METHYLATION ANALYSIS OF LINE-1 ELEMENTS IN CANINE MAST CELL TUMOURS

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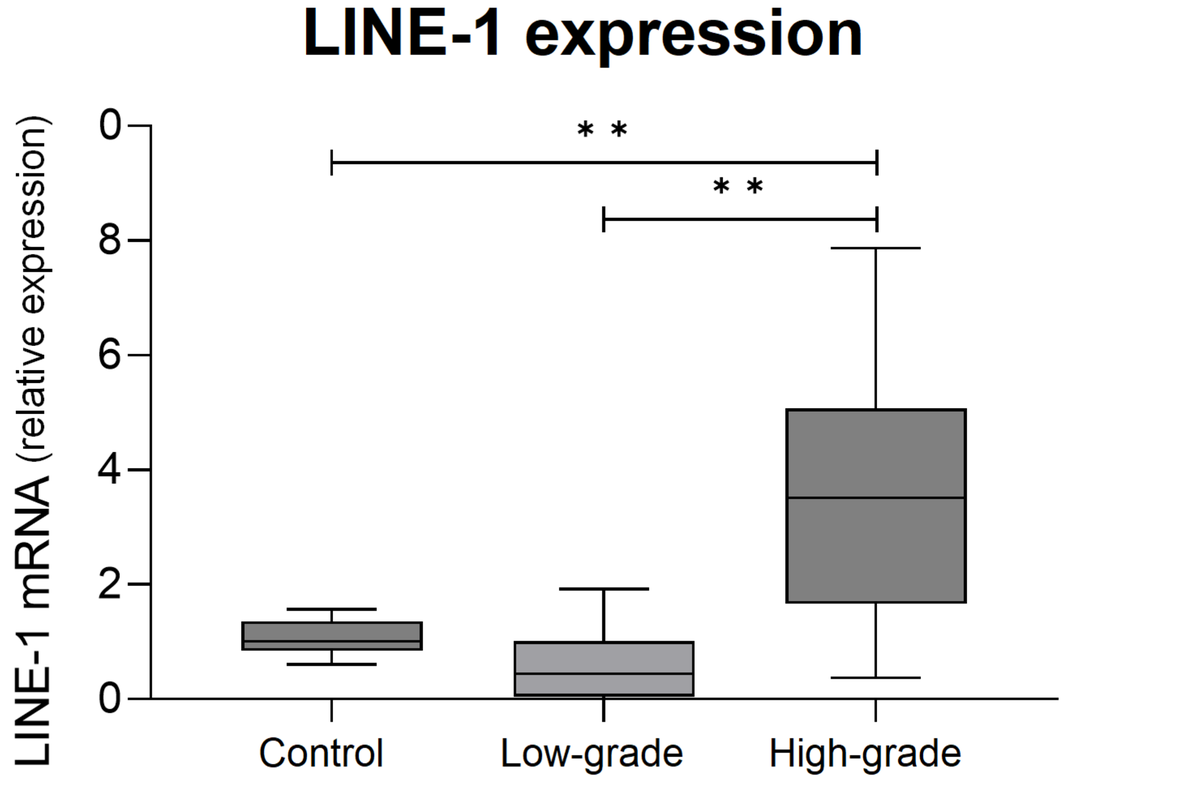
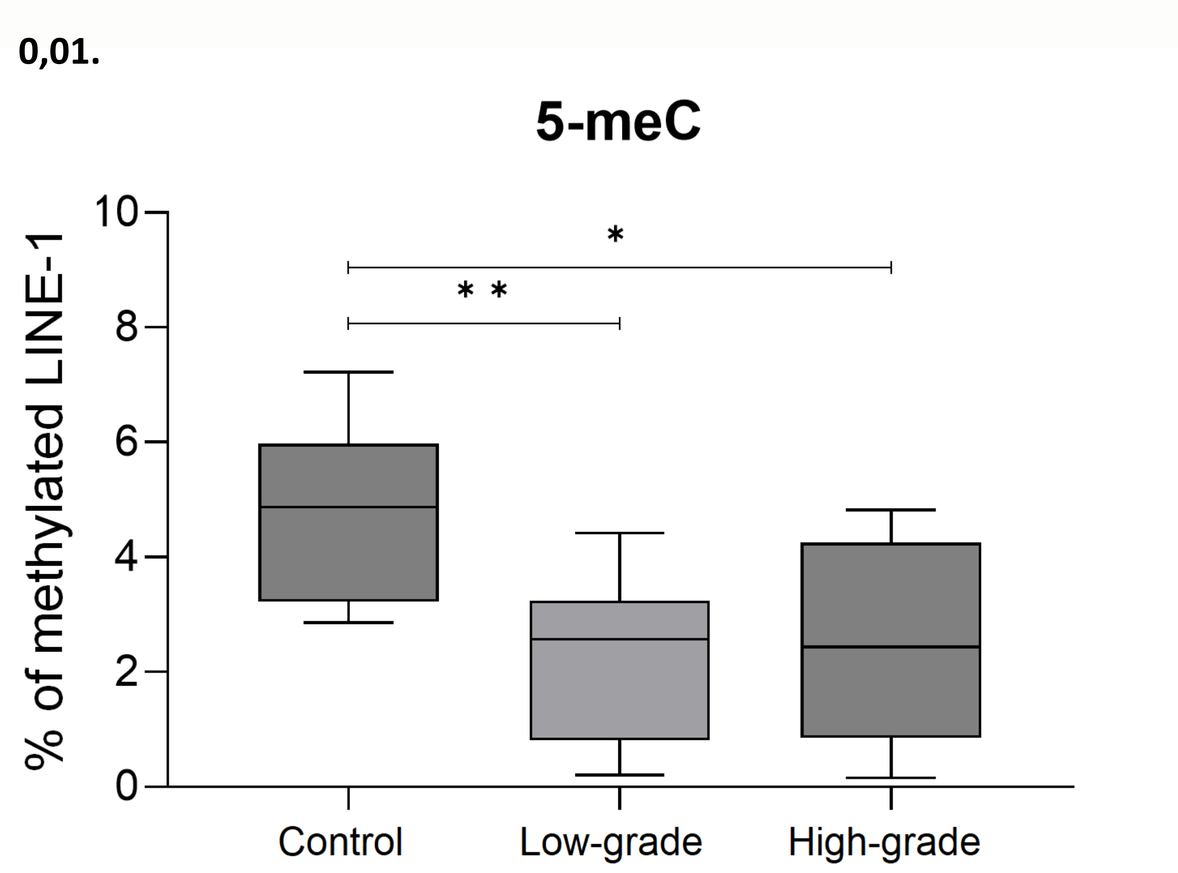
INTRODUCTION: Epigenetics is the study of changes in gene function that are meiotically or mitotically heritable but without implying DNA change. LINE-1 (long interspersed nuclear element-1) is a family of related class I transposable elements in the DNA that is dispersed throughout the entire genome. The study of its methylation pattern is used in the understanding of several neoplasms. Canine mast cell tumours represent a frequent cutaneous neoplasm presenting different degrees of malignancy, being classified according to their histological differentiation. In view of the current scientific scarcity in relation to the epigenetic pattern of neoplasms in domestic animals, this study aimed to verify whether there is correlation between the methylation pattern of LINE-1 with the different grades of canine mast cell tumours according to the Kiupel classification.

MATERIALS AND METHODS: DNA and RNA were extracted from paraffinized healthy and neoplastic (classified as low grade and high grade) tissue samples using the phenol/chloroform/isoamyl alcohol and TRIzol® methods, respectively. LINE-1 methylation pattern and gene expression were quantified by qPCR.

RESULTS: A higher LINE-1 gene expression was observed in neoplasms with a higher degree of malignancy in relation to healthy samples and neoplasms with a lower degree of malignancy. Hypomethylation of the LINE-1 promoter region was also noted in both neoplastic mast cell tumour grades in relation to the healthy tissue. Results are shown in graphs 1 and 2.



Graph 1. Relative genic expression of LINE-1. Performed One-way ANOVA, followed by Tukey's test. ** p < 0,01.



Graph 2. % of 5-methylcytosine's (5-meC) LINE-1 methylation. Performed One-way ANOVA, followed by Tukey's test. * p < 0,05; ** p < 0,01.

CONCLUSIONS: Tumours classified as more malignant present higher gene expression of LINE-1 and lower degree of methylation of the promoter region, evidencing the potential use of LINE-1 as a biomarker of genomic instability.