Circulating miRNAs as potential biomarkers of early myxomatous mitral valve disease in Cavalier King Charles Spaniels

Bagardi M.[1], Ghilardi S.[1], Zamarian V.[1,2], Locatelli C.[1], Galizzi A.[1], Cozzoli G.S.[1], Lecchi C.[1], Brambilla P.G.[1]

[1]University of Milan, Department of Veterinary Medicine

[2]Diabetes Research Institute, IRCCS San Raffaele Hospital, Milan, Italy

Abstract

Myxomatous mitral valve disease (MMVD) is the most common heart disease in dogs and in Cavalier King Charles Spaniels (CKCS) is an important cause of cardiac morbidity and death, even at a young age. One of the main challenges in the study of MMVD in CKCS is the identification of biomarkers that that allow to discriminate the classes of the mitral disease and obtain an early diagnosis. MicroRNAs (miRNAs) are small non-coding RNAs, identified as post-transcriptional regulators of gene expression, both in physiologic and pathologic conditions that meet diagnostic and prognostic expectancies as biomarkers of MMVD.

In this study, we quantified the expression of 5 circulating miRNAs as being involved in MMVD in the plasma (left-over samples) of CKCS classified as ACVIM classes A (healthy) and B1 (affected by MMVD, asympthomatic and without cardiac remodelling). Forty-four CKCS were included in the study: 11 A, 11 B1 younger than 3 years, 11 B1 between 3 and 7 years, and 11 B1 older than 7 years. Total RNA was isolated from plasma, retro-transcribed and then the expression levels of miR-128-3p, miR-1, miR-30-b, miR-103, miR-191 were evaluated by RT-qPCR using TaqMan probes. The comparative analysis demonstrated that the concentrations of circulating miR128-3p and miR-30b were significantly greater in B1<3y ($p=0.013$ and $p=0.001$) and B1>7y ($p=0.049$ and $p=0.006$) as compared with A. The age of the subjects did not affect miRNAs' expression ($p>0.05$). ROC curves showed that miR-30b and miR-128-3p can discriminate between A and B1 under 3 years CKCS ($AUC_{[miR-30b]}$ 0.88, cut-off 23.56, Se 82%, Sp 82% and $AUC_{[miR-128-3p]}$ 0.80, cut-off 32.24, Se 54%, Sp 100%), and between A and B1 over 7 years CKCS ($AUC_{[miR-30b]}$ 0.86, cut-off 23.56, Se 80%, Sp 82% and $AUC_{[miR-128-3p]}$ 0.80, cut-off 13.33, Se 70%, Sp 82%). Combining two miRNAs, namely miR-30b and miR-128-3p, in a panel increased the efficiency of distinguishing between A and B1 over 7 years CKCS ($AUC_{[average]}$ 0.88, cut-off 0.52, Se 80%, Sp 91%).

The expression of miR-30b and miR-128-3p allows to discriminate ACVIM A and young B1 (< 3 years) CKCS, in most cases without heart murmurs. These miRNAs may be candidates as novel biomarkers in the disease characterization and may provide the basis for further investigations in the follow-up of the examined subjects, aimed to the characterization of the evolution of the disease in the CKCS.