Journal of Life Science 2008 Vol. 18. No. 2. 279~283 ©JLS / ISSN 1225-9918

- Note -

Perianal Adenocarcinoma in Dog

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Received December 7, 2007 / Accepted December 27, 2007

A 12.6-year-old, male Shitzu was diagnosed with perianal adenocarcinoma. The presented mass was brown to black, 4×3×3 cm in size, and yellowish on cut section. Microscopic findings revealed that the mass composed of variable sized clusters of hepatoid cells with inconspicuous distinct. The tumor cells were polyhedral and pyknotic and exhibited high mitotic activity. Tumor cells intermingled with basaloid cells and primitive cells invaded the adjacent normal tissues. Basaloid cells exhibited positive immunoreactivity for Epidermal growth factor receptor (EGFR), human epidermal growth factor receptor (HER-2/neu), matrix metallopnateinase 9 (MMP-9), and perianal adenocarcinoma, protein kinase C alpha (PKC α). Generally, tumors of the perianal gland are common and benign 4.5 times more often than carcinoma in the dog, particularly in males. In the present report, we examined histopathological and immunohistochemical characteristics of a rare perianal adenocarcinoma in association with proteins involved tumor metastasis and adenocarcinoma development.

Key words : Epidermal growth factor receptor, human epidermal growth factor receptor, metastasis, matrix metallopnateinase 9, perianal adenocarcinoma, protein kinase C alpha

Introduction

Canine circumanal skin contains large sweat glands, large flasked-shaped sebaceous glands, and equally large and multilobed hepatoid glands, the last two derived from the epithelium of the hair follicles, but lacking patent ducts [8]. The hepatoid circumanal glands are dermal skin glands located mainly around the anal orifice of dogs and at a few extra-anal sites such as prepuce, groin, loin, and dorsal and ventral portions of the tail [7]. Tumours of the perianal glands (hepatoid or circumanal glands) are found only in dogs, frequently in old dogs and intact males, and are much more common in males than in females. They are more often benign than malignant [11,13]. They are commonly hyperplastic and subsequently may show progression to adenomas in older male dogs. Perianal adenocarcinomas are rare and the tumor cells from perianal adenocarcinomas usually exhibit variation in nuclear size, number, and size of nucleoli. It is difficult to differentiate perianal adenocarcinomas from adenomas cytologically and histologically. Thus, we report a differential diagnostic method of perianal adenocarcinoma using immunohistochemistry for epidermal growth factor receptor (EGFR), HER-2/neu, matrix metallopnateinase 9 (MMP-9), and protein kinase C alpha (PKC α), which are associated with tumor metastasis and development of adenocarcinoma.

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Materials and Methods

A 12.6-year-old, male Shiutzu was referred to an animal hospital because of anorexia and perianal gland solitary masses. The dog had a mass on the lung, hypertrophy of prostate and right ventricle, and retention of urine upon radiographic examination. However, the masses were not excised surgical. Tissue samples for light microscopy were fixed in 10% neutral buffered formalin, paraffin embedded, and stained with hematoxylin and eosin (H&E). For immunohistochemistry, tissue sections were deparaffinized in xylene, rehydrated in a graded alcohol series, incubated in a solution of 3% hydrogen peroxide in methanol for 30 min, and microwaves at 750 W for 10 minutes in a 10

mmol/l citric buffer (pH 6.0). Tissue sections were washed with PBS and then immunostained with a primary antibody; EGFR, HER-2/neu, MMP-9, and PKC α. The antigen-antibody complex was visualized with an avidin-biotin peroxidase complex solution using an ABC kit with 3,3-diaminobenzidine. Tissue sections were then rinsed in distilled water and counterstained with Mayer's hematoxylin.

Results

Grossly, the presented perianal mass was 2×1.2 cm, blackish with a rough surface, and was filled with homogenous whitish lobulated surface on cut section (Fig. 1). Microscopically, the tumor composed of variable sized clusters and pyknotic cells with some lesions (Fig. 2 Inset), and there was invasion of primitive cells into the adjacent tissue (Fig. 2). The immunohistochemical results revealed obtained with there were a number of MMP-9-positive pyknotic cells and basaloid cells (Fig. 3), and there is cytoplasmic membrane of neoplastic cell are by a distinct EGFR-positive cells (Fig. 4), and the neoplastic cell cytoplasmic are outlined by a distinct HER-2/neu-positive expression (Fig. 5), and there is PKC α expression in tumor cells was mainly revealed on the cytoplasm (Fig. 6).

Discussion

Tumor cell invasion and metastasis are regarded as multi-step phenomena involving the proteolytic degradation of the basement membrane (BM) and extracellular matrix (ECM), altered cell adhesion, and physical movement of tumor cells. Immunohistochemical analysis revealed a progressive increase of MMP-9 with severity of carcinoma, and MMP-9 showed strong staining in tumor invasion or surrounding tumor nests [3]. MMPs are a large family of zinc-dependent proteolytic enzymes, which are believed to play a key role in malignant behavior of cancer cells such as rapid growth, invasion, and metastasis by degrading ECM [3]. Increased expression of MMPs has been found in various carcinomas such as bladder, prostate, and breast [4,13]. Their level of expression has been correlated with tumor aggressiveness as suggested by histology grade [4], advanced clinical stage [5,6,10], poor survival [17,20], and increased relapse rate [11].

In previous studies, there was considerable discrepancy in the frequency and distribution of EGFR overexpression in carcinomas [16]. In the present study, basaloid cells had positive immunoreactivity for EGFR in immunohistochemistry. EGFR is a member of a family of four-cell surface membrane receptors (EGFR [Her-1], ERbB2 [Her-2/neu], ERbB3 [Her-3], and ERbB4 [Her-4]) [19]. Increased activity of EGFR has been shown to occur in a variety of solid tumors including glioblastoma, non-small cell lung carcinomas, many head/neck carcinomas, and carcinomas of the colon, breast, prostate, stomach, and ovary [2]. EGFR overexpression may result from various causes including gene amplification, decreased protein destruction, and increased messenger RNA production. It initiates a cascade of cellular reactions when a ligand binds to EGFR, which increase cell division and influence progression of malignancies through increased angiogenesis, metastasis, and inhibition of apoptosis [16]. Angiogenesis, by increasing blood supply to a tumor, allows greater and more rapid growth. Metastases are facilitated by EGFR overexpression through decreasing the adhesive properties of cancer cells allowing them to more easily migrate to distant sites. Finally, apoptosis or programmed cell death is compromised in some neoplasms overexpressing EGFR so that malignant cells survive longer and a tumor mass will increase.

PKC α comprises a family of serine/threonine kinases that plays a key role in the signal transduction pathways. It consists of at least 12 isoforms with different tissue expressions, substrate specificity, and subcellular localization that are related to specialized cell functions, including cell proliferation, differentiation, and apoptosis [14]. PKC α involved in transmembrane signally of cell surface receptors, promotes carcinogens and tumor progression by increase cellular and decreasing proliferation differentiating.

The HER-2/neu gene is a growth factor receptor tyrosine kinase encoded by the HER-2 proto-oncogene assigned to chromosome 17q21 and encodes a 1255 amino acid transmembrane glycoprotein with a molecular weight of 185 Kd [1], HER-2/neu overexpression has been detected in several solid tumor malignancies, but research has focused particularly on its role in breast cancer because of the high prevalence of the disease. Slamon *et al* first described the observation of HER-2/neu oncogene amplification in human breast cancer [18]. A number of studies have now confirmed that the HER-2/neu oncogene is either amplified or its product overexpressed in 10% to 40% of primary human breast cancer [9].

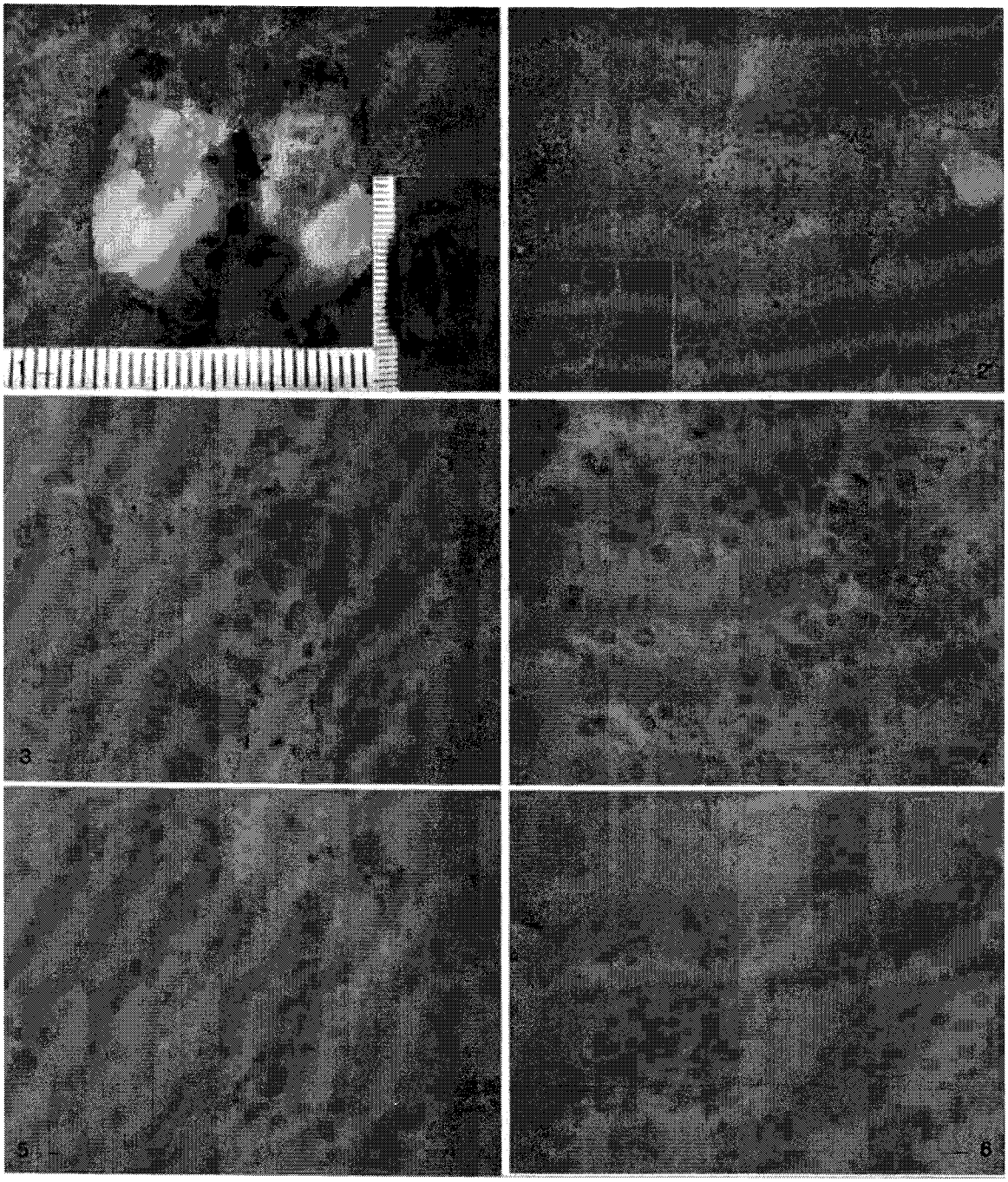

Fig. 1. Perianal mass, canine; The presented mass was blackish (inset) and the mass had homogenous whitish lobulated surface on cut section.

Fig. 2. Perianal gland carcinoma, canine; The tumor composed of variable sized clusters and tumor cells were pyknotic (Inset), Invasion of primitive cells into adjacent tissue. Hematoxylin and eosin stain. Bar: 25 um; Inset Bar: 50 um.

Fig. 3. Perianal gland carcinoma, canine; The pyknotic tumor cells and basaloid cells were positive immunoreactivity for MMP-9. ABC method. Mayer's hematoxylin counterstain. Bar: 50 um.

Fig. 4. Perianal gland carcinoma, canine; There is cytoplasmic membrane of invasive tumor cells were positive immunoreactivity for EGFR. ABC method. Mayer's hematoxylin counterstain. Bar: 50 um.

Fig. 5. Perianal gland carcinoma, canine; the neoplastic cell cytoplasmic are outlined by a distinct HER-2/neu-positive expression. ABC method. Mayer's hematoxylin counterstain. Bar: 50um.

Fig. 6. Perianal gland carcinoma, canine; there is neoplastic cell are by PKC α -positive cell. ABC method. Mayer's hematoxylin counterstain. Bar: 50 um.

Our results demonstrated that EGFR, HER-2/neu, MMP-9, and PKC α activity increased as the depth of invasion extended, indicating that these proteins participate in malignant characteristics of tumor. We considered that the hyperplastic prostate and the lesion of lung in the presented dog were related to the expression of these proteins. On the basis of our results, EGFR, HER-2/neu, MMP-9, and PKC α may help distinguish perianal adenoma from perianal adenocarcinoma with similar histopathological characteristics. In addition, HER-2/neu overexpression or amplification may provide valuable prognostic information about a variety of neoplams, including these perianal adenocarcinoma as well as mammary adenocarcinoma.

Acknowledgment

This research was supported by a grant (code: CBM 31-B3003-01-01-00) from the Center for Biological Modulators of the 21st Century Frontier R&D Program, the Ministry of Science and Technology, Korea.

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초록 : 개에서의 항문주위선 샘암종

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12.6살 된 수컷 Shitzu의 항문주위에 형성된 결절 조직의 생검을 실시하였다. 육안적 소견상 결절은 갈색과 검은색을 나타냈으며 절단면은 흰색의 균질한 물질로 가득 차 있었으며 일부에서는 괴사소견을 관찰 할 수 있었다. 광학적 현미경 상에서 종양세포들은 농축된 핵을 가지고 있으며 일부에서는 원시 종양세포들이 인접 조직을 침습하고 있는 것을 관찰할 수 있었다. 또한 면역조직 화학적 염색상에서 농축된 종양세포, 주변조직에 침습한 종양세포와 기저세포는 EGFR, HER-2/neu, MMP-9, PKC α 등에 양성반응이 나타난 것을 관찰되었다. 일반적으로 개에서의 양성 항문주위선종은 다른 종양보다 4.5배 이상 발생된다는 보고(특히 수캐)가 있으나 조직병리학적 소견과 면역화학적 염색소견상 전형적인 항문주위선 샘암종은 보기 드물게 나타나므로 향후의 종양진단과 종양연구에 도움이 될 것으로 사료된다.