NEOPLASTIC DISEASE

Epithelial Cell Adhesion Molecule Expression in Canine Tumours

D. H. Thamm [*,†,§], D. F. Hayes [‖], T. Meuten [‡], T. Laver [*] and D. G. Thomas [‖]

[*] Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, [†] Cell and Molecular Biology Graduate Program, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, [‡] Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, [§] University of Colorado Comprehensive Cancer Center, 1665 N. Ursula St., Aurora, CO and [‖] Comprehensive Cancer Center, University of Michigan, 1500 E. Medical Center Drive SPC 5942, Ann Arbor, MI, USA

Summary

Epithelial cell adhesion molecule (EpCAM) is expressed in most human normal and neoplastic tissues of epithelial derivation and may have an association with tumour cell aggressiveness, a stem cell-like phenotype and clinical outcome. Antibody-based strategies for the targeting and capture of EpCAM-expressing tumour cells are showing promise, both as diagnostic tools and potential therapies. The aim of this study was to assess EpCAM expression in canine tumours. EpCAM expression was assessed in tumour cell lines via gene expression profiling and in formalin-fixed and paraffin wax-embedded tissues from canine carcinomas representing various anatomical sites by immunohistochemistry. EpCAM mRNA expression was higher in cell lines from carcinomas than those derived from sarcomas or haemopoietic tumours. EpCAM was expressed by >2/3 of tumour cells in 71% of canine carcinomas evaluated, irrespective of histotype, with the exception of carcinomas of the adrenal gland. Canine sarcomas and haemopoietic tumours were uniformly negative. Most canine carcinomas express EpCAM and so could be suitable for the study of EpCAM-directed diagnostics and therapeutics.

© 2016 Elsevier Ltd. All rights reserved.

Keywords: carcinoma; dog; epithelial cell adhesion molecule; immunohistochemistry

Epithelial cell adhesion molecule (EpCAM, CD326) is a transmembrane glycoprotein, which mediates cell-to-cell contact and participates in juxtacrine signal transduction (Pavsic et al., 2014). EpCAM is expressed in most healthy adult epithelial-derived tissues, primarily on the basolateral epithelial membranes (Moldenhauer et al., 1987; Munz et al., 2008; Schmelzer and Reid, 2008). EpCAM is also expressed in embryonic and somatic stem cells, where it can participate in the regulation of Wnt pathway signalling and may play a role in the maintenance of pluripotency (Carpenter and Red Brewer, 2009; Gonzalez et al., 2009).

EpCAM is highly expressed in the majority of human carcinomas and expression is generally diffusely membranous and/or cytoplasmic, rather than being restricted to the basolateral membrane (Gastl et al., 2000; Gosens et al., 2007; van der Gun et al., 2010). Upregulation of EpCAM has been associated with downregulation of E-cadherin, reduction of cell-to-cell adhesion and promotion of cell motility, features consistent with epithelial—mesenchymal transition (Winter et al., 2003, 2007; van der Gun et al., 2010).

correspondence to: D. H. Thamm (e-mail: dthamm@colostate.edu).

The intensity of tumour-associated EpCAM expression has been associated with tumour aggressiveness and clinical outcome in a variety of human carcinomas, including lung, breast, head and neck, biliary and colorectal carcinomas, among others (Soysal *et al.*, 2013; Murakami *et al.*, 2014; Sulpice *et al.*, 2014; Kim *et al.*, 2015; Zhou *et al.*, 2015).

EpCAM-directed antibody therapies have been developed, and catumaxomab (Removab™, Neovii, Grafelfing, Germany), a bispecific anti-EpCAM monoclonal antibody received European Medicines Agency approval for the treatment of malignant ascites in patients with ovarian cancer in 2009 (Sebastian, 2010). Additionally, EpCAM antibody-based capture methods have been developed to allow the identification, quantification and characterization of circulating EpCAM-positive carcinoma cells from the circulation (Allard *et al.*, 2004; Bidard *et al.*, 2013; Smerage *et al.*, 2013). One of these detection systems, CellSearch® (Jansen Diagnostics, Raritan, New Jersey, USA), has received approval from the US Food and Drug Administration for patient monitoring and prognostication in human metastatic breast, colorectal and prostate cancer (Cristofanilli *et al.*, 2004; Smerage *et al.*, 2013; Huang *et al.*, 2015; Janni *et al.*, 2016; Zhang and Armstrong, 2016).

There is limited information available regarding the expression of EpCAM in normal or neoplastic canine tissues. EpCAM has been utilized as a neuroendocrine marker for the immunohistochemical identification of canine Merkel cells (Ramirez *et al.*, 2014) and recently as a putative stem cell marker in canine mammary carcinoma cells (Rybicka *et al.*, 2015). The aim of this study was to evaluate EpCAM expression in a collection of normal and neoplastic canine tissues.

The MCF7 human breast carcinoma, D17 canine osteosarcoma and MDCK immortalized canine kidney cell lines were obtained from the American Type Culture Collection (Manassas, Virginia, USA). The CMT 12 canine mammary carcinoma cell line was generously provided by Dr. L. Wolfe, Auburn University, Alabama, USA (Bird *et al.*, 2008). The Bliley canine transitional cell carcinoma cell line was derived in the laboratory of Dr. S. Dow, Colorado State University, Colorado, USA. The OSW canine T-cell lymphoma cell line was generously provided by Dr. W. Kisseberth from The Ohio State University, Ohio, USA (Kisseberth *et al.*, 2007). All were cultured under standard conditions. All canine cell lines were confirmed to be canine in origin and unique based on multispecies microsatellite polymerase chain reaction (PCR) as described (O'Donoghue *et al.*, 2011).

The details regarding characterization and gene expression profiling methodology for the FACC30 canine tumour cell line panel are published (Fowles *et al.*, 2016). Normalized intensities for the three Affymetrix Canine Genome 2.0 probesets corresponding to canine EpCAM sequences were compared between epithelial and non-epithelial-derived cell lines using a two-tailed, unpaired Mann—Whitney U test.

For western blotting analysis, protein was extracted with radioimmunoprecipitation assay buffer containing 1 mM activated sodium orthovanadate, 1 mM phenylmethylsulphonyl fluoride solubilized in isopropanol and protease inhibitor cocktail tablet according to the manufacturer's instructions (Complete Mini™, Roche Diagnostics, Mannheim, Germany). Total protein concentration was determined using a bicinchoninic acid protein assay kit (Thermo Scientific, Waltham, Massachusetts, USA) and run on a 4—12% NuPAGE Bis-Tris precast gel in a NOVEX Xcell SureLock™ mini-cell system (Invitrogen, Carlsbad, California) and transferred to a polyvinylidene fluoride membrane (Biorad, Hercules, California, USA). The membrane was then blocked using Superblock™ blocking buffer (Pierce, Rockford, Illinois, USA). The primary rabbit anti-human EpCAM antibody EPR677 (Abcam, Cambridge, Massachusetts, USA) was applied at a dilution of 1 in 2,000 and incubated overnight at 4°C. Membranes were then washed using Tris-buffered saline with Tween 20 and incubated with goat anti-rabbit horseradish peroxidase (Pierce) diluted in Superblock™ blocking buffer (Pierce). They were then developed using SuperSignal West Pico™ (Pierce) and imaged using a GelDoc™ imager (Kodak, Rochester, New York, USA).

For generation of cell blocks, cells were collected by scraping, washed in phosphate buffered saline (PBS), transferred to a 1.7 ml Eppendorf tube and pelleted by centrifugation. The cell pellet was resuspended in 1 ml 10% neutral buffered formalin (NBF) (Cancer Diagnostics Inc., Durham, North Carolina, USA) and fixed overnight at 4°C. Fixed cells were centrifuged, NBF was removed by aspiration and 1% agarose in PBS was added to the cell pellet. The embedded pellet was cooled to 4°C for 30 min before sectioning.

Paraffin wax-embedded normal and neoplastic canine tissues were retrieved from the Colorado State University Flint Animal Cancer Center tumour archive. All tissue samples were collected with Institutional Animal Care and Use Committee approval and written informed owner consent and all samples were anonymized. After pathological review, tissue microarrays (TMAs) were constructed from the most representative, non-necrotic areas