Expression Analysis of MicroRNAs in FFPE Samples of canine cutaneous and oral melanoma by RT-qPCR

Carlotta Catozzi[1][*], Cristina Lecchi[1], Chiara Gini[1], Fabrizio Ceciliani[1],
Riccardo Finotello[2], Lorenzo Ressel[3]

[1]*University of Milan, Department of Veterinary Medicine, Italy*

[2]*University of Liverpool, Department of Small Animal Clinical Science, Neston, United Kingdom*

[3]*University of Liverpool, Department of Veterinary Pathology and Public Health, Neston, United Kingdom*

Abstract

MicroRNA (miRNA), a class of small, non-coding RNA - regulating post-transcriptionally protein expression - are emerging as clinical biomarkers in many pathologies, including cancer (Peng and Croce, 2016). Since miRNA are supposed to represent fundamental key regulators, better understanding of melanoma tumour biology is essential to improve both disease grading and staging and, consequently, therapy options and prognosis. The aim of the study was to investigate whether miRNA expression can vary in canine melanoma samples derived from formalin-fixed-paraffin-embedded (FFPE) tissues. Experimental design of the study included three groups, each one composed of 7 animals: i) control healthy skin group ii) oral melanoma group iii) skin melanoma group. Two tissue slides were used for miRNA extraction. The expression levels of seven miRNA - miR-145, miR-146a, miR-425-5p, miR-223, miR-365, miR-155 and miR-134 - were detected and assessed by qPCR using TaqMan® probes (Veramo *et al*, 2017; Segura *et al.*, 2012; Wagner *et al.*, 2013; Sand *et al.*,2013). Five miRNA were significantly up-(n=3) or down-(n=2) regulated. In details, miR-146a and miR-155 abundance was increased as compared with control in both oral and skin melanoma (Fig 1 B,E) (p = 0.004 and 0.014 and p = 0.043 and 0.035 respectively), while the levels of miR-145 and miR-365 were lower (Fig 1 A,D) (p = 0.018 and 0.008 and p = 0.01 and 0.028, respectively). MiR-425-5p was upregulated (p = 0.039) only in skin melanoma (Fig. 1 C). Furthermore, functional analysis, carried out using miRNet web-based tool, showed that 76 genes related to cancer-associated pathways were possible target of these five microRNA (p = 6.95E-9); in particular, 21 target genes were associated with melanoma (p = 1.47E-5), including BRAF and CDK, E2F, FGF and PIK3 families. In conclusion, miR-145, miR-146a, miR-425-5p, miR-365 and miR-155 are differentially expressed in melanoma and healthy FFPE samples, suggesting that they may play a role in canine melanoma pathogenesis and/or progression.
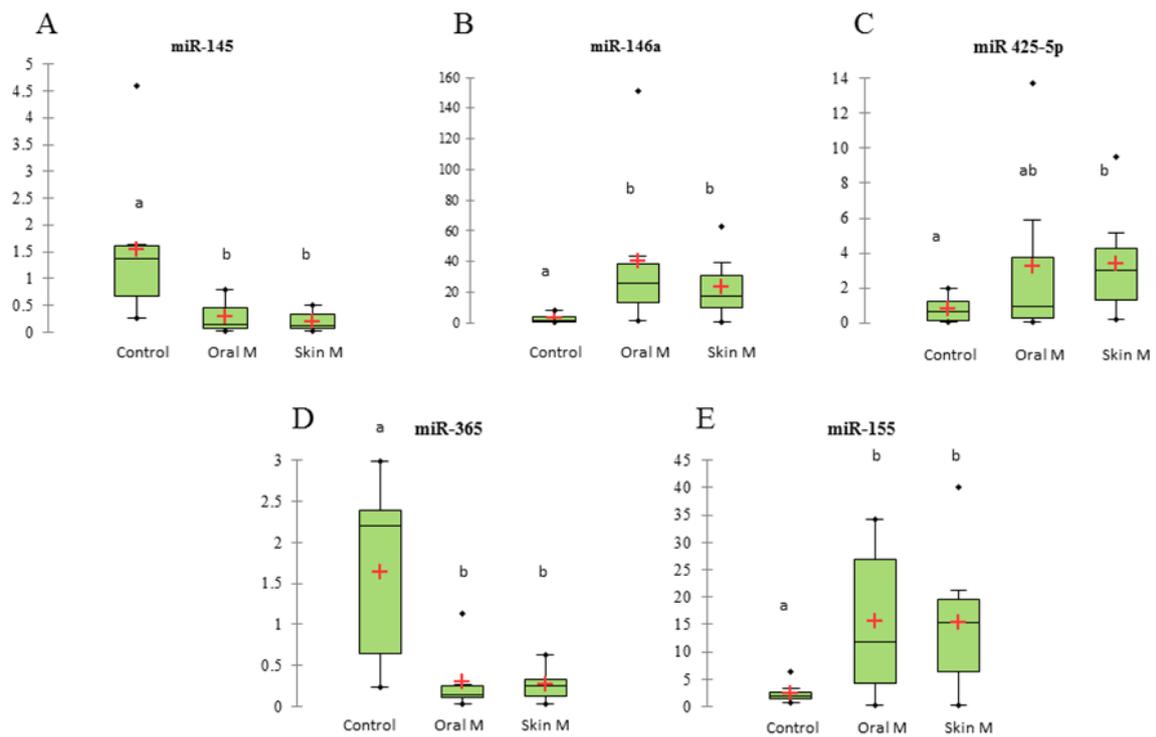
Fig 1: Boxplots of the median (black line), the mean (+), 25%- and 75%-quartile and range of the values measured, as well as statistical differences between groups are shown for miR-145, miR-146a, miR-425-5p, miR-365 and miR-155 in control, oral melanoma (oral M) and cutaneous melanoma (skin M) samples

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