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Research paper

The expression profile of sterile alpha motif and histidine-aspartate domain-containing protein 1 (SAMHD1) in feline tissues

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ABSTRACT

SAMHD1 restricts lentiviruses by limiting availability of deoxynucleoside triphosphates for reverse transcription. HIV-2 and SIV have virion-associated proteins to counteract SAMHD1. Cats have an ortholog to human SAMHD1 and the FIV is restricted by human SAMHD1, but expression of feline SAMHD1 is unknown. Using a whole-body tissue microarray consisting of 24 tissues for immunohistochemistry, SAMHD1 expression was identified in a wide range of cat tissues. SAMHD1 was most strongly expressed in skin and mucosal epithelium, and in hemolymphatic and spermatogenic tissues. Both nuclear and cytoplasmic expression was detected. Feline cell lines susceptible to FIV infection also highly expressed SAMHD1. Preferential expression of SAMHD1 at sites of viral entry and replication supports a role for feline SAMHD1 in restricting FIV.

1. Introduction

Sterile α motif (SAM) and histidine-aspartate domain (HD) is an enzyme termed SAMHD1 that hydrolyzes nucleotide triphosphates to triphosphate and a nucleoside. This activity reduces the cellular dNTP pool to a level below that required for efficient HIV-1 cDNA synthesis by viral reverse transcriptase (RT) in non-cycling myeloid cells (macrophages, dendritic cells) and resting $CD4^+$ T cells (Baldauf et al., 2012; Goldstone et al., 2011; Ji et al., 2014; Wei and Yu, 2015; Zhu et al., 2013). By degrading dNTPs, SAMHD1 also affects cell cycle progression and DNA replication (Rampazzo et al., 2010). Germline mutations in SAMHD1 are associated with the autoinflammatory condition Aicardi Goutières syndrome (AGS), and acquired mutations are common in chronic lymphocytic leukemia (CLL) in humans (Ji et al., 2014). SAMHD1 is especially effective in restricting the size of the dGTP pool (Franzolin et al., 2015). Among the nucleotide triphosphates, dGTP is the preferred substrate for SAMHD1, and hydrolysis of dGTP activates SAMHD1 to hydrolyze other dNTPs (Goldstone et al., 2011). SAMHD1 also promotes hydrolysis of the deoxycytidine analog cytarabine used for treatment of leukemia (Herold et al., 2017). With crystallographic studies it was noted that dGTP-binding residues in the allosteric site of SAMHD1 promote the formation of tetramers and conformational changes in the active site. Tetrameric SAMHD1 is the most active form of the dNTPase (Zhu et al., 2013).

SAMHD1 has N-terminal SAM and central HD domains (Ji et al., 2014). The dNTPase activity of SAMHD1 resides in the HD, while the SAM domain is considered to mediate protein–protein interaction (Ji et al., 2013). Human SAMHD1 was the first enzyme with triphosphohydrolase activity identified in eukaryotic cells (Goldstone et al., 2011). Beloglazova et al. (2013) also reported magnesium dependent 3′ → 5′exonuclease activity of purified full-length human SAMHD1 during *in vitro* digestion of single-stranded DNA and RNA (Beloglazova et al., 2013). SAMHD1 was detected in cells of many organs in humans, but expression was highly variable (Goldstone et al., 2011; Schmidt et al., 2015). Initially, it was thought that SAMHD1 is expressed only in cells of hematopoietic origin, but recent findings indicate more widespread distribution consistent with a role in multiple cell homeostasis (Franzolin et al., 2015; Schmidt et al., 2015).

The HIV-2 accessory protein Vpx induces proteasomal degradation of SAMHD1 to a trinucleotide level sufficient for lentiviral RT activity in macrophages (Hrecka et al., 2011; White et al., 2013b). Human SAMHD1 blocked infection of HIV-2, feline immunodeficiency virus (FIV), bovine immunodeficiency virus (BIV), equine infectious anemia virus (EIAV), N-tropic murine leukemia virus (N-MLV), and B-tropic murine leukemia virus (B-MLV) in human monocytic U937 cells (White et al., 2013a). FIV and HIV-1 are highly similar in genome structure, manifestation of infection and host cell restriction mechanisms (Wongsrikeao et al., 2011) but little is known about SAMHD1 in feline cells. The feline genome encodes a SAMHD1 ortholog but its *in vivo* function is unknown.

The objectives of this study were to establish assays for detection of feline SAMHD1, to characterize the expression profile of SAMHD1 in a broad array of feline tissues, and to assess gene and protein expression in feline cell lines.

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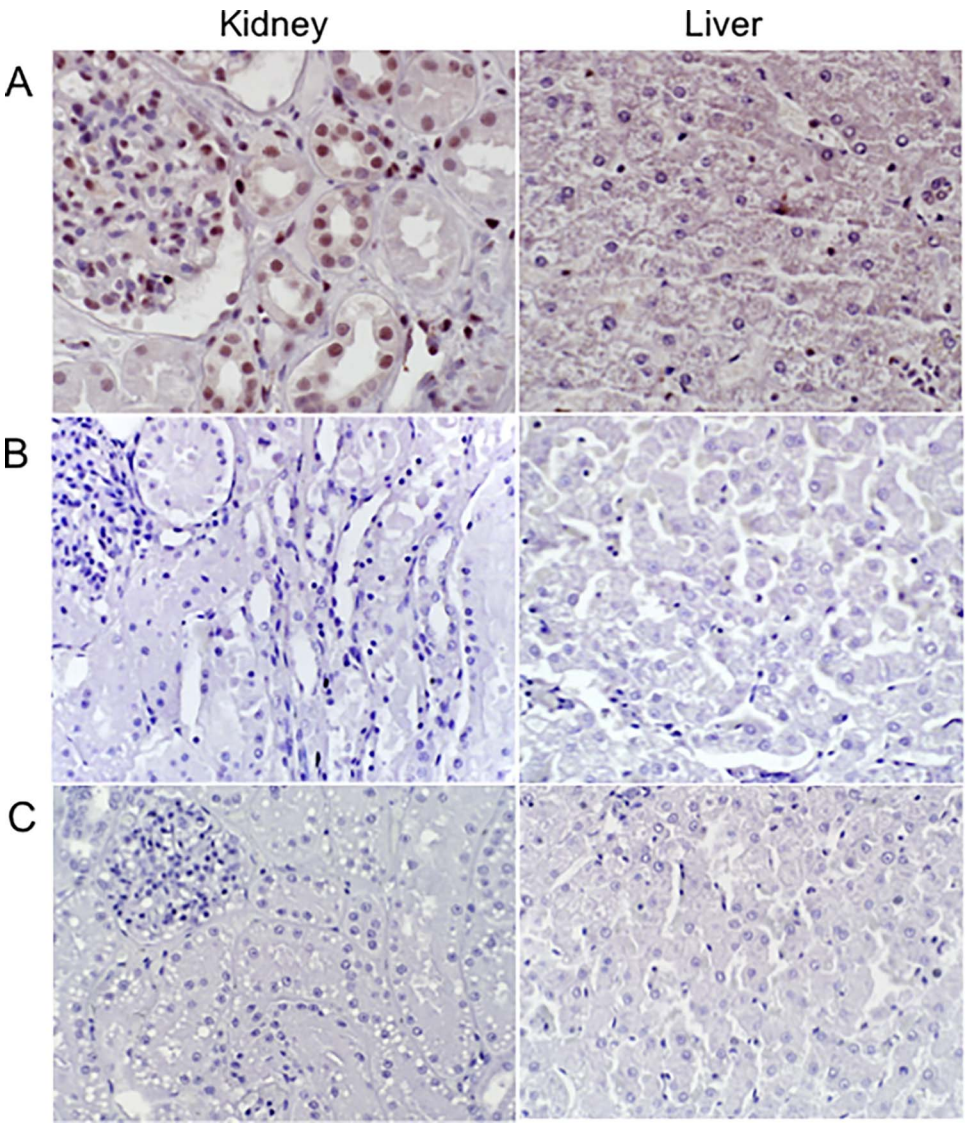

Fig. 1. Positive and negative control sections for immunohistochemical detection of SAMHD1. (A) Sections of human kidney and liver show strong nuclear SAMHD1 reactivity in proximal tubular cells, glomerular cells and leukocytes, and faint cytoplasmic reactivity in tubular epithelial cells and hepatocytes. (B) Feline tissues incubated without primary antibody yielded no specific staining. (C) Feline tissues incubated with an isotype control antibody also yielded no specific staining. Nova Red chromogen (brown), hematoxylin counterstain (blue). Original magnification × 400. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Immunohistochemistry

Tissues collected from three healthy adult male cats euthanized for reasons unrelated to this study were cut into 1 cm blocks, fixed overnight in 10% neutral buffered formaldehyde and embedded in paraffin. Tissue microarrays (TMA) encompassing adrenal gland, bone marrow, ear, epididymis, esophagus, fat, gall bladder, heart, intestine (small and large), kidney, liver, lung, pancreas, salivary gland (parotid and sublingual), skeletal muscle, skin, spleen, stomach, testis, trachea, tongue and urinary bladder were constructed by arraying 1.5 mm cores of each tissue in a separate block. The tissue microarrays containing 24 tissues were then sectioned at 3 µm with a rotary microtome. Sections were deparaffinized in xylene, antigens were retrieved by steaming slides in target retrieval solution (Dako, Mississauga, ON) with pH 6.1 at 95 °C for 10 or 30 min, or at 121 °C for 3 min. Subsequently, endogenous peroxidase was inhibited by 10 min incubation with dual enzyme blocker solution, and slides were incubated for 30 min at room temperature with serum free protein blocker (Dako). Slides were then incubated overnight with primary antibody (mouse monoclonal antibody 1A1, IgG2b; OriGene, Rockville, MD) diluted 1:100 in wash buffer. Next day slides were rinsed and incubated with Envision Dual Link System-HRP. Bound antibody was detected with Nova Red chromogen (Dako), and slides were counterstained with Harris modified hematoxylin (Thermo Fisher Scientific, Burlington, ON) for 90 s. Sections (TMA) from all cats were processed in a single batch. Positive control slides consisted of human kidney and liver sections prepared with identical antigen retrieval (10 min at 95 °C and pH 6.1) and antibody dilution (1:100). Negative control slides consisted of each feline TMA containing 24 tissues prepared in an identical manner with omission of primary antibody, and tissues incubated with an isotype-matched monoclonal antibody against human CXCR4 (clone 44717, IgG2b, R&D Systems, Minneapolis, MN). Images were acquired on an Olympus BX45 microscope using an Olympus camera DP71 and cellSens standard 1.12 software. The proportion and intensity of staining was graded as adapted from Allred et al. (1993) to indicate 0 = no staining; 1 = < 1%; 2 = 2–10%; 3 = 11–33%; 4 = 34–66%; 5 = > 66% of cells were positively stained. The intensity of staining in the nucleus and cytoplasm was graded as 0 = none; 1 = weak; 2 = intermediate; 3 = strong. Combined semiquantitative immunohistochemical (IHC) scores were calculated for each of nuclear and cytoplasmic staining by multiplying the percentage of labeled cells and the intensity score yielding potential scores ranging from 0 to 30 (Allred et al., 1993; Doane et al., 2006).