111]

Pharmacologic Functions of miRNAs and miRNA Therapeutics

Effects of miRNA on Drug Metabolism

Recent studies elucidated an important role of miRNAs in mediating and modulating the effects of various common drugs. It is not surprising that this field of research has generated considerable interest for the miRNA field, including its application in perioperative medicine.[112–115] Similar to any other target gene, miRNAs can target genes that code for enzymes that are important for drug metabolism. Based on so-called miRNA pharmacogenomic studies, differing miRNA expression levels can affect the ability of drugs to be activated, thereby altering a drug's efficiency or toxicity.[116–118] A conceivable scenario would be an miRNA target that serves as binding partner or transporter for a specific drug. In cases where the desired protein is reduced due to miRNA-mediated gene regulation, the drug efficiency will be equally reduced, resulting in insufficient therapy. In the opposite situation, miRNAs could exaggerate drug effects by repressing drug metabolism. Thereby, drug dosages could reach toxic levels.

A very prominent drug metabolizer is the cytochrome CYP3A4 protein. It is relevant for the turnover of more than 50% of commonly used drugs.[119] Human drug responses are highly dependent on the individual expression level of CYP3A4; therefore, the regulation of this master metabolizer is equally regulating the resulting drug-induced effects. So far, there are various reports about miRNAs regulating CYP3A4, but only a few miRNAs have been identified that are likely to have a profound regulatory impact on drug efficiency.[120] Studies in human liver biopsies revealed a significant linear correlation of four miRNAs, miR-577, miR-1, miR-532-3p, and hsa-miR-627 with reciprocal translational efficiency of CYP34A. Subsequent functional analysis confirmed direct targeting of CYP3A4 by miR-577, miR-1, miR-532-3p, and miR-627 and repression of protein synthesis of CYP3A4. Therefore, these miRNAs serve as top candidates contributing to the interindividual variability in CYP3A4 levels in human population. Possibly affecting a large group of patients, ongoing studies investigate the possibility that the pharmacologic efficiency of the anticoagulant warfarin could be affected by miRNAs. Due to its narrow therapeutic window, knowledge about additional regulatory levels of warfarin metabolism and action could help avoid adverse drug events. CYP1A1, one of the enzymes responsible for warfarin metabolism, contains a binding site for miR-125b and was experimentally shown to be regulated by miR-125b.[121] The vitamin K epoxide reductase complex subunit 1 (VKORC1) gene, the molecular target for warfarin, was also found to carry two binding sites for miRNAs, miR-133 and miR-137.[122] Studies in human liver samples from healthy subjects revealed a significant and inverse correlation between miR-133a and VKORC1 levels, indicating that miR-133a might be involved in regulation of VKORC1 expression. Results from different subsequent experimental approaches delivered evidence that miR-133a can regulate VKORC1 expression.[122] These findings illustrate a high likelihood that these miRNAs effecting warfarin efficiency are clinically relevant and make it highly plausible that anticoagulatory therapy in future could be optimized and made safer by monitoring or modulating specific miRNA levels.

Role of MiRNAs in Mediating Anesthetic Toxicity

A research direction that is currently under intense investigation and excitement for the field of perioperative medicine is the area of anesthetic neurotoxicity, relating to the potential neurotoxic effects of commonly used anesthetics, in particular in newborn and infants.[123,124] These studies associate exposure to common anesthetics with neurodegenerative and abnormal processes in the developing brain, which may affect learning and behavior in the course of later life.[125–128] Although initial studies were performed in immature rodent pups, recent studies also include evidence from nonhuman primates.[129,130] Interestingly, miRNAs have been investigated in this context. To date, a number of *in vitro* studies confirm miRNA expression changes after exposure to volatile anesthetics.[131–133] Other studies demonstrated effects of propofol on human stem cells, leading to alterations in miRNA expression levels.[15,134] In addition, some recent studies also indicate functional relevance for certain miRNAs in the pathogenesis or protection from neurotoxicity, respectively. Moreover, some miRNAs have already been identified to present potential targets that could be modulated to enhance the miRNA-mediated neuroprotective effects.[15,16,135] For example, studies examining the human embryonic stem cell–derived neurons *in vitro* identified a relevant role for miR-21 in anesthetic-induced neurotoxicity.[15] The findings of this study showed that propofol-induced

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cell death in human embryonic stem cell–derived neurons involved down-regulation of miR-21 and subsequently resulted in an increased expression of its target gene Sprouty 2 (SPRY2), a regulator of multiple receptor tyrosine kinases. SPRY2 acts as negative feedback regulator of multiple receptor tyrosine kinases causing attenuation of growth factor–mediated pathways, such as cell migration and cellular differentiation. Experimental overexpression of miR-21 significantly attenuated cell death of human embryonic stem cell–derived neurons induced by propofol. Consequently, reversing the propofol-induced down-regulation of miR-21 could play a pivotal role in protecting from anesthetic-induced neurotoxicity. At this point, confirmatory functional studies *in vivo* are the next step to take. For example, studies in mice with genetic deletion of miR-21 could help to further identify a functional role of miR-21 in anesthetic-induced cell death. Based on these findings, the prediction would be that miR-21 knockout mice receiving propofol are protected from neuronal cell death *via* targeting genes attenuating the mediation of anesthesia-induced neurotoxic effects. Moreover, studies examining miRNA expression levels in patients undergoing propofol anesthesia would be important to help transitioning this area of research from bench to bedside.

Similar to the study mentioned in the previous paragraph, investigations in rats at the age of 1 month who received ketamine anesthesia revealed ketamine-induced apoptosis of hippocampal neurons, memory deficiency, and significant down-regulation of miR-137.[16] Pretreatment with miR-137 *via* lentiviral-mediated miR-137 overexpression protected from hippocampal neurodegeneration and long-term memory dysfunction. Again, more functional studies in genetic models are needed to confirm these results. Subsequent steps will also need to include the identification of relevant miRNA target genes that could be modulated. Surprisingly, at present, there is only very limited availability of data from human studies focusing on the role miRNAs during anesthesia. For example, further analysis of miRNAs comparing expression patterns pre- and postanesthesia in patient populations exposed to different types of anesthetic agents will be of relevance to help study human miRNA responses to anesthetics *in vivo*.

Pharmacologic Approaches Targeting miRNAs

miRNA Inhibition. MicroRNAs represent a novel group of gene regulators that can be targeted for therapeutic treatment. From a pharmacologic perspective, targeting miRNAs, in particular suppressing them, can be achieved relatively easily and reliably. Based on a large number of successful experimental loss-of-function studies, current effort is focused on developing reliable methods to inhibit specific miRNAs *in vivo* to repress their unwanted function in disease. The inhibition of miRNA functions is usually obtained by using chemically modified oligonucleotides, named anti-miRs, or so-called "miRNA sponges." miRNA sponges represent RNA produced from transgenes within the cells, with complementary binding sites to the miRNA of interest. MiRNA sponges—blocking miRNAs by acting as competitive inhibitor—serve as excellent experimental devices. However, from a therapeutic perspective, anti-miRs represent the more promising approach (fig. 6). In fact, several modifications have been tested over the last

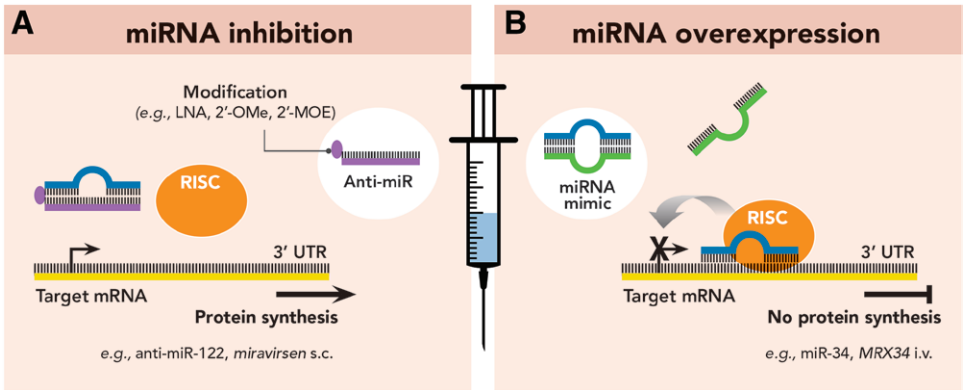

Fig. 6. Pharmacologic approaches to target microRNAs (miRNAs). miRNAs can be targeted for treatment. (*A*) Chemically modified RNA molecules can function as miRNA inhibitors (anti-miRs). They bind to specific endogenous miRNAs and inhibit their function. This will rescue a specific miRNA from miRNA-mediated regulation. The most successful chemical modification increasing the hybridization properties of the RNA molecule are locked nucleic acids (LNAs). An anti-miR that recently successfully entered a clinical phase II study is SPC 3649 that binds to and blocks the function of miR-122, an miRNA that is required for the hepatitis C virus replication.[103] (*B*) miRNA mimics are double-stranded miRNAs that intend to mimic the endogenous miRNA. They comprise the same nucleotide sequence as the desired endogenous miRNA and are designed to target the same mRNAs by binding in the 3 prime untranslated region (3′UTR) and recruiting RNA-induced silencing complex (RISC). A miR-34 mimic, *MRX34*, currently entered a phase I clinical trial (ClinicalTrials.gov identifier: NCT01829971) to be tested in patients with primary liver cancer or patients with liver metastasis from other cancers. In this study intended to investigate safety, pharmacokinetics, and pharmacodynamics of *MRX34*, the drug will be administered intravenously (IV). 2′-OMe = 2′-*O*-methyl; 2′-OME = 2′-*O*-methyoxyethyl; s.c. = subcutaneously.

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decade with the goal to optimize binding affinity, stability, and pharmacokinetics of designed oligonucleotides. Also, the desired therapeutic modifications should reduce any off-target effects and unwanted immune responses and avoid interference with the endogenous miRNA machinery. Examples for these modifications at the 2′ position of the sugar ring are 2′-*O*-methyl, 2′-*O*-methyoxyethyl, and LNA modifications (fig. 6). The most successful modification getting closest to fulfilling all the criteria mentioned in this paragraph, and providing strongest nuclease resistance as well as exhibiting highest RNA affinity, are sugar-modified LNAs. Here, the ribose is "locked" by a bridge connecting the 2′ oxygen and 4′ carbon in the RNA molecule. In addition, antisense oligonucleotides containing LNAs have been shown to be nontoxic and produce high *in vivo* efficiency.[136] Targeting disease-associated miRNAs implies a very potent approach because miRNAs usually have several targets within a pathway and could therefore modulate the underlying disease mechanisms efficiently *via* targeting several key proteins.

The miRNA-122 inhibitor named SPC3649 (*miravirsen*; Santaris Pharma, Denmark) also represents an LNA. SPC3649 robustly inhibited miR-122 function in liver cells *ex vivo*[137] and was further shown to inherit a high efficiency in antagonizing miR-122 in mice *in vivo*[138] and the same could later be confirmed in primates.[137] In addition, subsequent favorable results from the preclinical toxicology studies in monkeys paved the way for SPC3649 into clinics.[139] Today, SPC3649 represents the very first miRNA-targeted drug that progressed to clinical phase II of the first human trial modulating an miRNA.[103]

At this point, it is important for the clinicians to be aware of possible interactions of miRNA therapy with its targets. For example, the same miRNA that is worth blocking in one disease condition could be harmful if suppressed in another situation. In cancer, a prominent miRNA that was shown to act "good" or "bad" depending on the tumor entity is miR-125b.[140] Similar scenarios where positive and negative effects of one specific miRNA are conflicting can be easily imagined. For example, clinical trials have not revealed any adverse side effects for SPC 3649. However, in situations where patients are suffering from additional disease and/or undergoing a stress situation (*e.g.*, major surgery), unwanted effects might occur. The miRNA therapy that protects in a certain primary disease, here anti-miR-122 in HCV, could harm the patient by preventing a desired miRNA-mediated effect (*e.g.*, organ protection during liver surgery). For example, a study in genetic models demonstrates that deletion of miR-122 in mice resulted in liver pathologies including hepatosteatosis, hepatitis, and the development of tumors resembling HCC.[141] These findings indicating antiinflammatory and antitumorigenic functions of miR-122 should be taken under critical consideration in patients who are subjected to HCV therapy containing anti-miR-122 substances.[142]

miRNA Overexpression. Although progress in therapeutic inhibition of miRNAs has already reached clinical phase II (table 1), the approach of restoring miRNA functions is more difficult and challenging. The goal of miRNA overexpression is to restore the lost or reduced miRNA expression but at the same time to avoid side effects from nonphysiologic high miRNA levels. Moreover, the chemical structure of miRNA mimetics has to be as close to its physiologic appearance as possible to allow for normal loading into the RISC complex and resulting in the desired effects mediated by the miRNA of interest. The big challenge here is to meet desired opposing requirements: restore a close-to-physiologic-structured miRNA for optimal function and avoid nonphysiologic miRNA levels to prevent adverse side effects, but also modify the agent to ensure efficient and controlled cellular uptake. To reintroduce miRNAs, either synthetic miRNAs, so-called mimics, or viral vector–based miRNA, overexpression is used (fig. 6). miRNA mimics represent double-stranded miRNAs with chemical modifications improving their stability and increasing probability of their cellular uptake. The strand that is identical to the miRNA of interest is ensuring the RISC-loading process—similar as with the native miRNA. Therefore, its modification is minimized. In contrast, the partner strand is modified to improve the mimic's properties. To prevent RNA degradation and to enhance stability and cellular uptake, the mimics frequently have to be packaged into lipid emulsions.[143] Because in cancer, it was found that miRNAs are often lost or downregulated,[3] there is a high interest for developing miRNA replacement therapies. Although various cancer tissues have been screened and revealed specific aberrant miRNA expression patterns,[3] still only few miRNAs have been analyzed functionally in depth. One of those is miR-34a. MiR-34a plays a key role as master regulator of tumor suppression. Strikingly, this antitumor activity of miR-34 revealed profound effects in a mouse model of hepatocellular carcinoma after its systemic delivery.[144,145] Based on these and other studies, miR-34a recently became the pioneer miRNA in miRNA-based replacement therapies to enter the clinic. In 2013, a miR-34a analog (*MRX34*) has become the first miRNA mimic to enter a phase I clinical trial (ClinicalTrials.gov identifier: NCT01829971). *MRX34* is currently tested in patients with unresectable primary liver cancer or advanced or metastatic cancer with liver involvement or hematologic malignancies. According to the study design, *MRX34* is administered IV in two different regimes, twice a week for 3 weeks with 1 week off or daily for 1 week with 2 weeks off. The present trial is focused on safety, pharmacokinetics, and pharmacodynamics. Nevertheless, this clinical trial represents an important landmark in the development of miRNA-based therapeutics.

Oncologic surgery is represents a rapidly growing field of surgical interventions—mainly related to the fact that patients at advanced ages are more likely to experience neoplastic diseases. Cancer patients usually receive chemotherapies or

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radiotherapies before or after a surgical intervention. Therefore, it is feasible that in the imminent future, a patient who is undergoing tumor resection will be also receiving an miRNA-targeting medication. Therefore, physicians acting in the perioperative field will benefit from knowledge about the mechanisms and potential side effects of those new therapeutic approaches. They may benefit from knowledge of the target of the miRNA therapeutics and also their pharmacodynamics and pharmacokinetics. For example, although miR-34a inhibition could be a promising approach in aged patients suffering from heart failure or undergoing cardiac surgery,[72] the well-known tumor suppressor function of miR-34a could be affected in an unwanted direction. MiR-34a mimics have already been shown to qualify for a novel therapeutic approach *in vivo* and *in vitro*.[146] As such, patients who have suffered from multiple myeloma, now receiving anti-miR-34a for cardioprotection, may simultaneously be at higher risk for developing tumor relapse.

The biggest challenge in miRNA-based therapeutics, regardless if they are designed to restore or repress miRNA functions, is finding a safe and efficient strategy of delivery. This delivery method needs to assure that the miRNA therapeutic reaches its specific target cell and that its local concentration is sufficient to induce the desired effect in the target cells. Currently, the most promising method to deliver synthetic double strands is to use agents that form complexes with overexpressing mimics (*e.g.*, liposome nanoparticles).[143,147] This method is also used in the first miRNA restoring therapy that recently entered clinical phase I.

An additional important aspect of miRNA therapy, including anti-miRNA or miRNA mimetic, is drug monitoring. Although the plasma levels of miRNA-targeting therapeutics are cleared within a short time due to cellular uptake, their high metabolic stability provides long half-lives reaching weeks to months. Consequently, although their blood levels are undetectable, their therapeutic effects of miRNA targeting may still last.[148] Therefore, predictions about pharmacologic effects and their duration can only be controlled by analyzing target tissue concentrations. In addition, the "delayed nature" of the pharmacologic effects of the miRNA-targeting substances is complicating their monitoring. Although the particular miRNA is immediately antagonized or acting as analog, the downstream effects of derepression or repression of families of genes need time, as they predominantly depend on the half-life of a specific protein, and half-life of proteins are highly variable. In addition, important consideration for miRNA therapies is the route of treatment. At present, there are no successful oral applications for anti-miRs or miRNA mimics available, so that the two application routes currently used are IV or subcutaneous injections.

Challenges and Conclusions

The speed the miRNA field developed from bench to bedside is remarkable. The entrance into clinical trials of both, miRNA-replacement and miRNA-suppression, therapies are the most recent achievements in this rapidly evolving course. Initially, miRNA-based therapies were mainly seen in the cancer field. Here, most recent achievements are highlighted in successful antitumor treatment in models of murine non–small-cell lung cancer and hepatocellular cancer.[143,145,149] Further preclinical studies with systemic delivery of the tumor suppressor miR-34a in various cancer models revealed significant tumor growth suppression and survival advantage.[150,151] These results justified miR-34 to be the first miRNA mimic to reach the clinic, currently in a multicenter phase 1 clinical trial (table 1; ClinicalTrials.gov identifier: NCT01829971). Entering the term "miRNA" in the search query at ClinicalTrials.gov reveals a total of 257 hits. An extraction of some studies relevant for the perioperative medicine displayed in the table 1 highlights that miRNA-based therapies are about to reach various fields of human disease (table 1). Therefore, it is very likely that anesthesiologists will soon be involved in the care of patients receiving miRNA-based therapy and should be aware of the broad involvement of these small molecules and their impact on the posttranslational regulation of gene expression in diverse physiologic and pathologic conditions.

Although there are numerous bioinformatic studies reporting the changes in miRNA expression levels and correlating those with specific disease components, there are many examples where we still lack functional insight toward understanding the full impact of miRNA activities in the perioperative field. Although there are studies delineating miRNA expression in different disease states, we still need additional studies to address the functional consequences of these differentially expressed miRNAs. What specific downstream effects are mediated by aberrantly expressed miRNAs and what are the consequences with regard to neurologic and immunologic outcome and hemostasis? Critical factors for a successful miRNA study should include characterization of miRNA expression levels, followed by functional investigations with gain-of-function and loss-of-function studies. Identifying and further verifying a relevant target is the biggest challenge of any miRNA study, and consequently among the numerous miRNA studies, this key feature is only present in the minority of published reports. But the more we know about the downstream effects that are caused by aberrant expression of specific miRNAs, the more precise we can modulate them, monitor therapy, and consequently benefit from the potential miRNA-based therapies bear. It is conceivable that patients who are at risk for developing a specific disease (*e.g.*, AKI) could be treated with an miRNA mimic or inhibitor that would dampen the risk for developing this specific type of organ injury after elective surgery. Indeed, the fact that many surgical patients are examined weeks before an elective surgical intervention would allow for preoperative treatment with such medications. Similarly, we anticipate that additional mechanistic research on the role of miRNAs in mediating toxic side effects could ultimately

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lead to altered anesthetic approaches in patients with specific miRNA expression patterns. Taken together, we believe that the described emerging roles of miRNAs have many implications for perioperative medicine and will soon become an integral part of the daily practice of anesthesiology, critical care, and emergency medicine.

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Competing Interests

The authors declare no competing interests.

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