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Editorial

miR-16 induces endoplasmic reticulum stress in ischemic dilated cardiomyopathy

Los niveles de miR-16 inducen estrés de retículo en la cardiomiopatía dilatada isquémica

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Dilated cardiomyopathy (DCM) is the second cause of heart failure after ischemic heart disease, and the leading cause of heart transplant with a prevalence that ranges from 1/2500 up to 1/250.[1] DCM is 3 times more frequent in men and mainly affects young adults in their thirties and forties.[2] DCM can be divided into ischemic and nonischemic forms of the disease. Ischemic DCM (iDCM) is associated with coronary diseases and affects approximately to 50% of all patients,[1] while nonischemic etiologies have been subclassified into *a)* familiar DCM, caused by genetic conditions[3]; *b)* infectious DCM associated with myocarditis; and *c)* idiopathic DCM, since in almost half of non-iDCM, the exact etiology remains unknown.[2] Hence, uncovering the leading mechanisms that cause DCM is crucial for the correct management of the disease.

A major complication of DCM is the delay in diagnosing patients, most of them asymptomatic for long periods of time. Undiagnosed patients will suffer from severe contractile dysfunction (left ventricle ejection fraction $< 45\%$) and ventricular remodeling, which will substantially cause a worsening impact on quality of life. To develop early noninvasive specific biomarkers of DCM, the scientific community is now studying the expression of specific microRNAs (miRNAs) as potential indicators and therapeutic tools. In this regard, the article by Calderon-Dominguez et al.,[4] recently published in *Revista Española de Cardiología*, sheds light on the role of microRNA-16-5p (miR-16) in the pathophysiological mechanisms associated with iDCM. miRNAs are short noncoding RNAs with a length of of 20-22 nucleotides that posttranscriptionally silence the expression of specific genes. They can participate in the regulation of multiple cellular processes, and their involvement in the pathogenesis of several diseases has been widely studied over the past years.[5] In the cardiovascular system, miRNAs are highly involved in cardiovascular development and repair since they regulate the proliferation and differentiation of stem and progenitor cells. They are also involved in the correct function of cardiomyocytes, endothelial and smooth muscle cells, and in cell-to-cell communication.[6]

Several miRNAs are related to the deregulation of the main biological processes involved in cardiac homeostasis, including stress responses, autophagy, and apoptosis. For example, overexpression of miR-221 and miR-222 leads to autophagic inhibition through mTOR activation and pathological myocardium remodeling,[7,8] while in the ischemic heart, overexpression of miR-16 was related to an increase of apoptosis in the myocardium by targeting the antiapoptotic BCL2 protein.[9] Several studies have attempted to investigate the relationship between miRNAs and DCM. In this regard, Ikeda et al.[10] identified a different and specific expression pattern of miR-17-5p, miR-28, and miR-106a, in the left ventricle of DCM patients compared with healthy, ischemic cardiomyopathy, and aortic stenosis patients. Specific plasma miRNA signatures for ischemic and idiopathic DCM etiologies have also been described,[11] and a recent study identified a profile of circulating miRNAs expressed differentially in BAG3-related familiar DCM composed of miR-154-5p, miR-182-5p, miR-3191-3p, miR-6769b-3p, and miR-6855-5p.[12]

Although miRNA-based diagnostics seem to hold great promise, miRNA-based therapeutics are still far from being a reality, with the need arising for a deep understanding of the role of miRNA in disease pathophysiology. In their study, Calderon-Dominguez et al.[4] focused on miR-16. As mentioned above, many reports have associated a specific miRNA with different DCM etiologies.[11,12] This study supports a previous investigation analyzing circulating miRNAs as biomarkers in lamin A/C related DCM[13] and presents miR-16 as a new potential noninvasive biomarker for specific iDCM diagnosis. Several pieces of evidence suggest a link between miR-16 and the pathophysiology of DCM, since both circulating and tissue-specific miR-16 worsens myocardial infarction injury, at least by inducing apoptosis and decreasing angiogenesis.[14]

To correlate the expression of miR-16 with iDCM, the authors analyzed the plasma of healthy individuals (n = 76), iDCM patients (n = 60), and patients with BAG3 related familial DCM (n = 32). Only those individuals older than 18 were considered eligible for the study, and the average participant's age was 38.3 ± 11.8, 68.2 ± 8.3, and 42.3 ± 15.3 for the control, the iDCM and the familial DCM groups, respectively. In the study, miR-16 expression was 1.34 times higher in the plasma of iDCM patients compared with healthy participants but, surprisingly, the authors found no differences between familial DCM vs control groups, suggesting that the increase in circulating miR-16 expression may be iDCM specific. The analysis showed a sensitivity of 75.0% and a specificity of 56.2% for identifying

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iDCM and healthy patients, and multivariate logistic regression analysis showed that echocardiography parameters including the sphericity index, the maximum systolic waveform of the tissue Doppler imaging, together with plasma miR-16 concentration, were associated with iDCM.

The main objective of the study was to elucidate the role of miR-16 in the heart. By overexpressing miR-16 in the human cardiomyocyte cell line AC16, the authors found a 64% reduction in cell viability and a significant increase of heart failure specific biomarkers that included brain natriuretic propeptide and myocardial troponin T. Moreover, miR-16 overexpression induced cardiomyocyte apoptosis by reducing the proapoptotic BCL-2 mRNA levels and increasing caspase-3 activation. Also, the authors found an increase in c/EBP homologous protein (CHOP) mRNA levels, the final apoptotic effector of the unfolded protein response (UPR). These results suggest that miR-16 could induce endoplasmic reticulum (ER) stress. Moreover, in the setting of iDCM, lack of oxygen dramatically increases cardiomyocytes oxidative stress, which may damage cell protein components, leading to ER stress.[15] To relieve ER stress, cells initiate 2 main signaling pathways, the UPR and ER-associated degradation (ERAD), which comprises ubiquitin-proteasome-dependent ERAD and autophagy-lysosome-dependent ERAD. However, chronic ER stress or a dysregulated UPR can lead to premature apoptosis and exaggerated inflammatory response.[16] Indeed, several reports suggested that autophagy is activated in DCM.[17]

To study the molecular implications of their findings in depth, the authors analyzed the ER stress response by testing the gene expression of UPR and ERAD effectors. Three different pathways compose the UPR: inositol-requiring enzyme 1 (IRE1), activating transcription factor-6 (ATF6), and the protein kinase RNA-like ER kinase (PERK)-eukaryotic translation initiation factor (eIF-2α). miR-16 overexpression promoted the UPR activation by increasing the PERK/CHOP mRNA expression: PERK, ATF4, CHOP, and the ERAD response (EDEM and OS-9 gene expression). In contrast, GRP78 (protein and mRNA levels), ATF6, and XBP1 branches were downregulated. The authors suggest that this downregulation may be due to *ATF6* being a direct target of miR-16 or the general inhibition of protein translation by the PERK/CHOP pathway. Nevertheless, the unusual UPR activation merits further investigation. Cardiomyocytes overexpressing miR-16 also expressed high levels of proinflammatory cytokine IL-1β mRNA, suggesting a link between miR-16 activated-PERK/CHOP pathway and inflammation.

Next, they studied autophagy since in-silico analysis found the autophagy-related *ATFG14* gene as a potential target of miR-16. Protein and mRNA levels of ATFG14 were significantly downregulated in miR-16 overexpressing cardiomyocytes, suggesting downregulation of autophagy. However, the increased presence of autophagosomes and elevated autophagy flux measured by the LC 3BII/I ratio indicated autophagy activation, whereas p62 and beclin proteins were not increased in these scenarios. Understanding the mechanism behind miR-16 induced autophagy may help to support the future use of miR-16 as a diagnostic tool.

Finally, the authors also attempted to clarify the timing of events that led to cardiomyocyte apoptosis and autophagy, and they found that the UPR pathway-gene expression changes occur within 6 hours after miR-16 overexpression, in which CHOP mRNA levels were increased. However, upregulation of autophagy-dependent genes was not observed until 24 hours, suggesting that the UPR precedes autophagy in this cell line. It is possible to speculate that the UPR pathway alone cannot cope with the ER stress, and autophagy is activated to restore protein homeostasis.

In summary, the work by Calderon-Dominguez et al.[4] uncovers a previously uncharacterized effect of miR-16 in DCM of ischemic origin. miRNA overexpression increases several pathways, which result in apoptosis and autophagy, presenting a novel molecular mechanism for miR-16 in regulating protein homeostasis on this cardiomyopathy.

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CONFLICTS OF INTEREST

None declared.

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