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Vespa

LTA4H expression in canine oral melanomas: methodological set up and preliminary results.

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Abstract

Leukotriene A4 hydrolase (LTA4H) is a hydrolytic enzyme which converts leukotriene A4 into leukotriene B4 inside the arachidonic acid cascade. Besides playing a well-known role in inflammation, it has also been investigated for possible implications in different types of tumors, including canine uveal melanoma (Chen et al., 2004; Malho et al., 2013). In the present study, we set up RT-PCR and immunohistochemical protocols to investigate the expression of LTA4H gene and protein, respectively, in formalin fixed paraffin embedded (FFPE) specimens of canine melanomas. 16 samples of canine oral melanomas were histopathologically evaluated and divided in two subgroups based on morphological criteria of malignancy. RT-PCR protocols for the target gene LTA4H were set up on frozen and FFPE samples of the same tumor. Immunohistochemical investigation of LTA4H protein was performed with a mouse monoclonal antibody anti-LTA4H (clone 1E9). Preliminary tests were carried out to define the protocol: with and without bleaching, with different unmaskings, with different serial dilutions of the primary antibody. RT-PCR set up resulted in comparable good efficiency on both frozen and FFPE samples. Final immunohistochemical protocol included: hydrogen peroxide bleaching, water bath unmasking and 1:100 dilution of the primary antibody. Positive immunostaining for LTA4H was present in neoplastic cells of all melanoma samples, with variable localization and intensity. These preliminary results encourage future applications of the studied techniques to quantify differential expression of LTA4H in canine melanomas both at molecular and histological level. Laboratory results will be compared with follow up data, in order to verify if LTA4H can be proposed as a valuable prognostic marker.

References

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