Characterization of fibroblasts in BPV1-associated equine sarcoids

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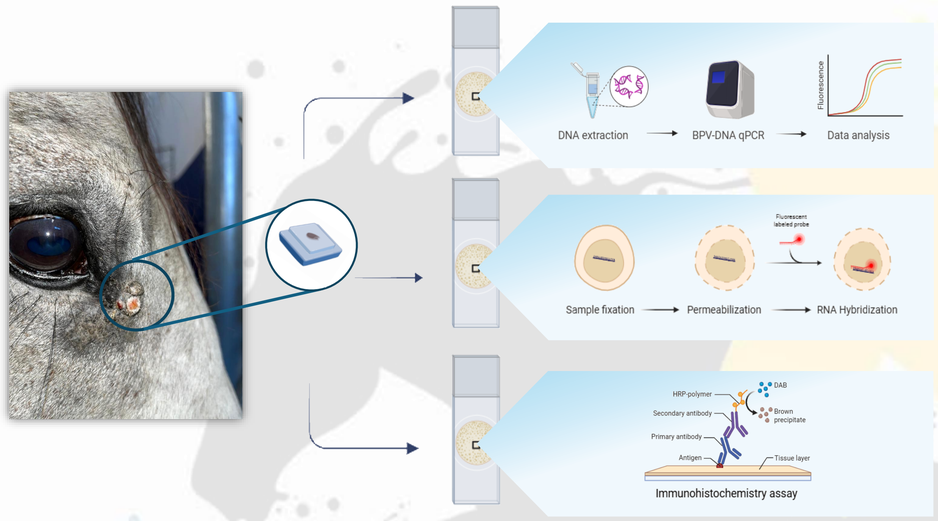
INTRODUCTION

Equine sarcoids are the most commonly diagnosed skin tumors in horses, marked by locally invasive growth and a high recurrence rate following surgical removal [1]. These neoplasms are associated with δ bovine papillomavirus (BPV) types 1, 2, and 13, and are characterized by activated fibroblasts capable of invading and degrading the extracellular matrix. To date, few studies have explored their phenotypic characteristics beyond matrix metalloproteinase activity [2].

The aim of this study was to further characterize sarcoid fibroblasts by evaluating the expression of hypoxia-inducible factor 1-alpha (HIF-1α), alpha smooth muscle actin (αSMA), and S100 protein.



MATERIALS & METHODS

A retrospective study was performed on 33 specimens: 23 equine sarcoids and 10 samples from normal control skin. Histopathological evaluation was performed and the presence of BPV-1, -2, and -13 DNA was assessed by qPCR in all samples. *In situ* Hybridization (ISH) was performed in order to detect E6/E7 oncogene mRNA expression. Expression of HIF-1α, αSMA, and S100 protein was assessed by immunohistochemistry (IHC). For the evaluation of HIF-1α, αSMA, and S100 expression, a semiquantitative score was given under blind evaluation by two pathologists. [3]. For each sample the number of positively-labelled cells was established by counting 1000 cells in 5 fields at 200X magnification. Results were expressed as percentage and scored as follows: 0 (≤ 10% positive cells); 1 (10-40 % positive cells); 2 (40-60 % positive cells); 3 (>60 % positive cells). The intensity of immunostaining was graded from: n.a. (not assessable); − (negative staining); +/− (weak immunolabelling); + (moderate immunolabelling); ++ (strong immunolabelling).

RESULTS

All selected sarcoid samples were reviewed and the diagnosis confirmed (**Fig. 1a and 1b**). All tumour samples were positive for BPV-1 DNA and showed expression of the E6/E7 oncogenes (**Fig. 1c and 1d**). Control samples were all negative for any BPVs DNA. HIF-1α immunostaining was detected in 18/26 equine sarcoid samples; 8/26 sarcoid samples were not assessable. 10/26 sarcoid samples (39%) showed a moderate and finely granular cytoplasmic staining in 40–60% of neoplastic fibroblasts and endothelial cells (score 2); the remaining 3/26 sarcoid samples (11.5%) showed a strong cytoplasmic staining for HIF-1α in ≥60% of neoplastic fibroblasts and endothelial cells (score 3) (**Fig. 2a and 2b**). 5/26 sarcoid samples (19%) showed from weak to moderate immunostaining in 10–40% of neoplastic fibroblasts. S100 immunostaining was evaluated in 20/26 sarcoid specimens; 6/26 sarcoid samples were not assessable. 1/26 sarcoid samples (3.8%) was negative for S100 (score 0). 3/26 sarcoid samples (11.5%) exhibited a moderate to strong cytoplasmic and nuclear immunolabelling in 40–60% of neoplastic fibroblasts (score 1), while 13/26 sarcoid samples (50%) showed a moderate to strong staining in ≥60% of neoplastic fibroblasts (**Fig. 2c and 2d**). All sarcoid samples tested, except for one, were negative for αSMA.

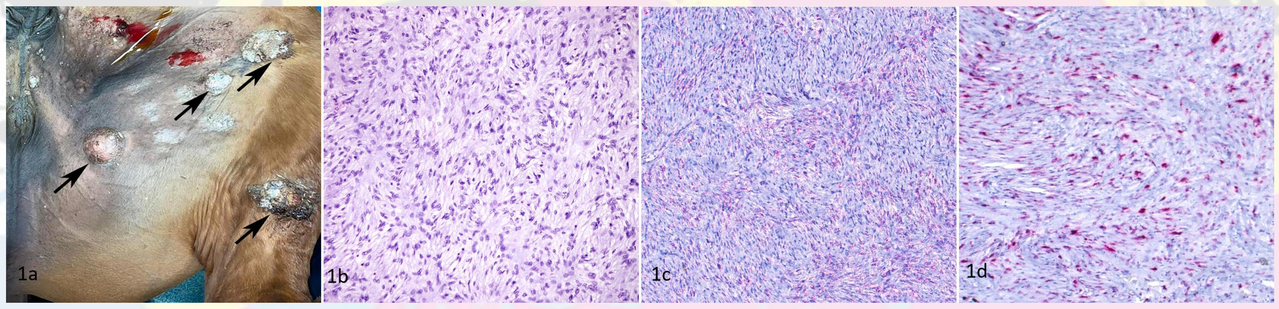
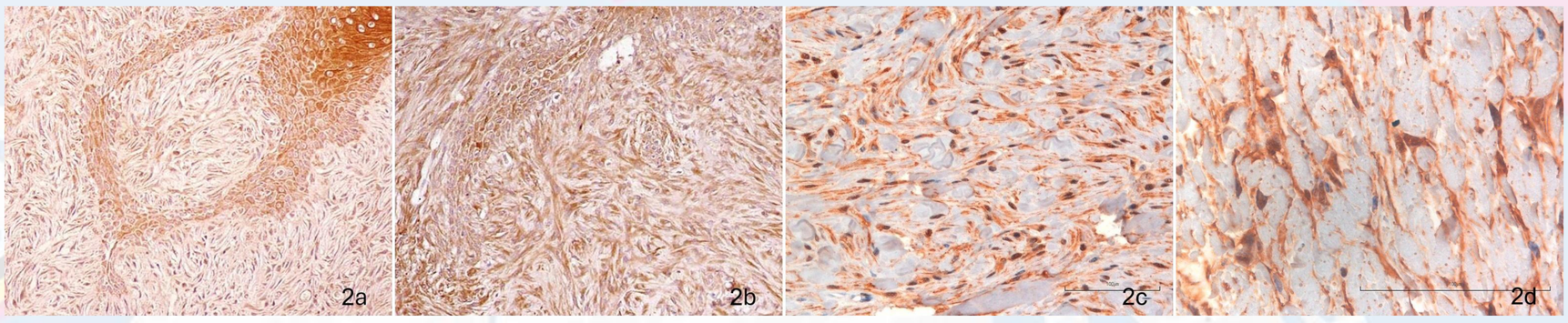


Figure 1: (1a) Nodular sarcoids, horse. (1b) Equine sarcoid. Hematoxylin and eosin (H&E). Microscopically sarcoids might appear as areas of high cellularity characterized by spindle to stellate cells embedded in finely fibrillar to dense collagenous stroma and organized in parallel and perpendicular rows. (1c) Equine sarcoid. ISH for BPV1 E6/E7 oncogenes. The labelling is represented by magenta dots located in the cytoplasm of neoplastic fibroblasts. (1d) Equine Sarcoid. ISH. Dots disseminated within the nuclei of neoplastic cells.

Figure 2: Strong Immunohistochemical expression of HIF-1α (2a, 200x; 2b, 400x) and S100 (2c, 200x; 2d, 400x) (Score 3, ++).



CONCLUSIONS

Based on the histological features, anatomical localization, and lack of αSMA expression, the S100-positive spindle cells are interpreted as activated fibroblasts. Although S100 is commonly associated with neural lineages, its expression in various cell types, including fibroblasts, has been reported [4]. The absence of αSMA and strong HIF-1α expression support the presence of a hypoxic microenvironment, which may promote fibroblast activation and tumour persistence without inducing myofibroblastic differentiation. Interestingly, S100 expression has been described as a target gene of HIF-1α in tumour progression in several tumours [5-7]. Overall, our findings suggest that S100 may also be a hypoxia-induced target gene in BPV-associated sarcoids, indicating potential cooperation between viral oncogenes and HIF-1α in tumour progression.

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