METHYLATION ANALYSIS OF LINE-1 ELEMENTS AND MORPHOLOGICAL NUCLEAR PARAMETERS IN THE DIFFERENTIAL DIAGNOSIS OF CUTANEOUS MELANOCYTIC TUMOURS IN DOGS

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INTRODUCTION

Recently, epigenetic changes have been proposed as biomarkers for cancer detection, tumour prognosis, and prediction to treatment response. LINE-1 (L1) retrotransposons are widespread repetitive elements in the human genome. These L1 source elements are usually transcriptionally repressed, but epigenetic changes that occur in tumours may promote their expression and allow them to retrotranspose. There are few reports regarding DNA methylation in canine malignant melanoma. Melanocytic neoplasms account for 4-20% of all cutaneous neoplasms in dogs. The borderline between malignant melanomas and melanocytomas may be broad, and the cytologic characteristics contain a degree of uncertainty. The aim of this retrospective study was to evaluate nuclear morphological parameters and epigenetic alterations in order to distinguish between the benign and malignant neoplastic populations.

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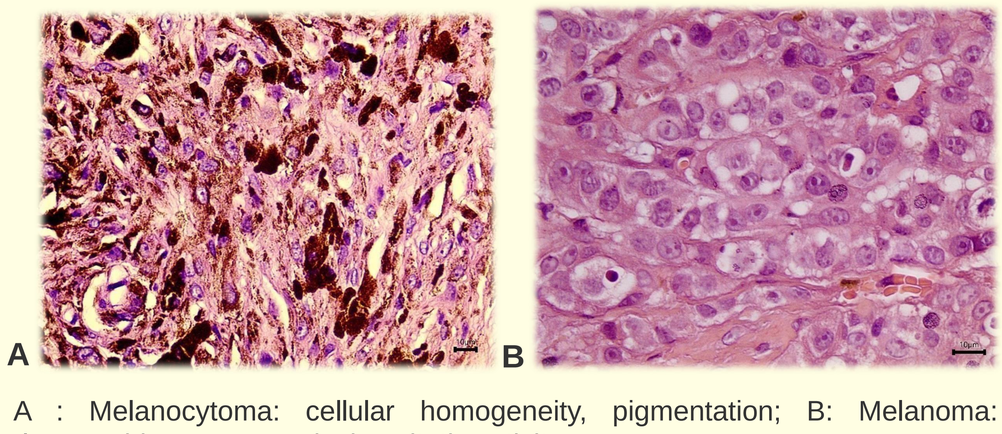
MATERIAL AND METHODS

- Samples: 8 melanocytomas and 10 melanomas
- Nuclear score: percentual of atypic nuclei
- Nucleolar frequency: percentual of cells with evident nucleoli
- Morphometric analysis, using a digital computerized analysis system (Metamorph®), evaluating urothelial nuclear area and perimeter. For each lesion, 200 neoplastic cells were measured in “hot spots”. The nuclei, stained with HE were outlined by tracing their margins with the help of a computer mouse.
- DNA extractions were performed and LINE-1 methylation patterns were quantified by qPCR after previous treatment with T4-BGT, Mspl and Hpall enzymes.

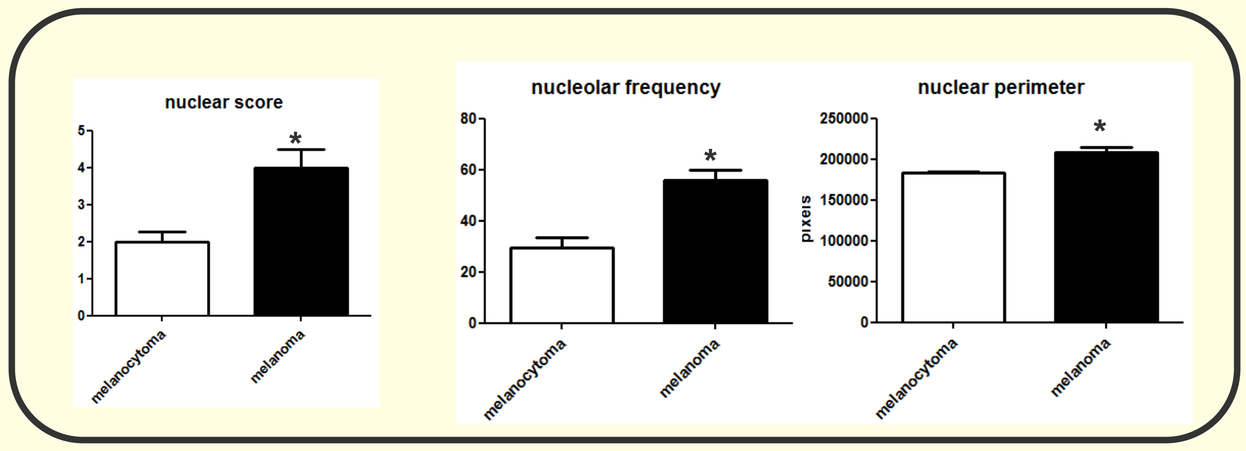
RESULTS

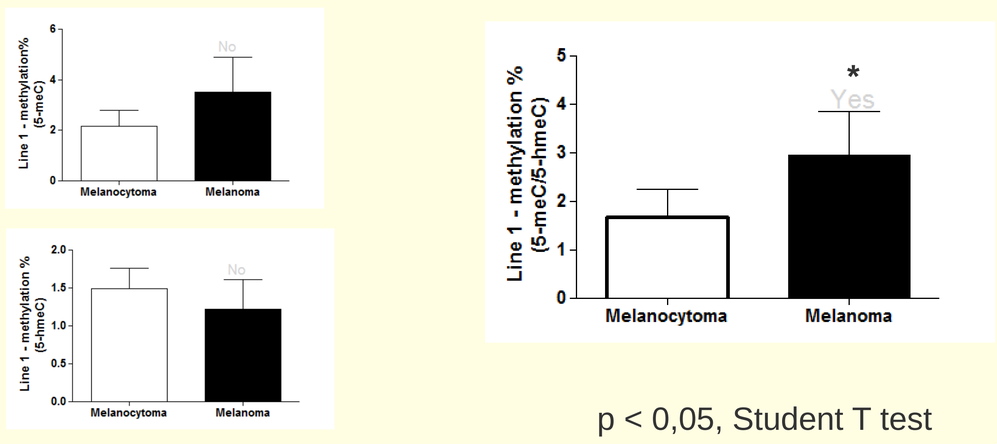
Of the 18 dogs included in this study, 10 dogs were male (55,6%) and 8 were female (44.4%). Eleven were mongrels (11 of 18, 61.1%). Schnauzer(two of 18, 11,1%), and rottweiler (two of 18, 11.1%) were the most prevalent breeds. The breeds of the remaining dogs were labrador retriever, staffordshire terrier and english pointer. The average age was 9.3 years (range from six to 13 years) in melanocytomas and 11,5 years (range from eight to 16 years) in melanomas.

Nucleolar frequency, nuclear score and nuclear perimeter showed a statistically significant difference between benign and malignant tumours ($p<0,004$, Student T test).
Epigenetic evaluation identified a significant difference between the groups with the 5-meC/5-hmeC ratio, with hypermethylation in melanomas.



A : Melanocytoma: cellular homogeneity, pigmentation; B: Melanoma: cellular pleomorphism, anysocariosis, mitotic activity





p < 0,05, Student T test

CONCLUSIONS

The results indicate that increase in nucleolar frequency, nuclear score, nuclear perimeter and LINE-1 hypermethylation are associated with a malignant behaviour in canine cutaneous melanocytic neoplasms.

REFERENCES

Araújo, É.S.S.,Kashiwabara, A.Y., Achatz, M.I.W.,Moredo, L.F., De Sá, B. C. S., Duprat, J. P.,Krepischi,A. C. V. 2015. LINE-1 hypermethylation in peripheral blood of cutaneous melanoma patients is associated with metastasis. Melanoma Research, 25(2): 173– 177

Donizy, P., Biecek, P., Halon, A., Maciejczyk, A., Matkowski, R. 2017. Nucleoli cytomorphology in cutaneous melanoma cells - a new prognostic approach to an old concept. Diagn Pathol. 12(1): 88.

Ishizaki, T., Yamazaki, J.,Aoshima, K.,Kimura, T. 2020.Genome-wide DNA methylation analysis identifies promoter hypermethylation in canine malignant melanoma. Res Vet Sci. 132:521-526.

Scattone, N.V., Epiphanio, T.M.F., Caddrobi, K.G., Ferrão, J.S.P., Hernandez-Blazquez, F.J., Loureiro, A.P.M., Massoco, C.O., Dagli, M.L.Z. 2021. Quantification of Global DNA Methylation in Canine Melanotic and Amelanotic Oral Mucosal Melanomas and Peripheral Blood Leukocytes From the Same Patients With OMM: First Study. Front Vet Sci, v. 8, p.68.