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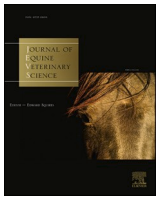

Overexpression of the key metabolic protein Carnitine Palmitoyl Transferase 1A (CPT1A) in equine sarcoid

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ABSTRACT

The equine sarcoid is the most common skin neoplasia of fibroblastic origin in horses, characterized by an excessive accumulation of extracellular matrix produced by sarcoid fibroblasts under hypoxic condition. Neoplastic cells can adapt to hypoxia by using alternative energy sources, particularly those that arise from fatty acid oxidation (FAO). The Carnitine Palmitoyl Transferase 1A (CPT1A) belongs to Carnitine System (CS) and promotes the entrance of fatty acids into the mitochondria for β-oxidation. In this study, CPT1A expression was comparatively addressed in 25 equine sarcoids and 5 normal skin samples using immunohistochemistry (IHC). Specificity of CPT1A antibody was validated by Western Blotting (WB). In normal skin samples IHC staining was weak and mainly confined to basal epidermis and few dermal fibroblasts. Sarcoid fibroblast exhibited a strong cytoplasmic and nuclear signal in 60% of the tumor samples. Cytoplasmic CPT1A expression in sarcoid fibroblasts indicates that the protein is actively involved in metabolic reprogramming processes. Nuclear CPT1A expression suggests that the protein may also be involved in the regulation of neoplastic proliferation.

1. Introduction

Equine sarcoids are the most common skin neoplasia of fibroblastic origin in horses, characterized by locally invasiveness, rare regression and high recurrence following surgery [1-3].

Although the etiology is multifactorial involving genetics and skin trauma, delta bovine papillomaviruses (BPV type 1, 2 and 13) are widely accepted as essential extrinsic factors contributing to equine sarcoid development [4,5]. This has been partly explained by the detection of BPV oncogenes and capsid gene transcripts (E2, E5, E7, and L1) in naturally occurring equine sarcoid that prove evidence for a direct involvement of BPV in equine sarcoids development [1-5]. Among BPV oncoproteins, E5 is considered the major BPV transforming protein, as it binds and activates the platelet-derived growth factor b receptor (PDGF β -R) leading to a stimulation of intracellular growth signal in infected cells [6-8]. Direct contact with cattle or horses carrying BPV or through bites of a virus-carrying fly or contaminated surfaces, are presumably the most common routes of viral transmission [9].

Currently, immunotherapeutic approaches and a new vaccine have shown promising results for sarcoids management [10,11]. However, these therapeutic approaches are still in a preliminary phase, and a more comprehensive understanding of equine sarcoid pathogenesis is crucial for the development of novel and more effective therapies.

We have previously shown that equine sarcoid are characterized by hyperproliferation of dermal fibroblasts with production of excessive connective tissue [12], leading to a hypoxic condition [13,14] with consequent activation of autophagy that could support the process thought the recycling of essential nutrients for energy supply [15]. There is growing evidence that in the case of limited energy resources and metabolically unfavorable conditions, such as hypoxia, neoplastic cells adjust their metabolism by activating alternative pathways to utilize different energy sources which allow them to survive and proliferate [16-18]. One of the alternative pathways which can be activated is fatty acid oxidation (FAO) of lipids from adipose tissue adjacent to neoplasia, lipoproteins, and phospholipids, mediated by the Carnitine System (CS) [19,20]. Among the proteins which are involved in CS, the Carnitine Palmitoyl Transferase 1 (CPT1), together with Carnitine Palmitoyl Transferase (CPT2) and Carnitine/acyl-Carnitine Translocase, has a

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crucial role in controlling the fatty acid metabolism, through the transport of fatty acids from the cytoplasm into the mitochondria to undergo β-oxidation [20]. The canonical members of the CPT1 family include three isoforms CPT1A, CPT1B and CPT1C, which are ubiquitous enzymes with higher expression levels on the outer mitochondrial membrane of most tissues [20]. CPT1A controls the rate limiting process in fatty acid β-oxidation, during which acyl-coenzyme A (acyl-CoA) esters are converted into acyl-carnitines [21]. It is reported that CPT1A is involved not only in metabolic regulation, but also in nearly all aspect of tumorigenesis, such as proliferation, tumor growth, invasion, and metastasis [21-23]. The upregulation of CPT1A has been reported in animal [24-27] and human tumors often associated with poor prognosis [28-32].

To the best of our knowledge no data are yet available on the expression of CPT1A in equine sarcoids. Therefore, the aim of this study was to evaluate the expression level of CPT1A in a subset of BPV positive equine sarcoids by IHC.

2. Materials and methods

2.1. Samples

2.1.1. Equine sarcoids and normal skin samples

Equine sarcoids were obtained from affected horses, which underwent surgery routinely, adhering to a high standard (best practice) of veterinary care, after informed written consent of the owners, and according to Directives 2010/63/EU (art. 1 c. 4) and 2010/63/ EU.

For this study twenty five equine sarcoids (S1-S25) clinically identified on their gross morphology according to Knottenbelt [3] as fibroblastic or verrucous, were individually and routine surgically collected from affected horses coming from farms located in Campania region (Italy) (Table 1). Five normal skin samples (N1-N5) collected during necropsy from healthy horses were used as controls. All samples were 10% formalin-fixed, paraffin-embedded for routine histological processing, and stained with hematoxylin and eosin (HE). Four sarcoids (S1-S4), two normal skin samples (N1–N2) and one perilesional skin (PS) were immediately frozen at −80 °C and analyzed by Western blotting. Sarcoid samples were previously used and resulted BPV1/2 positive, while normal skin samples were BPV negative [6].

2.1.2. Cell culture

2.1.2.1. HeLa cells. HeLa cells were kindly provided by Dr. Nunzio Antonio Cacciola (Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Italy). The cells were. grown in Dulbecco's Modified Eagle Medium (DMEM) medium (Gibco, NY, USA) and were supplemented with 10% fetal bovine serum (FBS, Gibco), 100 U/mL penicillin (Gibco), and 100 μg/mL streptomycin (Gibco) in a controlled humidified cell culture incubator (37°C, 5% CO2, and 95% humidity). The cell lines were routinely tested for mycoplasma contamination using the Mycoplasma PCR Detection Kit (#G238, SIAL s. r.l., Rome, Italy).

2.1.2.2. Primary cell lines from equine sarcoid. Four primary cell lines were obtained by four different horses affected by sarcoid, routine surgically by a freelance veterinarian at the Veterinary Hospital (table 1S).

This study was approved by the animal studies subcommittee of IZSPLV review board (25.05.22).

2.2. Isolation of the primary cell lines

Tissue was collected from the cases described above (Table 1S) and delivered to the laboratory in DMEM supplemented with Penicillin/ Streptomycin (P/S) (500 U/mL) at 4°C.

Primary cell lines were obtained using the method described by Vangipuram et al., [33]. Briefly, the biopsies were washing three times with saline solution supplemented with P/S (10,000 U/mL) and cut into smaller ones (2 mm2 in size). Then, 3 biopsy pieces were seeded into 25 cm2 cell culture flask with 1 ml of medium (RPMI-1640+20% FBS+100 U/ml penicillin, and 0.1 mg/ml streptomycin). Then, cells were incubated at 37°C with 5% CO2 for 7 days and sub-cultured as previous described [33]. Cell lines were maintained for over 10 passages, and the established primary cell lines consisting of CAFs were designated as "SAR1", "SAR2", "SAR3" and "SAR4" cells.

Table 1
Immunoscoring for CPT1A in equine sarcoid and normal skin samples.

Samples	Location	Intensity Staining Score*	Percentage positive Score*	Nuclear (N)/ Cytoplasmatic immunostaing (C)*
S1	neck	++	3	C
S2	limb	++	3	N
S3	Pectoral region	++	3	N
S4	limb	-	1	C
S5	head	++	3	N/C
S6	abdomen	++	3	N/C
S7	head	++	3	C
S8	Pectoralregion	+	2	C
S9	limb	++	3	N
S10	(para)-Genital	+	2	C
S11	Limb	-	1	C
S12	abdomen	++	3	C
S13	Pectoralregion	+	2	C
S14	head	++	3	N
S15	limb	++	3	N
S16	(para)-Genital	-	1	C
S17	Limb	++	3	C
S18	abdomen	-	1	C
S19	Pectoral region	++	3	C
S20	neck	++	3	N/C
S21	limb	++	3	N/C
S22	(para)-Genital	++	3	C
S23	Limb	+	2	C
S24	abdomen	+	2	C
S25	Pectoral region	-	1	C
N1	head	+	1	C
N2	abdomen	+	1	C
N3	neck	+	1	C
N4	limb	+	1	C
N5	Pectoral region	+	1	.C

*N: Normal skin sample; S: Sarcoid sample;; * Intensity staining score: − Negative staining; +/− Weak immunolabelling; + Moderate immunolabelling; ++ Strong immunolabelling; Percentage positive score: 0-50% positive cells (score 1); 50-70% positive cells (score 2); 70-100% positive cells (score 3); Nuclear (N) and cytoplasm (C) immunostaining

2.3. Characterization of the primary cell lines: Real Time PCR to BPV detection

All cell lines were tested for BPV using the methods described by Maggi and co-workers [34]. Briefly, total DNA was extracted from 4*106 cells using the Maxwell® RSC Viral Total Nucleic Acid Purification Kit (Promega, Milan, Italy), according to the manufacturer's instruction. The presence of BPV1/2/13 genome was investigated using 100 ng of DNA and the specific primer and probe sequences described by Maggi [34]. For each analytical session Internal controls were used. Real-time PCR was performed using a CFX96 Real-Time System (BioRad, Milan, Italy) and a threshold cycle of 38 was set as the cut off for sample positivity.

2.4. Characterization of the primary cell lines: IF

For the characterization of fibroblasts and evaluation of their purity, we tested vimentin expression by immunofluorescence. Briefly, cell lines were fixed with 4% paraformaldehyde (Cell Signalling, 12606S) and permeabilized with 0.1% Triton X-100 (Sigma-Aldrich, Merk, 93443) than blocked with 3% Bovine Serum Albumin (Sigma-Aldrich, Merk, B6917). After this step cells were incubated with anti-vimentin antibody (clone V-9; DAKO 1:50 dilution) and subsequently with a goat anti-mouse IgG antibody Alexa FluorTM 594 (ThermoFisher Scientific, Milan, Italy; 1:500 dilution). Fluorescence images were obtained with a Nexcope NE920 fluorescence microscope (TiesseLab, Milan, Italy).

2.5. Histological and immunohistochemical analysis

All samples were 10% formalin-fixed, paraffin-embedded and stained with hematoxylin and eosin (HE) for histopathological diagnosis. Immunohistochemistry was performed using the streptavidin-biotin peroxidase method. All sections were deparaffinized in alcohol decreasing. solutions, and endogenous peroxidase activity was blocked by incubation in 0.3% H2O2 in methanol for 20 min. Antigen retrieval was performed by pre-treating with microwave heating (twice for 5 min each at 750 W) in citrate buffer, pH 6.0. The slides were washed three times with phosphate buffered saline (PBS, pH 7.4, 0.01 M), then incubated for 1 h at roomtemperature with normal goat serum (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted at 20% in PBS. As primary antibody, a mouse monoclonal antibody, raised against Carnitine palmitoyl-transferase 1A (CPT1A) (Abcam -AB128568) diluted 1:200 in PBS was used and applied overnight at 4 °C. Control sections (equine normal skin and sarcoid) were incubated with PBS and with rabbit IgG (purified rabbit IgG P120-201-Bethyl Laboratories, Inc.) instead of the primary antibody using the same concentration as the primary antibodies. Then, sections were incubated with MACH 1 probe (Biocare Medical, LLC, Concord, CA, USA) for 20 min at room temperature and with MACH-1 Universal HPR-Polymer (Biocare Medical, LLC, Pathogens 2020, 9, 58 7 of 11 Concord, CA, USA) for 30 min at room temperature. Sections were counterstained with hematoxylin, and the immunolabelling was revealed with diaminobenzidine tetrahydrochloride.

For each sample, the number of immunolabeled cells was established by counting 1000 cells in 10 fields at 400X magnification (40X objective 10X ocular) by two independent observers. Results were expressed as percentage, and scored as follows: 0-50% positive cells (score 1); 50-70% positive cells (score 2); 70-100% positive cells (score 3). Moreover, the intensity of immunostaining was graded, as performed in previous studies [12-15]: n.a. (not assessable), weak immunostaining (-), moderate immunostaining (+), strong immunostaining (++).

2.6. Biochemical analysis

Hela cell line, four primary cell lines obtained from equine sarcoids (Sar1, Sar2, Sar 3, Sa4), two biopsies from healthy skin (N1, N2), one from perilesional skin (PS) and four from equine sarcoid samples (S1-S4) were subjected to homogenization, protein extraction and Western blotting. Tissues samples were lysed in RIPA Lysis and Extraction Buffer (Thermo Scientific) added protease inhibitor cocktail (cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail, Roche) and phosphatase inhibitor cocktail (PhosSTOP, Roche). Hela cell line was lysed for 30 min in RIPA lysis buffer, supplemented with protease and phosphatase inhibitors. Tissue homogenates and cell lysates were clarified by centrifugation and protein concentration was measured by Bradford assay (Bio-Rad Laboratories). For western blotting, 30 μg protein lysate was heated at 95°C in 4X premixed Laemmli sample buffer (Bio-Rad Laboratories), analysed by SDS polyacrylamide gel electrophoresis (PAGE) and transferred onto nitrocellulose membranes using Trans-Blot Turbo Transfer System (Bio-Rad Laboratories). Hela whole cell lysate was run along with equine samples as control according to antibody datasheet. Membranes were blocked with EveryBlot Blocking Buffer (Bio-Rad Laboratories) for 30 min at room temperature and subsequently incubated overnight at 4°C with anti-CPT1A antibody (Anti-CPT1A antibody [8F6AE9] ab128568, Abcam) at 1:1000 dilution and anti-GAPDH antibody (GAPDH (0411) sc-47724) as house-keeping protein. The membranes were washed three times with TBS-Tween 0.1%, and were incubated, for 1 hour at room temperature, with horseradish peroxidase conjugated secondary antibodies, according to the species of primary antibodies. After further washing steps, specific protein bands (88 Kda) were revealed using luminol-based chemiluminescent substrate (Clarity Western ECL, Bio-Rad Laboratories) and read under the ChemiDoc Gel Scanner (Bio-Rad Laboratories).

3. Results

All immunohistochemical results are summarized in table 1.

All normal skin samples showed epidermis without signs of pathological alterations and dermis without inflammatory cells that could suggest a bacterial or viral infection (not shown).

Hematoxylin and eosin staining of examined sarcoids showed the typical histopathological features of equine sarcoids: koilocytosis and epidermal hyperplasia accompanied by rete pegs and dermal proliferation of fibroblasts in an abundant extracellular matrix, oriented perpendicular to the basilar epidermal layer in a ‘picket fence’ pattern. There was often an inflammatory infiltrate, consisting of lymphocytes and plasma cells (not shown).

Immunohistochemical analysis of normal skin samples showed positive immunostaing for CPT1 in a low number of basal epidermal cells and in few dermal fibroblasts (score 1), which showed moderate brown staining in the cytoplasm (+) (Figure S1).

Immunohistochemical staining showed sarcoid samples with cytoplasmic (16/25) or nuclear (5/25) staining pattern for CPT1; occasionally (4/25) nuclear and cytoplasmic immunostaining were observed in the same samples. Immunostaing intensity was strong (++) in 6/16 sarcoids with cytoplasmic patterns (Fig. 1 a- Fig, 1 b), in all sarcoids with nuclear patterns (Fig. 2 a- Fig. 2 b) and with nuclear and cytoplasmic patterns. The immunostaining was observed in most sarcoid fibroblasts (SFs) (70-100%-score 3), which were arranged in picket fence pattern at dermal-epidermal junction (Fig. 1 a, Fig 1 b, Fig. 2 a) and organized in bundles in the dermal tissue (Fig. 2 b). 4/25 sarcoid samples showed cytoplasmic moderate immunostaing (+) in 50-70% of SFS (score 2); the remaining 5/25 sarcoid samples showed weak cytoplasmic immunostaining (-) in a minor percentage of immunolabeled SFs (0-50% -score 1).

RT-qPCR revealed the positivity to BPV1-L1 in all samples (Table 2S). BPV2 and BPV13 were no detected. Moreover, fibroblastic-like morphology and positivity to Vimentin were showed in SAR1 (Fig.2S), SAR2 (Fig.3S), SAR3 (Fig.4S), and SAR4 (Fig.5S) cell lines.

By Western blot, a band of the expected molecular size for CPT1A (88 kDa) was identified in Hela cells and primary cell lines from equine sarcoids used as positive control (Fig. 3 A) as well as in the tested samples (Fig. 3 B) as suggested by antibody datasheet and literature data, confirming the specificity of the antibody.

4. Discussion

Hypoxic microenvironment is a common phenomenon in many tumors that stimulates a complex cell signaling network in cancer cells, including the HIF-1α, leading to decrease or increase of hypoxic effects [35,36]. It is well known the role of HIF-1α in regulation the expression of several genes involved in energy metabolism [36,37]. Among enzyme involved in Fatty acids oxidation (FAO), CPT1A plays a significant role in metabolic adaptation of cancer cells [16].

We have previously showed that abnormal vessel structures and increase of collagen deposition [12-14] in equine sarcoid could lead to a

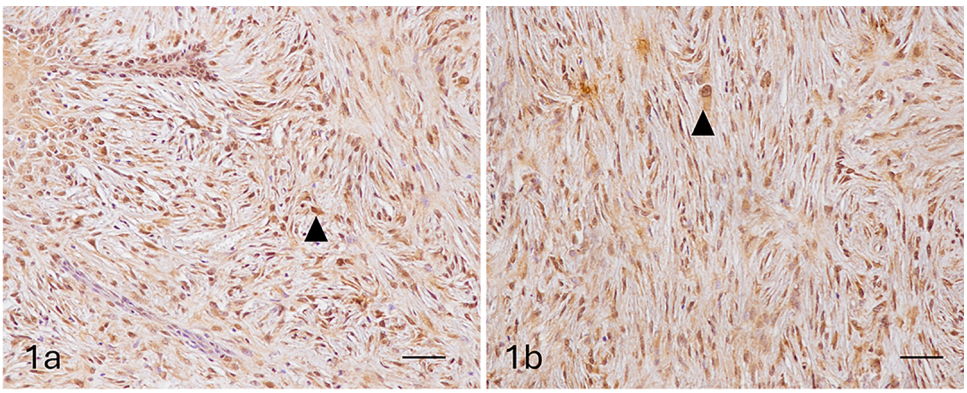

Fig. 1. Equine sarcoid. Strong cytoplasmic immunostaining for CPT1 in most sarcoid fibroblasts under the epidermis (A, B arrow) (20X; Scale bar: 100 μm; Streptavidin-biotin-peroxidase stain).

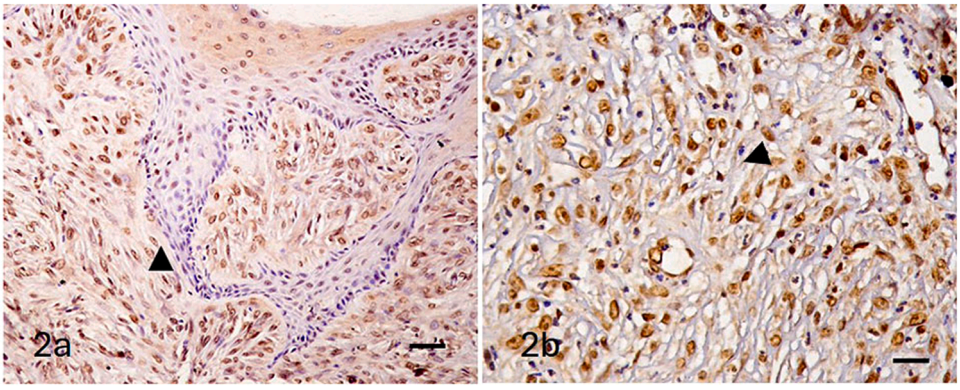

Fig. 2. Equine sarcoid. Strong nuclear immunostaining for CPT1 in most sarcoid fibroblasts under the epidermis (A, arrowhead; 20X;) and in dermis (B arrow) (40X; Scale bar: 100 μm; Streptavidin-biotin-peroxidase stain).

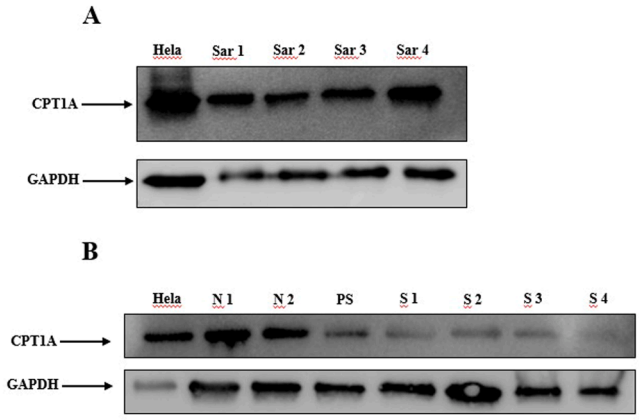

Fig. 3. Western blot analysis for CPT1A in primary cell lines obtained from sarcoid lesion, skin samples, perilesional skin and equine sarcoids. A band of the expected molecular size was detected in all samples (Hela: Hela cell line; Sar1-Sar4: primary cell lines; N1-N2: normal skin; PS: Perilesional skin; S1-S4: equine sarcoids). Protein lysate from Hela cell line was run on the gel to demonstrate the specificity of the antibody. Anti-GAPDH antibody was utilized to enable normalization.

hypoxic condition that induce an upregulation of HIF-1α [13].

In this study we have observed that CPT1A was strongly expressed in sarcoid samples compared to normal skins, suggesting its implication in metabolic reprogramming of these tumors, as already showed in canine mammary tumors [24,25,27] and human tumors [28,32]. We have found that 60% of sarcoid samples showed strong immunostaining for CPT1A in the nucleus and/or in the cytoplasm of sarcoid fibroblasts, while the remaining sarcoid samples showed moderate (20%) and weak (20%) cytoplasmic immunostaining. Cytoplasmic immunostaing is related to the physiologically resides of CPT1A at the outer mitochondrial membrane where transports long-chain fatty acids from the cytoplasm into the mitochondria for β-oxidation [16]. The overexpression of CPT1A in sarcoid tissues compared to normal skin could be related to the increase of CPT1A transcription in response to hypoxia. This could explain why sarcoid fibroblasts continue to survive, to produce collagen and to proliferate despite being in hypoxic conditions. The increase of CPT 1A expression was not found in normal tissue samples, suggesting that regulation of CPT1A depends on the specific circumstances of the tumor hypoxic microenvironment. Moreover, immunohistochemical experiments showed a peculiar nuclear expression of CPT1A in sarcoid fibroblasts, that was not observed in normal tissues. Nuclear localization of CPT1A has been reported in human tumors, and it was considered as the product of the transcript variant 2 of CPT1A, that doesn't retain the classical transferase activity, but is strongly involved in the epigenetic regulation of cancer survival, cell death escaping and tumor invasion pathways [22,23]. In the nucleus of neoplastic cells, CPT 1A was found strictly associated with the Histone Deacetylase Complex (HDAC), suggesting that it could have a role as regulatory protein of tumoral tissues, by modulation of HDAC-mediated deacetylation of specific genes involved in the control of cell cycle/growth and apoptosis [22,23]. Moreover, in the present study we have found an overexpression of CPT1A in BPV1 and BPV2 positive equine sarcoids, compared to BPV negative normal skin controls. It is well known that viruses depend on the availability of host metabolic constituents to replicate and accomplish their life cycles. An upregulation of CPT1A expression was reported

also in nasopharyngeal carcinoma, an Epstein–Barr virus (EBV)-associated cancer and in Human Papillomavirus (HPV16; HPV18) associated tumors [38-41] suggesting that viral infections could have a role in metabolic regulation of tumor cells, remodeling glucose metabolism and participating in lipid and glutamine regulation [38-41]. This research lays the groundwork for further investigations, focused on the direct interaction between CPT1A and BPV1/2 oncoproteins to investigate the possible role of BPV in metabolic regulation of equine sarcoid.

5. Conclusion

The study provides evidence for the first time that CPT1A is strongly expressed in equine sarcoids, not only in the cytoplasm of sarcoid fibroblast, suggesting its implication in metabolic reprogramming, but also in the nucleus of sarcoid fibroblasts, where it could be involved in regulation of neoplastic proliferation.

Results of this study paves the way to further studies focused on the better understanding of metabolic processes in equine sarcoid and could represent an initial step for the possibility to use metabolic pathway as target for innovative therapeutic strategies.

Ethics in publishing statement

This research presents an accurate account of the work performed, all data presented are accurate and methodologies detailed enough to permit others to replicate the work.

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All authors have been personally and actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content.

CRediT authorship contribution statement

Manuela Martano: Writing – review & editing, Writing – original draft, Validation, Conceptualization. **Karen Power:** Writing – original draft, Validation, Methodology, Investigation. **Bianca Cuccaro:** Methodology, Investigation. **Elisabetta Razzuoli:** Methodology, Investigation. **Paola Maiolino:** Writing – review & editing, Supervision. **Brunella Restucci:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jevs.2024.105205.

References

[1] Pascoe RR, Summers PM. Clinical survey of tumours and tumour like lesions in horses in southeast. Equine Vet J 1981;13:325–9.
[2] Ragland WL, Keown GH, Spencer GR, sarcoid Equine. Equine Vet J 1970;2:2–11.
[3] Knottenbelt DC. A suggested clinical classification of the equine sarcoid. Clin Techn Equ Pract 2005;4:278–95.
[4] Borzacchiello G, Corteggio A. Equine sarcoid: state of the art. Ippol 2009;20:7–14.
[5] Lunardi M, Alcântara BK, Otonel RAA, Rodrigues BW, Alfieri AF, Alfieri AA. Bovine papillomavirus type 13 DNA in equine sarcoids. J Clin Microbiol 2013;51:2167–71.
[6] Borzacchiello G, Russo V, Della Salda L, Roperto S, Roperto F. Expression of platelet-derived growth factor-β receptor and bovine papillomavirus E5 and E7 Oncoproteins in equine sarcoid. J Comp Pathol 2008;139(4):231–7.
[7] Cochrane AC. Models in vivo of wound healing in the horse and the role of growth factors. Vet Dermatol 1997;8:259–72.
[8] Semik EG. Molecular approaches to equine sarcoids. Equine Vet J 2021;53:221–30.
[9] Chambers G, Ellsmore VA, O'Brien PM, Reid SW, Love S, Campo MS, Nasir L. Association of bovine papillomavirus with the equine sarcoid. J Gen Virol 2003;84:1055–62.
[10] Jindra C, Hainisch EK, Brandt S. Immunotherapy of equine sarcoids—from early approaches to innovative vaccines. Vaccines (Basel) 2023;11:769.
[11] Jindra C, Hainisch EK, Rümmele A, Wolschek M, Muster T, Brandt S. Influenza virus vector iNS1 expressing bovine papillomavirus 1 (BPV1) antigens efficiently induces tumour regression in equine sarcoid patients. PLoS One 2021;16:11–22.
[12] Martano M, Corteggio A, Restucci B, De Biase ME, Borzacchiello G, Maiolino P. Extracellular matrix remodeling in equine sarcoid: an immunohistochemical and molecular study. BMC Vet Res 2016:12–24.
[13] Martano M, Altamura G, Power K, Restucci B, Carella F, Borzacchiello G. Evaluation of hypoxia-inducible factor-1 alpha (HIF-1α) in equine sarcoid: an immunohistochemical and biochemical study. Pathogens 2020;9:58.
[14] Martano M, Power K, Restucci B, Pagano I, Altamura G, Borzacchiello G, Maiolino P. Expression of vascular endothelial growth factor (VEGF) in equine sarcoid. BMC Vet Res 2018;14:266.
[15] Martano M, Altamura G, Power K, Liguori P, Restucci B, Borzacchiello G. Beclin 1, LC3 and P62 expression in equine sarcoids. Animals (Basel) 2021;12:20.
[16] Melone ABM, Valentino A, Margarucci S, Galderisi U, Giordano A, Peluso G. The carnitine system and cancer metabolic plasticity. Cell Death Dis 2018;9:228.
[17] Nomura DK, Cravatt BF. Lipid metabolism in cancer. Biochim Biophys Acta 2013;1831:1497–8.
[18] Li B, Sui L. Metabolic reprogramming in cervical cancer and metabolomics perspectives. Nutr Metab 2021;18:93.
[19] Eales KL, Hollinshead KER, Tennant DA. Hypoxia and metabolic adaptation of cancer cells. Oncogenesis 2016;5:e190.
[20] Qu Q, Zeng F, Liu X, Wang QJ, Deng F. Fatty acid oxidation and carnitine palmitoyl transferase I: Emerging therapeutic targets in cancer. Cell Death Dis 2016;7:e2226.
[21] Song S, Shi Z. CPT1A in cancer: tumorigenic roles and therapeutic implications. Biocell 2023;47:2207–15.
[22] Mazzarelli P, Pucci S, Bonanno E, Sesti F, Calvani M, Spagnoli LG. Carnitine palmitoyltransferase I in human carcinomas: a novel role in histone deacetylation? Cancer Biol Ther 2007;6:1606–13.
[23] Pucci S, Zonetti MJ, Fisco T, Polidoro C, Bocchinfuso G, Palleschi A, Novelli G, Spagnoli LG, Mazzarelli P. Carnitine palmitoyl transferase-1A (CPT1A): a new tumor specific target in human breast cancer. Oncotarget 2016;7:19982–96.
[24] Cacciola NA, Sgadari M, Petillo O, Margarucci S, Martano M, Cocchia N. Carnitine palmitoyltransferase 1A expression profile in canine mammary tumors. Vet J 2020;257:105453.
[25] Cacciola NA, Sgadari M, Sepe F, Petillo O, Margarucci S, Martano M. Metabolic flexibility in canine mammary tumors: implications of the carnitine system. Animals (Basel) 2021;11:2969.
[26] Cacciola NA, Sepe F, Fioriniello S, Petillo O, Margarucci S, Scivicco M. The Carnitine Palmitoyltransferase 1A inhibitor teglicar shows promising antitumour activity against canine mammary cancer cells by inducing apoptosis. Pharmaceuticals (Basel) 2023;16:1987.
[27] Sgadari M, Cacciola NA, Power K, Martano M, Restucci B. Sirtuin 1 expression in canine mammary tumors: a pilot study. Animals (Basel) 2023;13:2609.
[28] Das M, Giannoudis A, Sharma V. The role of CPT1A as a biomarker of breast cancer progression: a bioinformatic approach. Sci Rep 2022;12:16441.
[29] Xiong X, Wen Y, Fairchild R, Zaytseva YY, Weiss HL, Evers BM, Gao T. Upregulation of CPT1A is essential for the tumor-promoting effect of adipocytes in colon cancer. Cell Death Dis 2020;11:736.
[30] Samudio I, Harmancey R, Fiegl M, Kantarjian H, Konopleva M, Korchin B, et al. Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction. J, Clin, Invest 2010;20:142–56.
[31] Schlaepfer IR, Rider L, Rodrigues LU, Gijon MA, Pac CT, Romero L, et al. Lipid catabolism via CPT1 as a therapeutic target for prostate cancer. Mol Cancer Ther 2014;13:2361–71.
[32] Vithayathil JH, Kumar S, Sung S, Dobrolecki PL, Putluri LEV, et al. Fatty acid oxidation-driven src links mitochondrial energy reprogramming and oncogenic properties in triple-negative breast cancer. Cell Rep 2016;14:2154–65.
[33] Vangipuram M, Ting D, Kim S, Diaz R, Schüle B. Skin punch biopsy explant culture for derivation of primary human fibroblast. JoVE 2013;77:e3779.
[34] Maggi R, De Paolis L, De Santis D, Vellone VG, De Ciucis CG, Fruscione F, et al. Bovine papillomavirus type 1 infection in an equine congenital papilloma. Pathogens 2023;12:1059.
[35] Muz B, de la Puente P, Azab F, Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. Hypoxia 2015;3:83–92.

[36] Mylonis, I., Simos, G., Paraskeva, E., Hypoxia-inducible factors and the regulation of lipid metabolism cells, 8 (2019) 214.
[37] Taylor CT, Scholz CC. The effect of HIF on metabolism and immunity. Nat, Rev, Nephrol 2022;18:573–87.
[38] Li B, Sui L. Metabolic reprogramming in cervical cancer and metabolomics perspectives. Nutr Metab (Lond) 2021;18:93.
[39] Cruz-Gregorio A, Aranda-Rivera AK, Ortega-Lozano AJ, Pedraza-Chaverri J, Mendoza-Hoffmann F. Lipid metabolism and oxidative stress in HPV-related cancers, Free Radic. Biol Med 2021;172:226–36.
[40] Abudula A, Rouzi XL, Yang Y, Hasimu A. Tissue-based metabolomics reveals potential biomarkers for cervical carcinoma and HPV infection. Bosn J Basic Med Sci 2020;20:78–87.
[41] Tang M, Dong X, Xiao L, Tan Z, Luo X, Yang L, et al. CPT1A-mediated fatty acid oxidation promotes cell proliferation via nucleoside metabolism in nasopharyngeal carcinoma. Cell Death Dis 2022;13:331.