

Expression of stem cell biomarker aldehyde dehydrogenase 1 (ALDH1) in canine mammary tumor

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ABSTRACT

The study was conducted to investigate the immunohistochemical expression of a stem cell biomarker, ALDH1 in CMTs. ALDH1 is a cytosolic enzyme responsible for oxidizing intracellular aldehydes leading to the oxidation of retinol to retinoic acid, a key step involved in early stem cell differentiation. It was noticed that the cells expressing this marker possess ability to self renew. In present investigation, we assessed the expression of ALDH1 by immunohistochemistry on paraffin embedded tissue sections from 31 confirmed clinical cases of canine mammary tumors (CMT). The results of immunohistochemistry were analyzed semiquantitatively by calculating percentage score and staining intensity using light microscope and comparing it with the histological types. ALDH1 expression was detected in 26 (83%) of CMTs and it was observed in benign lesions, invasive cells and cells undergoing epithelial to mesenchymal transition (EMT). The high expression patterns of ALDH1 in various histological types of CMT indicated poor prognosis.

Key words: ALDH1, Canine mammary tumor, Immunohistochemistry, Stem cell

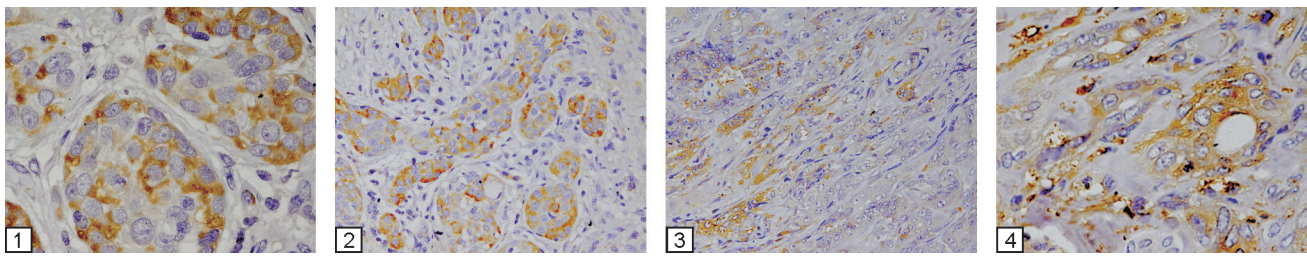
The origin of cancer is thought to be from embryo-like cells, as some cancer cells histologically resemble the embryonic tissues (Hendrix *et al.* 2007). The adult tissue contains embryonic remnants that remain dormant throughout life, but can be activated to develop in cancer forming cells (Hendrix *et al.* 2007). Some *in vitro* and *in vivo* studies indicated the existence of single stem cell or stem cell population that gave rise to diverse subpopulations of cancer cells with varied phenotypic and biologic behavior (Fillmore and Kuperwasser 2008). A new cancer stem cell (CSCs) biomarker aldehyde dehydrogenase1 (ALDH1) a cytosolic enzyme responsible for the oxidation of aldehydes accumulating within the cell, was identified. High ALDH1 activity was observed in normal and progenitor stem cells, which is required for early stem cell differentiation (Al-Hajj and Clark 2004). Furthermore, in cases of haematopoeitic malignancies, oncologists have observed higher activity of ALDH1 in bone marrow stem cells. Soon after this, ALDH1 immunoreactive cancer cells were also detected in solid tumors which supported the concept cancer stem cell theory (Ginestier *et al.* 2007). Among many biomarkers, ALDH-1 appears to be a more effective predictive marker for identifying CSCs. Existing studies

also confirmed ALDH-1 expression in primary lesions of breast cancer and its significant association with poor clinical prognosis (Crocker *et al.* 2009). Aldehyde dehydrogenase expressing (ALDH+) breast cells include malignant stem-like cell populations that maintain and cause progression of cancer (Clarke *et al.* 2006), and it was suggested that this enzyme is also involved in stem cell preservation and initiation of differentiation (Ma and Allan 2011). The CSC hypothesis was proposed to explain breast cancer heterogeneity and risk of recurrence. Although, ALDH1 was used for identification of human breast CSCs (Ginestier *et al.* 2007, Douville *et al.* 2009), however, the information regarding its expression pattern in canine mammary tumors is meager. CMTs are the most important tumors of canines and account for 25 and 50 % of all tumors occurring in canines (Misdorp 2003 to Sorensen 2003). Many accumulating evidences point that approximately 30 to 50% of the canine mammary tumours are malignant (Misdorp 2003). Therefore, the present paper discusses the immunohistochemical expression of ALDH1 in cases of CMT.

MATERIALS AND METHODS

The study was conducted on 31 cases of canine mammary gland tumors presented to the Small Animal Clinics of the Department of Teaching Veterinary Clinical Complex, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. Detailed histopathological examination was done and all

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Figs 1–4. **1.** Cytoplasmic immunoreactivity of ALDH1 in a case of carcinoma. **2.** Cytoplasmic immunoreactivity of ALDH1 in peripheral/basal cells of lobules. **3.** Cytoplasmic immunoreactivity of ALDH1 in invasive cells. **4.** Cytoplasmic immunoreactivity of ALDH1 in invasive cells

the 31 cases of CMT were classified on the basis of latest WHO classification of CMT. In addition, the lesions in the cases of CMT were further classified into early and late lesions. Lesions such as alveolar hyperplasia, atypical hyperplasia, intraductal papillomas, ductal carcinoma *in situ* (DCIS), and lobular carcinoma *in situ* were grouped as early lesions, whereas lesions such as invasive ductal carcinoma (IDC), invasive lobular carcinomas and epithelial–mesenchymal transitions (EMT) were grouped as advanced lesions as per earlier observations (Antuofermo *et al.* 2007, Ely *et al.* 2001, Kretschmer *et al.* 2011, Mediana 2008, Schmitt *et al.* 1999).

Immunohistochemistry: For immunohistochemical studies, 4–5 μ thick paraffin embedded tissue sections were taken on positively charged microscopic slides. Epitope retrieval was performed by using citrate buffer at 95°C for 5 minutes. Rabbit primary antibody against ALDH1 was used and immunohistochemical staining was performed by using advanced SSTM One- step polymer–HRP IHC detection system. The immunoreactivity of ALDH1 was expressed as percentage score (P) and intensity score (I) in the cells showing positive reaction (Tanei *et al.* 2009). Intensity scores were assessed as 0, 1, 2 and 3 and percentage scores were assessed depending upon percentage of cells showing immunoreactivity for ALDH1 as 3+ (>50%), 2+ (<50%, >10%), 1+ (<10%, >5%), and negative (<5%). To confirm EMT, we studied immunohistochemical expression of cytokeratin cocktail, vimentin and E-cadherin. It has been documented earlier that if the neoplastic cells express cytokeratin, vimentin but lose E-Cadherin then it indicates that they have lost epithelial characters and have acquired a mesenchymal phenotype (Chaw *et al.* 2012, Kallergi *et al.* 2011).

RESULTS AND DISCUSSION

Following latest WHO classification of mammary tumors of dogs, all the 31 cases were classified into simple carcinomas (2 cases), complex carcinomas (14 cases), carcinosarcomas (13 cases) and sarcomas (2 cases). Twenty six cases (83%) out of 31 CMT, expressed ALDH1. ALDH1 expression was detected both in the cytoplasm as well as nucleus of the affected cells (Figs 1–4).

In most of the cases, cytoplasmic expression of ALDH1 was more evident than that in the nucleus. Basal epithelium in general showed more immunoreactivity for ALDH1. The

cells undergoing EMT also showed positive reactivity for ALDH1. In few cases, ALDH1 immunoreactivity was also detected in the tumor emboli. In addition, some stromal cells and connective tissue cells also showed some ALDH1 immunoreactivity. Thus, ALDH1 was expressed in benign lesions, and also in more anaplastic and invasive cells in case of advanced lesions. The details of IHC results are summarized in Tables 1,2.

In the present study, 26 cases out of the 31 (83%), exhibited positive immunoreactivity for ALDH1. Although the pattern of immunolocalization was mainly cytoplasmic, however, nuclear expression was also noticed in benign lesions and simple carcinomas. These findings indicate that ALDH1 might have higher activity in early lesions. It was documented that the basal cells from duct or lobule possess stem cell like properties as these cells continuously divide

Table 1. Cellular location of ALDH1 in CMTs

Type of tumor	No. of cases showing ALDH1 expression	Immunore activity
Simple carcinoma (n=2)	2	Nuclear and cytoplasmic
Complex carcinoma (n=14)	12	Cytoplasmic
Carcino-sarcoma (n=13)	10	Cytoplasmic
Sarcoma (n=2)	2	Cytoplasmic

Table 2. ALDH1 expression in different histological types of CMTs

Type of tumor	Intensity	No. of cases	Percentage score	No. of cases
Simple carcinoma (2)	++	1	2	0
	+++	1	3	2
Complex carcinoma (14)	0	2	0	2
	+	5	1	2
	++	5	2	5
	+++	2	3	5
Carcino sarcoma (13)	0	3	0	3
	+	0	1	1
	++	6	2	2
	+++	4	3	7
Sarcoma (2)	+	2	2	2
		Total 31		Total 31

and give rise to new lobular and ductular cells in lactation phase (Visvader 2009). The expression of stem cell biomarker ALDH1 in these basal cell compartments might support above documentation and this may be a possible source of differentiated, undifferentiated cancer cells forming the bulk of tumor. Human oncologists reportedly reproduced mammary gland tumors in laboratory animals by inoculating ALDH1 immunoreactive cells isolated from the human breast cancer patients into the laboratory animal models (Ginestier *et al.* 2007). A new concept that is being extensively explored in oncology especially in human breast cancers is EMT. EMT is a phenomenon in which the epithelial cells in tumor acquire properties of mesenchymal cells (Savagner 2010). Expression of ALDH1 indicates the role of ALDH1 positive cells in EMT. ALDH1 might help in switching of cell from one type to another which is the property of the stem cells.

The expression of ALDH1 was evident in invasive cells in cases of carcinoma and carcinosarcoma in the present study. It indicated possible role of ALDH1 in invasion and tumor progression from benign to invasive. Invasion and metastasis are unfavorable signs when cancerogenesis is concerned. In addition, cells expressing ALDH1 have been reported to have high tumor regeneration potential (Burger 2009). Thus high expression of ALDH1 in most of the tumor cells may indicate poor prognosis. Furthermore, not all the cancer cells behave as CSCs most of the cells are differentiated cancer cells forming just a tumor bulk. However, high number of CSCs can be a direct indication of proliferative potential of the tumor. It was demonstrated in human breast cancer patients that the acquired drug resistance was associated with the transcriptional activation of ALDH-1 expression in the cells (Tanei *et al.* 2009). Therefore, these ALDH1 reactive cells may serve as potential targets for cancer chemotherapy to destroy CSCs to regress of primary as well as metastatic tumor.

Findings from current study indicated that stem cell biomarker ALDH1 is highly expressed and is a marker of poor prognosis in CMT cases.

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