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MicroRNAs in veterinary cardiology

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ABSTRACT: *The use of biomarkers is an important recent development in veterinary medicine. Biomarkers allow non-invasive quantification of substances with diagnostic and prognostic potential in several diseases. The microRNAs are small, non-coding RNAs that regulate gene expression and are expressed in different forms in many diseases. Reduced or over-expression of microRNAs showed to be part of the pathogenesis of some heart diseases in humans and animals. Diagnostic and therapeutic value of measuring microRNAs in veterinary cardiology is increased because abnormal expression can be managed by the use of antagonists (in the case of overexpression) and mimicking (in the case of underexpression). Thus, this literature review aimed to compile scientific evidence of dysregulation of microRNAs expression in different cardiac diseases being one of the promises in the therapeutic field and diagnosis of veterinary cardiology. MicroRNAs not only have potential as a biomarker but may also help in elucidation of aspects of the pathogenesis of a variety of diseases.*

Key words: molecular biology, genetic, biomarker, heart failure.

MicroRNAs em cardiologia veterinária

RESUMO: *Os biomarcadores vêm crescendo na medicina veterinária, pois permitem, de forma não invasiva, a quantificação de substâncias com potencial para avaliação prognóstica e diagnóstica em diversas doenças. Os microRNAs são pequenos RNAs não codificantes que regulam a expressão de genes e são expressos de diferentes formas em diversas doenças. A sua super ou subexpressão já foi evidenciada como parte da patogênese de cardiopatias em seres humanos e animais. O controle dessa anormalidade de expressão pode ser obtido pela utilização de antagonistas (em casos de superexpressão) e mimetizadores (em casos de subexpressão). Dessa forma, esta revisão de literatura tem como objetivo compilar as evidências científicas da desregulação da expressão de microRNAs nas diferentes doenças cardíacas, sendo essa uma das promessas no campo terapêutico e diagnóstico da cardiologia veterinária, permitindo não só a sua utilização como biomarcador, mas também com aspecto elucidativo da patogênese de diversas doenças.*

Palavras-chave: biologia molecular, genética, biomarcador, insuficiência cardíaca.

INTRODUCTION

MicroRNAs are small, non-coding, endogenous RNAs that modulate a large and complex set of gene expression. MicroRNAs are potentially involved in a wide range of biological and pathological events. They are composed of approximately 19 to 25 nucleotides (ZHANG et al., 2015), transcribed from intranuclear DNA and transported to the cytoplasm, where a pre-microRNA is cleaved into mature microRNAs (Figure 1) (CREEMERS et al., 2012). Increasingly, new evidence points to the role of microRNAs in the pathogenesis of several diseases. In recent years, there has been an increased interest in genetic and genomic markers of heart diseases

and the evaluation of microRNAs expression may provide information for their use as biomarkers, since such molecules act in many ways on cardiomyocytes, with roles from basic maintenance to the promotion of remodeling in congestive heart failure (IKEDA e PU, 2010; ORENES-PIÑERO et al., 2015).

Given that the pathogenesis of some diseases involves under or overexpression of microRNAs (Table 1), the evaluation of microRNA expression may be used in therapeutic planning. Thus, therapy aims to antagonize microRNAs that are overexpressed and mimic the action of those that are underexpressed. However, the low specificity of the gene in question may provide problems for therapeutic targeting (XIAO et al., 2007; ROMAINE et al., 2015).

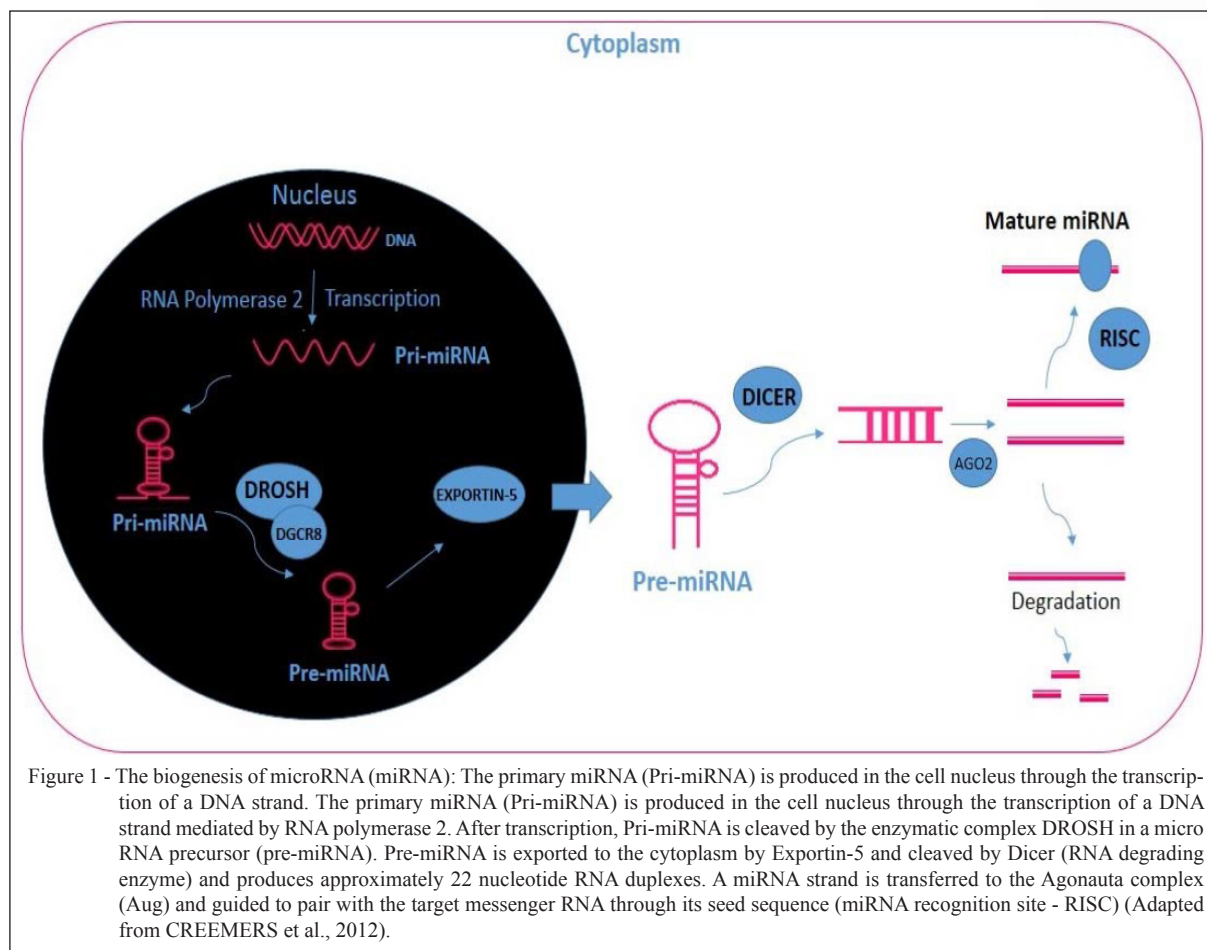


Figure 1 - The biogenesis of microRNA (miRNA): The primary miRNA (Pri-miRNA) is produced in the cell nucleus through the transcription of a DNA strand. The primary miRNA (Pri-miRNA) is produced in the cell nucleus through the transcription of a DNA strand mediated by RNA polymerase 2. After transcription, Pri-miRNA is cleaved by the enzymatic complex DROSH in a micro RNA precursor (pre-miRNA). Pre-miRNA is exported to the cytoplasm by Exportin-5 and cleaved by Dicer (RNA degrading enzyme) and produces approximately 22 nucleotide RNA duplexes. A miRNA strand is transferred to the Agonauta complex (Aug) and guided to pair with the target messenger RNA through its seed sequence (miRNA recognition site - RISC) (Adapted from CREEMERS et al., 2012).

In veterinary cardiology, the study of new biomarkers is important because in general, the diseases are progressive and many have a genetic basis, such as feline hypertrophic cardiomyopathy (WEBER et al., 2015). The study of the microRNAs can facilitate the understanding of the etiopathogenesis, diagnosis and collaborate with the treatment of these diseases (XIAO et al., 2007; BOSWOOD, 2009). This article reviews the literature to demonstrate the dysregulation of the expression of microRNAs in various cardiac diseases in veterinary medicine and its potential as a biomarker and therapeutic approach.

DEVELOPMENT

Most studies that have evaluated microRNA expression have performed serum or tissue quantification (FUJIWARA-IGARASHI et al., 2015; WEBER et al., 2015; DIRKSEN et al., 2016). Interestingly, some studies have tried to measure microRNA expression in other locations including cardiac cells (ORENES-

PIÑERO et al., 2015; POWERS et al., 2015). CHEN et al. (2014), for example, reported that some microRNAs were expressed differently in the left atrium and ventricle in dogs with CHF, and identified eight microRNAs that were selectively expressed in fibroblasts and three in cardiomyocytes. Understanding where these microRNAs are located may facilitate understanding of their role in the pathogenesis of various heart conditions.

The possibilities offered by microRNAs make it one of the most exciting areas of modern veterinary medicine. Changes in expression are correlated with pathogenesis of several diseases, such as viral infections, inflammatory, neoplastic, metabolic and autoimmune diseases and they may have utility as biomarkers (FUJIWARA-IGARASHI et al., 2015; WEBER et al., 2015). Several cardiac biomarkers have already been shown to be useful in the evaluation of a variety of diseases (BOSWOOD, 2009) and, similarly, the evaluation of microRNA expression in heart diseases may well provide a noninvasive technique for gathering information to aid diagnosis and prognosis which could

Table 1 - Summary of the literature review evidencing dysregulation of the expression of microRNAs in various diseases.

Disease	n	Overexpression	Underexpression	Author
MVD	18 dogs	cfa-miR-103 cfa-miR-98 cfa-let-7c cfa-let-7b	cfa-miR-487b cfa-miR-582	LI et al., 2012
MVD	24 dogs	miR-133b miR-125 miR-126 miR-21	miR-30b miR-29b	HULANICKA et al., 2014
Secondary DCM	10 dogs	miR-21 miR-221 miR-582	miR-151	POWERS et al., 2015
HCM	23 cats	miR-381-3p miR 486-3p miR5700 miR-513a-3p miR-320E	-	WEBER et al., 2015
Induced concentric cardiac hypertrophy	6 mice	miR-21 miR-31 miR-34b-3p miR-132 miR-142 miR-214 miR-222 miR-1	miR-30b-5p miR-30c miR-30e miR-133a miR-133b miR-150 miR486-5p miR-499-5p	BAGNALL et al., 2015
Atrial fibrillation	12 dogs	miR 206 miR-224	miR-137 miR-203	ZHANG et al., 2015

N = number of subjects in the study, MVD = mitral valve disease, DCM = dilated cardiomyopathy, HCM = hypertrophic cardiomyopathy.

lead to the development of new therapeutic protocols. In cardiology, microRNA expression has an important role in the development of heart failure and cardiac remodeling (CONDORELLI et al., 2014). Congestive heart failure (CHF) triggers a series of changes in the expression of various microRNAs, such as miR-1, miR-21, miR-23, miR-133, miR-199, miR-208 and miR-320, but the complexity of the expression system makes understanding the action of individual microRNAs a great challenge (TOPKARA & MANN, 2011).

MicroRNAs have also been used as biomarkers in other areas of veterinary medicine. A

recent study investigated the use of microRNAs, in particular miR-122, as a biomarker to assess hepatocyte damage in Labrador Retriever dogs (DIRKSEN et al., 2016). Evaluation of 5 of 277 microRNAs demonstrated different expression in dogs with lymphoma, with let-7b, miR-223, miR-25 and miR-92a being under expressed while miR-423A was overexpressed compared with the control group (FUJIWARA-IGARASHI et al., 2015).

The study conducted by THUM et al. (2008), demonstrated that the use of miR-21 antagonists in rats with concentric cardiac hypertrophy induced by pressure overload, significantly reduced remodeling, improving

systolic function. Furthermore, histopathologic evaluation of the myocardium demonstrated reduction of fibrosis in treated animals. MicroRNA mimics can be used to treat diseases where there is subexpression microRNAs. MicroRNA mimics are synthesized from nucleic acids, and have the same effect as the microRNAs being mimicked (XIAO et al., 2007).

Despite a promise in veterinary cardiology exists, there are some obstacles to the use of microRNAs in the clinical routine, such as their instability in the sample and a degradation of the molecules, which may affect the results of the expression analyzes. For this, there are several extraction protocols aiming to minimize these effects (EIKMANS et al., 2013).

Degenerative mitral valve disease

Mitral valve disease (MVD) is a degenerative heart disease with a high incidence in small dogs (ATKINS et al., 2009). Although the echocardiographic and histopathologic findings and some biomarkers (e.g. natriuretic peptides) have already been well documented, genetic and molecular changes need to be better investigated (REYNOLDS et al., 2012).

Altered expressions of 11 microRNAs have been shown in MVD, and since these changes vary according to disease progression they also provide information on gene regulation in this disease. Symptomatic dogs (American College of Veterinary Internal Medicine (ACVIM) C and D) appear to exhibit different gene expression from asymptomatic dogs (ACVIM B1 and B2). In symptomatic animals there is underexpression of microRNAs cfa-miR-487b and cfa-miR-582 and overexpression of cfa-miR-103, cfa-miR-98, cfa-let-7c and cfa-let-7b (LI et al., 2012). This changes in gene expression may explain the genetic component of disease progression.

Another research, in Dachshunds showed that the expression of microRNAs is different between dogs with MVD in different phases. The miR-30b was under expressed in dogs with class B and C disease when compared to those with class A, while the microRNAs miR-133b, miR-125, miR-126, miR-21, miR-29b and miR-30b were less expressed in dogs with class C disease. These results emphasized the potential of miR-133b as a marker for stage C congestive heart failure (HULANICKA et al., 2014). In another study, that used an experimental model of mitral regurgitation in pigs, levels of miR-21 microRNAs and miR-133a expression were associated with the severity of mitral regurgitation (CIRERA et al., 2013).

There is evidence that the signaling pathway of transforming growth factor β (TGF- β) has an important role in the pathogenesis of MVD (DISATIAN

et al., 2009). MicroRNA 302 inhibits the TGF- β II receptors consequently decreasing the production of fibronectin and spondine which cause fibrosis. Reduced expression of miR-302 in disease and its role in the production of these fibrotic substances supports the theory of its close involvement in the pathogenesis of MVD (LI et al., 2015). Other microRNAs, such as miR-30 and miR-133 also regulated the action of TGF, a key molecule in the fibrosis process, and may represent an attractive target for the treatment of heart disease (HULANICKA et al., 2014).

The study of diagnostic and therapeutic approaches in MVD is extremely important because it is the most common cardiac disease in dogs, and has a progressive nature. The microRNAs are intended to be used as biomarkers, contributing to the diagnostic procedure and a better understanding of the pathophysiology of the disease, and may in the future help in the therapeutic approach with antagonists and agonists substances.

Cardiomyopathies in dogs

Expressions of different microRNAs have also been evaluated in hypertrophic cardiomyopathy and primary and secondary dilated cardiomyopathy (DCM) (STEUEDEMANN et al., 2013; JEANSON-LEH et al., 2014; WEBER et al., 2015).

DCM is the primary cardiopathy of large dogs and has high rates of morbidity and mortality (TIDHOLM et al., 2001). Studies in man have shown that several microRNAs are involved in the pathogenesis of DCM and are used as biomarkers for the disease in human patients, such as miR-423-5p microRNA for diagnosis of heart failure secondary to DCM (IKEDA & PU, 2010; FAN et al., 2013). A study with Doberman Pinscher dogs evaluated the expression of 401 microRNAs in serum and reported no differences in expression in any animal with DCM (STEUEDEMANN et al., 2013). This may be explained in part by the small number of animals in the study or by the type of sample analyzed. In contrast, several studies in people have used cardiac tissue samples instead of blood samples, since microRNAs are expressed differently in various tissues and cells (SATO et al., 2010; POWERS et al., 2015).

MicroRNA 208 is encoded by the heavy chain myosin gene and is closely involved in myosin regulation and myocardial fibrosis, as well as being involved in the progression of DCM in people (IKEDA & PU, 2010; SATO et al., 2010). One study documented increased expression of miR-208, miR-208b and miR-499 in myocardial samples obtained by biopsy in human patients with dilated cardiomyopathy

compared to the samples from the control group. Levels of α -heavy chain myosin messenger RNA were lower in DCM patients, whereas the β -heavy chains levels were higher and were associated with greater volumes of collagen in the myocardium (SATO et al., 2010). In other research, ZHOU et al. (2010) observed a significant increase in the risk of dilated cardiomyopathy in people with overexpression of some pre-microRNAs, as a consequence of common polymorphism of a single nucleotide, including miR-196a2 and HSA-miR-499, which influenced several genes with different functions.

An experimental model in dogs with induced dilated cardiomyopathy demonstrated that the expression of 20 microRNAs differed from that in control animals. There were differences in right ventricular (RV) expression of miR-211, miR-221, miR-582 and miR-151 compared to the left ventricle, a fact that may play a fundamental role in RV insufficiency in CHF (POWERS et al., 2015). Another study observed the expression of microRNAs in dogs with cardiomyopathy secondary to Duchenne muscular dystrophy. In this disease the miR-1, miR-133, miR-206, miR-378, miR-208, miR-208b, miR-499, miR-539 and miR-95 microRNAs are deregulated (JEANSON-LEH et al., 2014).

Feline cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is the most common cardiomyopathy in cats, although its pathogenesis is not fully understood, it is known to have a strong genetic background. According to WEBER et al. (2015), 49 microRNAs are related to cardiac hypertrophy in feline species. For example, greater expression of miR-381-3p, miR-486-3p, miR-5700, miR-513a-3p and miR-320E has already been recognized in cats with stable congestive heart failure secondary to HCM compared to the control group. In contrast, in mice with concentric left ventricle hypertrophy there is a decrease in the expression of miR-1 and miR-133 in the early phase of the disease, while in the terminal phase 16 microRNAs are deregulated. This dysregulation is associated with cardiac hypertrophy, calcium signaling, fibrosis and TGF- β signaling pathway (BAGNALL et al., 2015).

Arrhythmias

Heart failure results in remodeling of the atrium and left ventricle, creating substrates for the appearance of arrhythmias. Atrial fibrillation (AF) is a common arrhythmia in dogs and is histologically associated with atrial fibrosis (LI et al., 2012). In arrhythmias the expression of microRNAs can also be altered. Changes in miR-1 for

example, may lead to the development of arrhythmias, because miR-1 is closely related to genes regulating the ion channels and alterations in its expression may alter the ionic balance. Other microRNAs, such as miR-21, are overexpressed in cardiac remodeling, and miR-208 and miR-29, which are expressed primarily in fibroblasts, also have arrhythmogenic potential (ORENES-PIÑERO et al., 2015).

According to ZHANG et al. (2015), micro-cfa-miR-206 is overexpressed 9.5 times more in dogs with AF when compared to dogs without arrhythmia. Expression of 16 other microRNAs is also dysregulated in dogs with AF. Another study that included 42 dogs showed that miR-133 and miR-30, which are antifibrotic markers, were underexpressed in dogs with atrial fibrillation (LI et al., 2012). In human patients, RADTKE et al. (2014) showed that microRNA 208 is associated with fibrosis of the conduction tissue and allows the clinical categorization of individuals with atrial fibrillation, due to the observation of similar levels of microRNA 208 in individuals with paroxysmal AF and longstanding persistent AF, whereas significantly higher levels were found in patients with persistent AF.

In 260 dogs with pacemaker-induced ventricular tachycardia, the evaluation of microRNAs in the atrial and ventricular extracellular matrix revealed alterations in the expression of 99 microRNAs in the left atrium and 36 in the left ventricle. There was lower expression of miR-1 in the atrial myocardium in animals with tachycardia when compared to the control group, although no alteration in its expression was identified in the left ventricle, nor in expression of miR-26, miR-29b and miR-30a (CHEN et al., 2014).

The different expression of the microRNAs in arrhythmias can facilitate the understanding of the origin, duration and severity of these rhythm disturbances, besides having potential to clarify unknown pathways of arrhythmogenesis.

CONCLUSION

The understanding of the mechanisms involved in the expression of microRNAs and their role in the pathogenesis of the various cardiopathies is a promising new field in veterinary medicine. As a diagnostic biomarker microRNAs can provide relevant information on the development and progression of the disease, but they can also aid in determination of prognosis and the design of a possible therapeutic strategy. However, there are a number of obstacles that could potentially reduce the applicability of these molecules, including the stability of RNAs and the operational procedures

involved extraction and quantification. It is likely that further studies will extend the application of these molecules in veterinary cardiology.

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