Veterinary Dermatology

Vet Dermatol 2019 DOI: 10.1111/vde.12780

Letter to the Editor

Evaluation of the expression and distribution of Prune-1 in the skin of healthy dogs

Dear Editors,

Prune-1 is a member of the DHH (Asp-His-His) phosphoesterase protein superfamily involved in the regulation of cell proliferation and migration.[1–3] In humans, the gene *Prune-1* is located in the 1q21.3 chromosomal region (epidermal differentiation complex) making it a good candidate for skin proliferation/differentiation and regulation. In a transgenic mouse model characterized by cutaneous hyper-proliferation, H-prune-1 has been associated with epidermal proliferation and keratinocyte differentiation, increased expression of pro-inflammatory cytokines and alteration of the skin barrier.[4] To date, although much is known about the role that Prune-1 plays in metastatic carcinomas in humans and mice, its role in healthy and/or inflammatory skin conditions in humans or dogs has not been published. In one study, the skin of transgenic mice showed an increased expression of Prune-M1 protein, which was associated with cutaneous inflammation and epidermal proliferation.[4] These results may suggest a potential role for Prune-M1 orthologues in the pathogenesis of inflammatory skin diseases. These data point toward a potential role of Prune-1 in keratinocyte homeostasis and its involvement as a master-regulator in cutaneous inflammation; this has not been studied previously in dogs. We have started to explore the presence of Prune-1 in canine skin using a commercially available mouse antibody. The goal of this pilot study was to investigate the presence and distribution of Prune-1 in the abdominal skin of healthy dogs.

Ten privately owned healthy adult dogs were included in this study. One 8 mm punch biopsy skin sample from abdominal skin was collected, fixed in 10% buffered formalin (≤48 h) and processed for indirect immunofluorescence as described previously.[5] Sodium citrate pH6 buffer was necessary before incubation with the primary antibody (cat#: ab88613, AbCam; Cambridge, MA, USA) (1:100 dilution). Mouse isotype (cat#: NB60-986, Novus Biologicals; Littleton, CO, USA) at the equivalent concentration was used as negative control. The sections were washed and then incubated with an anti-mouse antibody bound with a red fluorochrome at 1:1,000 dilution. Finally, the sections were mounted and counterstained with DAPI (Vector Laboratories; Burlingame, CA, USA). Five representative fields at ×200–400 magnification were evaluated for each section and pictures recorded. The antibody used in this study was chosen based on multiple alignment sequencing, gene homology (91% and 83% with human and murine counterparts, respectively) and epitope hydrophobicity between human and canine Prune-1 sequence (https://www.ncbi.nlm.nih.gov/BLAST/)

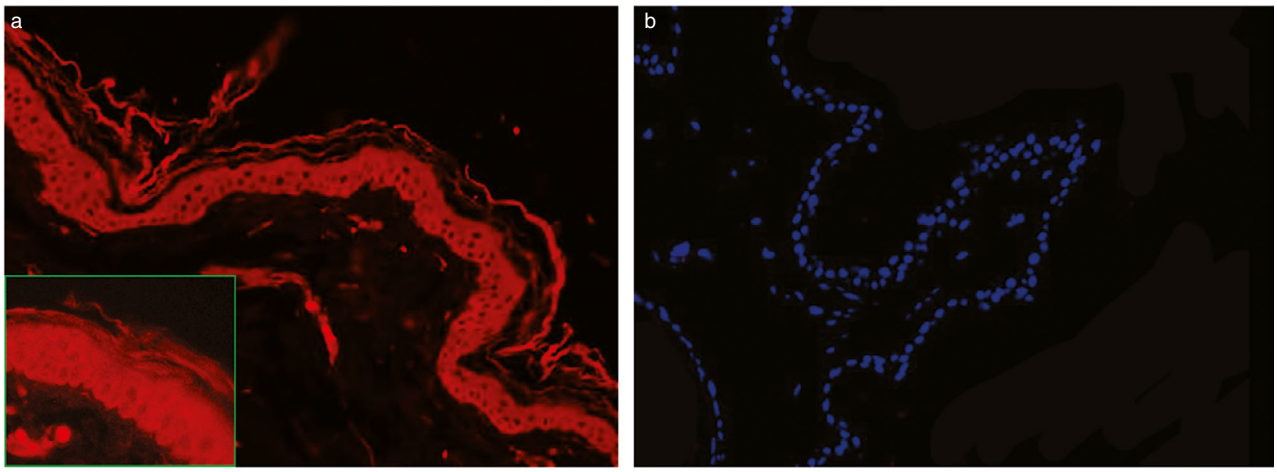

Figure 1. Photomicrographs of canine abdominal skin showing indirect immunofluorescence for Prune-1.
(a) Healthy skin. (b) Negative control – with mouse antibody isotype. Insert is a higher magnification (x 400) view of (a). Note the diffuse and homogeneous fluorescent signal present in the epidermis with few fluorescent cells detected in the superficial dermis. Prune-1, red fluorescence; DAPI, blue counterstain fluorescence. Magnification x200.

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confirming the highly conserved structure of this protein in different species.[6] Because no specific anti-canine Prune-1 antibodies are commercially available, further validation of the antibodies used in this study may be needed to confirm their specificity.

The analysis of epidermal photomicrographs showed the cytoplasmic presence of suspected Prune-1 in all layers of the epidermis (Figure 1). In addition, a few cells present in the superficial dermis, most likely lymphoid or myeloid in origin, also were (presumptively) positive for Prune-1.

Although encouraging, further studies are needed using more specific staining to better identify the origin of the dermal cells. In particular, potential staining of dermal mast cells versus lymphocytes may be a possibility and should be investigated further in future studies using double labelling with tryptase and/or CD3 staining.

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Sources of funding

This study was self-funded

Conflict of interest

No conflicts of interest have been declared.

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