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Short Communication

Immunohistochemical localisation of 14-3-3 σ protein in normal canine tissues

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ABSTRACT

The 14-3-3 σ protein, also called stratifin, belongs to the highly conserved family of 14-3-3 acid proteins, which are involved in the modulation of diverse signal transduction pathways. Loss of 14-3-3 σ expression has been observed in several types of human cancers, suggesting that it may have a role as a tumour suppressor gene. The 14-3-3 σ protein has been localised in normal human tissues exclusively in various epithelial cell types. The aim of the study was to investigate the expression and the distribution pattern of 14-3-3 σ by immunohistochemical analysis in normal canine tissues. Immunohistochemical expression of 14-3-3 σ was demonstrated in several normal canine tissues with some minor differences of distribution pattern compared with human tissues. It appears that 14-3-3 σ is a very specific epithelial cell marker in normal canine tissues.

The 14-3-3 proteins are members of a family of highly conserved, small, acid polypeptides of 28–33 kDa that are found in all eukaryotic species (Hermeking, 2003). They have a crucial role in a wide variety of cellular responses, including cell cycle progression, DNA damage checkpoints and apoptosis (Hermeking, 2003). Of the seven isoforms, 14-3-3 σ, also called stratifin, has been most directly linked to tumour development (Hermeking, 2003; Lodygin and Hermeking, 2006). It is a protein kinase-dependent activator of tyrosine and tryptophan hydroxylase and endogenous inhibitor of protein kinase C (Ichimura et al., 1988).

The 14-3-3 σ protein is a cell regulator and causes G2/M arrest by sequestering the cdc2–cyclin B1 complex in the cytoplasm and preventing its nuclear localisation, which is required for progression through mitosis (Chan et al., 1999). Furthermore, 14-3-3 σ has been shown to bind to G1-specific cyclin-dependent kinase 2 (cdk2), suggesting that it may also regulate G1/S progression (Laronga et al., 2000). Loss of 14-3-3 σ expression results in malignant transformation in vitro and supports tumour formation in vivo, suggesting a role as a tumour suppressor gene (Hermeking, 2003; Lodygin and Hermeking, 2006).

In spite of the ubiquitous distribution of most of the 14-3-3 isoforms, the expression of 14-3-3 σ is restricted to epithelial cells and increases during epithelial differentiation (Hermeking, 2003). These findings prompted us to analyse the distribution of 14-3-3 σ protein in normal canine tissues. So far and to the best of our knowledge, no previous assessment of 14-3-3 σ immunoexpression in canine tissues has been reported.

Normal canine tissues were obtained from four necropsy cases (two males, two females). Both males and one female dog were euthanased because of traffic accidents and had multiple fractures, and the second female died during recovery following caesarean surgery.

For the immunohistochemical study, tissue sections were dewaxed in xylene, taken through ethanol, blocked for endogenous peroxidase in methanol (10 min), and subjected to a high-temperature antigen retrieval technique (3 min of pressure cooking in citrate buffer pH 6.0). A goat polyclonal antibody specific for the N-terminus of the 14-3-3 σ isoform (N-14; Santa Cruz Biotechnology; 1:60; 16 h at 4 °C) and the avidin–biotin–peroxidase complex (Vector Laboratories) were employed (Simpson et al., 2004). Human skin was used as positive control, whereas negative controls were obtained by omitting the primary antibody.

The immunohistochemical results are summarised in Table 1. 14-3-3 σ immunoreactive cells were found in different locations including the epidermis (Fig. 1a), sebaceous glands (Fig. 1b), salivary gland (Fig. 1c), oesophagus (Fig. 1d), gall bladder (Fig. 2a), urinary bladder (Fig. 2b), prostate gland (Fig. 2c), mammary gland (Fig. 2d and e) and pancreatic islets (Fig. 2f). The expression was restricted to cells of epithelial origin.

The 14-3-3 protein family acts as adaptors or 'chaperone molecules', which are able to move freely from the cytoplasm to the nucleus and vice versa, showing the dynamic role of these proteins in normal cells (Muslin et al., 1996). So far, the most common function of 14-3-3 proteins appears to be sequestration of proteins in

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Table 1
Expression of 14-3-3 σ in normal canine tissues.

Tissue	Cytoplasm[a]	Nuclei[a]
Skin		
Epidermis/hair follicles	+++	–
Sebaceous glands		
Sebocytes	+	–
Reserve cells	+++	–
Apocrine glands		
Acini	+	–
Myoepithelium	++	–
Digestive system		
Oral cavity (Squamous epithelium)	+++	–
Salivary glands		
Acini	–	–
Myoepithelium	++	–/+
Ducts	+++	–
Esophagus		
Surface epithelium	+	+
Glands	+	–
Stomach		
Gastric pits	+	–
Corpus glands	–	–
Pyloric glands	–	–
Intestine		
Surface epithelium	–	–
Mucosal crypts	–	–
Brunneŕs glands	–	–
Liver		
Hepatocytes	–	–
Bile ducts	–	–
Gall bladder	++	–
Pancreas		
Acini	–	–
Ducts	–	–
Islet	+++	–
Respiratory tract		
Trachea		
Pseudostratified ciliated columnar epithelium	–	–
Basal cells	+	–
Bronchi		
Cylindrical ciliated epithelium	–	–
Basal cells	+	–
Alveolar epithelium	–	–
Glands	–	–
Urinary tract		
Kidney	–	–
Ureter	+++	+
Urinary bladder		
Superficial cells	–/+	–
Basal cells	+++	+
Urethra	+++	+
Endocrine glands		
Pituitary glands	–	–
Thyroid/parathyroid	–	–
Adrenal gland		
Cortex	–	–
Medulla	++	–
Male genital		
Seminiferous tubules	–	–
Excretory ducts	–	–
Epididymis	–	–
Prostate		
Secretory cells	+	–
Ducts	+++	+
Female genital		
Ovary		
Surface epithelium	–	–
Rete ovarii	–	–
Endometrium		
Surface epithelium	+	–
Glands	+	–
Vagina (Squamous epithelium)	+++	–
Mammary glands		
Secretory cells[b]	+ // –/+	–
Myoepithelium	+++	–
Ducts	–/+	–
Nervous system		
Cerebrum	–	–
Cerebelum	–	–
Ependymal cells	–	–

[a] – = No stained cells; –/+ = solitary positive cells; + = faint reaction; ++ = medium reaction; +++ = strong reaction.
[b] Lactating mammary gland, +; No-Lactating mammary gland, –/+.

the cytoplasm, leading to inhibition of their function (Hermeking, 2003). The 14-3-3 σ protein is a recently recognised tumour suppressor (Hermeking, 2003; Lodygin and Hermeking, 2006), the expression of which is down-regulated by CpG methylation in several types of human cancer (Hermeking, 2003; Lodygin and Hermeking, 2006). It is expressed in squamous epithelia in locations such as periductal and periglandular cells of various glands, and epithelial cells of the gastrointestinal tract, urinary tract and endometrium (Nakajima et al., 2003). Our findings indicate that 14-3-3 σ expression in normal canine tissues is generally similar to what has been described in humans. However, some minor differences in 14-3-3 σ expression between human and canine normal tissues were also observed.

In humans, the 14-3-3 σ protein has been detected by immunohistochemistry within the cytoplasm and nuclei of various cell types. Thus, strong immunoreactivity has been reported in the cytoplasm of epithelial cells of the skin, tongue, oesophagus, trachea, gall bladder, urinary bladder and cervix (Nakajima et al., 2003; Mhawech et al., 2005). Furthermore, a weak nuclear immunoreactivity has been reported in the epithelium of the oesophagus and tongue (Nakajima et al., 2003). In our study, canine skin, tongue, trachea, gall bladder, ureter, urinary bladder, urethra and vagina showed a pattern of reaction similar to those described in human tissues. However, the oesophageal epithelium showed a weak cytoplasmic immunostaining with an intense nuclear reaction in the basal cells.

In humans, 14-3-3 σ expression has been described in renal tubular epithelial cells (Nakajima et al., 2003; Mhawech et al., 2005). In contrast, 14-3-3 σ was apparently not expressed in canine kidneys. In the canine mammary gland, epithelial and myoepithelial cells were 14-3-3 σ positive with a predominant staining of the myoepithelial cells. Recently, 14-3-3 σ has been proposed as a novel and specific myoepithelial cell marker of the human mammary gland (Simpson et al., 2004). In contrast, other authors have described 14-3-3 σ immunoreaction within the cytoplasm of both luminal and myoepithelial cells of ducts and lobules with a stronger intensity in the myoepithelial cells (Simooka et al., 2004). Further studies are necessary to elucidate the real role of this protein in the canine mammary gland and its utility in the identification of myoepithelial cells.

Endocrine glands in humans show a complete lack of 14-3-3 σ expression (Nakajima et al., 2003). In canine tissues, a moderate and strong reaction was detected in the medulla of adrenal glands and in pancreatic islets, respectively. In the canine salivary gland, 14-3-3 σ was expressed in the simple cuboidal epithelium of ducts and myoepithelial cells, but glandular cells were totally negative. In human salivary glands, a similar pattern has been described with the exception of glandular cells, which expressed 14-3-3 σ at low levels (Nakajima et al., 2003). In the human prostate, 14-3-3 σ is expressed in periductal and periglandular cells (Nakajima et al., 2003) and luminal cells (Nakajima et al., 2003; Mhawech et al., 2005). In our study, no immunostaining was detected in periductal or periglandular cells and the immunoreaction was localised within the cytoplasm of both secretory and ductal cells with medium and intense intensity, respectively.