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Morphological and Immunohistochemical Characterization of Spontaneous Mammary Tumours in European Hedgehogs (*Erinaceus europaeus*)

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Summary

Mammary tumour samples (11 surgical and five post-mortem) from 16 adult European hedgehogs submitted between 1980 and 2004 were examined. Histologically, the tumours were classified as simple tubulo-papillary carcinomas with local invasive growth. In six cases, tumour cell emboli were present in blood vessels or lymphatic vessels, or both. However, metastasis to regional lymph nodes was found only in one hedgehog. Malignant neoplastic epithelial cells were immunolabelled by antibodies specific for various cytokeratins (CKs), including CK1–8, 10, 13–16, 19 and 20. CK expression did not differ from that in normal mammary gland tissue. CK20 was expressed in the mammary tissue of hedgehogs, in contrast to that of dogs and cats; CK7 immunolabelling, however, which commonly occurs in mammary epithelial cells, was negative. CK20 expression, together with the lack of CK7 as determined by a protein-specific antibody, represented an important difference from the CK profile shown by mammary epithelial cells of other mammalian species, including the dog and cat.

Keywords: cytokeratin; *Erinaceus europaeus*; European hedgehog; mammary tumour; tumour

Introduction

Spontaneous neoplasms in European hedgehogs (*Erinaceus europaeus*), although rarely described, include chromophobe pituitary adenoma (Campbell and Smith, 1966), papillomatous warts (Poduschka, 1981), hepatocellular carcinomas (S. Berns, personal communication), oral squamous cell carcinomas associated with dental plaque (Poduschka and Poduschka, 1986), and lacrimal ductal carcinoma (Kuonen *et al.*, 2002). Between 1980 and 2004, 17 additional neoplasms were recorded, including two fibrosarcomas, one malignant peripheral nerve sheath tumour, one undifferentiated brain tumour, one anaplastic round cell blastoma in the lung, one anaplastic sarcoma, one osteosarcoma, two malignant lymphomas, one squamous cell carcinoma, one carcinoma originating from adnexal glands, one fibroma, one papilloma, two fibropapillomas, one lipoma and one seminoma (Döpke, 2002, and unpublished).

In the African hedgehog (*Atelerix albiventris*), neoplasms have been recorded more frequently. Reports include the following: cutaneous epithelial or mesenchymal tumours, including malignant mast cell tumours, subcutaneous neurofibroma, subcutaneous schwannoma, squamous cell carcinoma, fibrosarcoma, sarcoma of periosteal origin, malignant peripheral nerve sheath tumour, and fibrous histiocytoma (Hruban *et al.*, 1992; Rivera and Janovitz, 1992; Peauroi *et al.*, 1994; Raymond *et al.*, 1997; Raymond and White, 1999; Raymond and Garner, 2000, 2001; Ramos-Vara, 2001); tumours of bones, including osteoma, osteochondroma and osteosarcoma (Peauroi *et al.*, 1994; Raymond and Garner, 2001); neoplasms originating from blood vessels or the haemato-lymphatic system, including haemangioma, haemoangiosarcoma, plasmacytoma, myelogenous leukaemia, and lymphosarcoma (Hruban *et al.*, 1992;

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Peauroi *et al.*, 1994; Ramos-Vara *et al.*, 1998; Raymond *et al.*, 1998; Raymond and White, 1999; Helmer, 2000; Raymond and Garner, 2000, 2001); endocrine tumours, including malignant neuro-endocrine tumour of the upper gastrointestinal tract, adrenocortical carcinoma, thyroid adenoma, thyroid adenocarcinoma, thyroid c-cell carcinoma, parathyroid adenoma, pituitary adenoma, and islet cell tumour (Peauroi *et al.*, 1994; Raymond and Garner, 2001; Miller *et al.*, 2002); and hepatocellular carcinoma and uterine adenocarcinoma, uterine leiomyoma and leiomyosarcoma (Campbell, 1999; Raymond and Garner, 2000, 2001).

Mammary gland tumours in African hedgehogs (*Atelerix albiventris*) occurred as single or multiple nodules and consisted of both benign and malignant variants (Raymond and Garner, 2000). Such tumours were identified as papillary adenoma or solid, tubular, and papillary carcinomas (Raymond and Garner, 2000), or as unclassified adenocarcinomas (Wellehan *et al.*, 2003). Malignant neoplasms showed local infiltrative growth and occasionally vascular invasion. There are no reports of the immunohistochemical characterization of mammary tumours in this species. Some affected animals suffered from additional single or multiple neoplasms, including mastocytoma, cutaneous haemangiosarcoma, oral squamous cell carcinoma, uterine leiomyosarcoma, ovarian granulosa cell tumour or uterine adenocarcinoma (Raymond and Garner, 2000, Wellehan *et al.*, 2003).

The purpose of this study was to investigate spontaneous mammary gland tumours in European hedgehogs, both morphologically and immunohistochemically.

Materials and Methods

Samples

From January 1980 to December 2004, 48 surgical tissue samples from European hedgehogs were submitted to the Department of Pathology at the University of Veterinary Medicine, Hannover for histopathological examination. In addition, during this period 404 necropsies were performed on European hedgehogs (200 male, 188 female, 16 of unknown gender). Mammary tumours were found in some of the surgical tissue samples ($n = 11$; cases 1–11) and necropsies ($n = 5$; cases 12–16). The case histories, gross findings, paraffin wax blocks and tissue sections were available from the archives of the Department of Pathology. The samples of tumorous and non-tumorous mammary gland tissue, and in addition samples of regional lymph node tissue from cases 5, 6, 8 and 14, had been processed by routine methods, embedded in paraffin wax, sectioned at 5 µm, and stained with haematoxylin and eosin (HE).

Immunohistochemistry (IHC)

This was performed with various murine monoclonal antibodies (all from DakoCytomation, Hamburg, Germany, unless otherwise stated) specific for the following seven markers: (1) Cytokeratin (CK) (clone AE1/AE3 against CK1–8, 10, 13–16 and 19, diluted 1 in 500 in phosphate-buffered saline [PBS, pH 7.1]; clone LP34 against CK5, 6 and 18, diluted 1 in 100 in PBS; clone 34βE12 against CK1, 5, 10 and 14, diluted 1 in 500 in PBS; clone D5/16B4 against CK5 and 6, clone OV-TL12/30 against CK7, clone DE-K10 against CK10 and clone Ks20.8 against CK20, all diluted 1 in 100 in PBS). (2) Vimentin (clone V9, diluted 1 in 100 in PBS). (3) α-Smooth-muscle-actin (clone 1A4, diluted 1 in 250 in PBS). (4) Desmin (clone D33, diluted 1 in 100 in PBS). (5) Macrophage antigen (clone Mac 387, diluted 1 in 200 in PBS). (6) p53 (clone DO-7, diluted 1 in 50 in PBS). (7) Ki-67 protein (clone MIB1 [Dianova Co., Hamburg, Germany], diluted 1 in 100 in PBS). In addition, rabbit polyclonal antibody specific for factor VIIIRa (DakoCytomation), diluted 1 in 200 in PBS, was used. Briefly, after dewaxing, tissue sections were immersed in H_2O_2 0.5% in methanol for 20 min. Non-specific binding was blocked with inactivated goat serum, diluted 1 in 5 in PBS. This was then replaced by the primary antibody before incubation in a moist chamber at 4 °C overnight. After washing, tissue sections were incubated with a biotin-labelled goat anti-mouse or rabbit IgG (Vector Laboratories, Burlingame, CA, USA). The avidin–biotin–peroxidase method (Vector Laboratories) was then applied according to the manufacturer's instructions. The chromogen used was 3, 3′-diaminobenzidine-tetrahydrochloride (Sigma Chemie, Taufkirchen, Germany) 0.05% with H_2O_2 0.03% as substrate in 0.1 M Tris-buffered saline (Tris-hydroxymethyl-aminomethane; Merck, Darmstadt, Germany), pH 7.6. Tissue sections were counterstained with Mayer's haematoxylin and mounted. Skin, musculature, lymph node and normal mammary tissue of a European hedgehog as well as skin with mammary gland tissue from a dog were used as positive controls. For negative control purposes, the primary antibody was replaced by ascitic fluid from non-immunized Balb/cJ mice (Biologo, Kronshagen, Germany) or rabbit serum (Sigma Chemie).

Grading of Tumours

Mammary tumours were classified according to the WHO Histological Classification of Mammary Tumors of the Dog and the Cat (Misdorp *et al.*, 1999). Grading of carcinomas was performed with respect to the formation of papillary proliferations, tubules, and the degree of nuclear and cellular pleomorphism. The mitotic rate was evaluated quantitatively by counting the