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# Validation of anti-FXR1 antibodies in the canine species and application to an immunohistochemical study of canine oral melanomas

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## Abstract

FXR1 (Fragile X mental retardation-related protein 1) is a cytoplasmic RNA binding protein (Siomi *et al.*, 1995), which genetic expression has been related to metastatic potential in human melanoma. The aims of the present study were to validate two commercially available clones of polyclonal anti-human FXR1 antibody in dogs and to use them to investigate FXR1 expression in a group of canine oral melanomas. Anti-FXR1 antibody was never validated before in the canine species.

Two different commercially available polyclonal anti-FXR1 antibodies (raised in goat and rabbit, respectively) were used. FXR1 protein in canine serum was identified by western blot after SDS-PAGE, using human serum as control. FXR1 immunohistochemical expression was tested in a series of normal tissues, that are expected to express FXR1, and in 31 cases of oral melanomas. The final immunohistochemical protocol used heat-induced unmasking and overnight incubation.

FXR1 protein bands in canine serum were detected by validated antibodies. The rabbit antibody specifically identified a band around 65 kDa (Figure 1A), whereas the goat antibody reacted also with other not specific bands. FXR1 immunohistochemical staining was positive in all tested organs, with different levels of expression. FXR1 was also expressed in 31/31 tested melanomas, with variable intensity and percentage of positive cells (Figure 1B). Equal results were achieved with the two antibodies in 8 cases of melanoma, whereas there were variable differences in 22, and one case stained only with goat antibody. The rabbit antibody gave less background staining. This study validated anti-FXR1 antibodies for use in the canine species. This protein was identified in several normal tissues, as well as in the tested neoplasms. Significance of different level of expression is undergoing evaluation with further studies.

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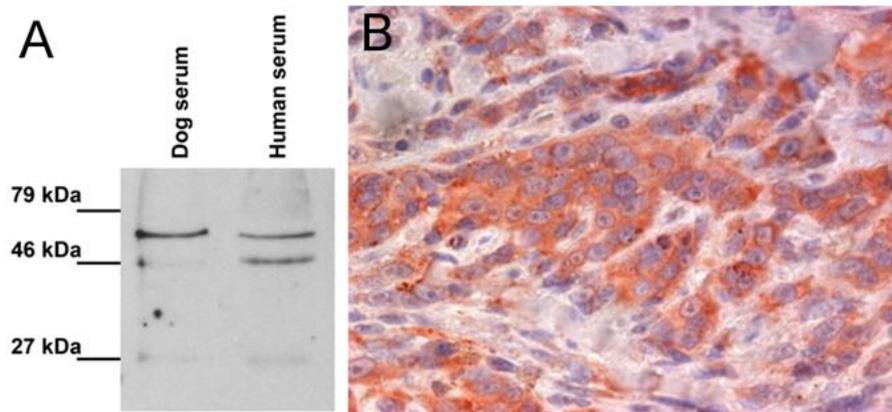


Figure 1. A) Western blot, rabbit anti-FXR1 antibody, positive bands around 65 kDa. B) Immunohistochemistry, oral melanoma, cytoplasmic positivity of neoplastic cells to FXR1. Rabbit anti-FXR1 antibody, AEC chromogen, 40x.

## References

Siomi, M.C., Siomi, H., Sauer, W.H., Srinivasan, S., Nussbaum, R.L., Dreyfuss, G., 1995. FXR1, an autosomal homolog of the fragile X mental retardation gene. *EMBO J.* 14: 2401-2408. *Journal of Microbiological Methods.* 40, 175-179.