Metastatic Urothelial Carcinoma with Systemic Amyloidosis in a Male Pseudohermaphrodite Goat

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Abstracts: Carcass of an 8-year-old mixed breed, male pseudohermaphrodite goat with a history of chronic wound at genitalia, dysuria and pyuria was investigated for the cause of death. Necropsy examination revealed multiple tumor masses or nodules at base of urinary bladder, proximal urethra, kidney, liver, spleen, heart, lung and adrenal gland. Histopathologically, multiple cords or nests of epithelial tumor cells with high mitotic figures were found. Areas of tumor with glandular-, squamous- and chondroid metaplasia were detected. Amyloid deposition was observed in liver, kidney and spleen. Immunohistochemistry using antibody to pancytokeratin, vimentin, cytokeratin 7 and cytokeratin 20 were performed. The tumor cells were positive for pancytokeratin but negative to vimentin, cytokeratin 7 and cytokeratin 20. Metastatic urothelial carcinoma or transitional cell carcinoma was diagnosed based on clinical history, macroscopic findings and characteristic microscopic features including immunohistochemical results. Urothelial carcinoma in goat is rare and has never been reported before in veterinary literatures. This article described clinico-pathological features of metastatic urothelial carcinoma with systemic secondary amyloidosis, glandular-, squamous- and chondroid metaplasia in a male pseudohermaphrodite goat, which is considered very rare case. The developmental abnormalities of gonads and reproductive tract, such as male pseudohermaphroditism in this animal, might be predisposing factor of urothelial cancer development. Veterinarians dealing with such condition have to be aware of the possible outcome as reported in this article.

Keywords: Urothelial carcinoma, Amyloid, Pathology, Goat

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มะเร็งของเซลล์บุผิวทางเดินปัสสาวะชนิดแพร่กระจาย และภาวะอะไมลอยโดซีส
ในแพะเพศผู้ที่เป็นกระเทยเทียม

บทคัดย่อ: ชำาแพะเพศผู้ที่เป็นกระเทยเทียม พันธุ์ผสม อายุประมาณ 8 ปี มีประวัติเป็นแพะเรื้อรัง บริเวณอวัยวะเพศ พบจุดสีน้ำตาลและมีรูเปิด ถูกสัมผัสจากรูสุดท้ายต่อมดีกิจ ผลการชันสูตรจากพบก้อนเนื้องอกกระจายบริเวณ โคนกระเพาะปัสสาวะ ส่วนต้นของท่อนำปัสสาวะ ไต ม้าม หัวใจ ปอด และ ต่อมหมวกไต ผลการตรวจทางจุลพยาธิวิทยาพบเซลล์เนื้องอกลักษณะเหมือนเซลล์บุผิวของเซลล์มะเร็งในแพะเพศผู้ที่เป็นกระเทยเทียม พบเซลล์ระยะแบ่งตัวได้มาก กลุ่มเซลล์เนื้องอกมีการจัดรูปส่วนกล้าที่มีการเปลี่ยนชนิดของเซลล์ด้านในห่อเนื้อเป็นเซลล์สร้างกระดูกอ่อน และพบบริเวณที่มีการเปลี่ยนรูปไปเป็นเซลล์สร้างกระดูกอ่อน พบการสะสมของสารอะไมลอยด์ที่ตับ ไต ม้าม หัวใจ ปอด และ ต่อมหมวกไต.

ผลการตรวจทางอิมมูโนฮีสโตเคมีพบเซลล์เนื้องอกให้ผลบวกต่อ โปรตีนโครงสร้างชนิด pancytokeratin และให้ผลลบต่อ โปรตีนโครงสร้างชนิด vimentin, cytokeratin 7 และ cytokeratin 20 จากข้อมูลข้างต้น ถัดไปก่อนจะอธิบายถึง ผลการตรวจทางอิมมูโนฮีสโตเคมีที่สังเกตได้ รายจากนั้นสถาปัตย์ม้าม ปอด ต่อมหมวกไต และ ลักษณะของเนื้องอก ที่สังเกตได้ ผลการตรวจทางอิมมูโนฮีสโตเคมีที่สังเกตได้ เนื้องอกผ่านการตรวจทางอิมมูโนฮีสโตเคมีที่สังเกตได้ ผลการตรวจทางอิมมูโนฮีสโตเคมีที่สังเกตได้.

ผลการตรวจทางอิมมูโนฮีสโตเคมีที่สังเกตได้ ผลการตรวจทางอิมมูโนฮีสโตเคมีที่สังเกตได้.

จากข้อมูลข้างต้น ถัดไปในนั้น ถ้ามีการเปลี่ยนแปลงลักษณะของเนื้องอก ที่สังเกตได้ ผลการตรวจทางอิมมูโนฮีสโตเคมีที่สังเกตได้.

ผลการตรวจทางอิมมูโนฮีสโตเคมีที่สังเกตได้ ผลการตรวจทางอิมมูโนฮีสโตเคมีที่สังเกตได้.

ผลการตรวจทางอิมมูโนฮีสโตเคมีที่สังเกตได้ ผลการตรวจทางอิมมูノノホシトケミ

รีวิว: มะเร็งทางเดินปัสสาวะ อะไมลอยโดซีส บำรุงพยาธิวิทยา

Introduction

Neoplasm of lower urinary tract is uncommon in goat. It is more common in cattle, dogs and cats, and occurs most frequently in urinary bladder (Raoofi et al., 2007). Urothelial carcinoma (UC) or transitional cell carcinoma (TCC) is the most common urinary bladder neoplasm in dogs.
with high metastatic tendency (Ramos-Vara et al., 2003; Mamom, 2008). The most common site of this cancer is prostatic urethra in male dogs and trigone area of urinary bladder in female dogs. In cattle, urothelial neoplasms have been reported associated with prolonged grazing the cow on pastures rich in bracken fern (*Pteridium spp.*) which result in the clinical syndrome of chronic enzootic hematuria (Ambrosio et al., 2001; Roperto et al., 2005). Bovine papillomavirus type 2 (BPV-2) is believed to play an important role in carcinogenesis of urothelial neoplasm (Roperto et al., 2005). In human medicine, urothelial carcinoma is a common disease of old age and more common among men than women (Shinagare et al., 2011). This cancer is able to metastasize especially to lymph nodes, bone, lung, liver, peritoneum and pleura (Patterson-Kane et al., 2000; Mamom, 2008; Maes et al., 2011; Shinagare et al., 2011). A wide range of metaplastic changes including mucoid degeneration, squamous-, chondroid-, osseous- and glandular metaplasia have been reported in urothelial carcinoma both in humans and animals (Patterson-Kane et al., 2000; Ramos-Vara et al., 2003; Perez-Montiel et al., 2006; Lopez-Beltran et al., 2007; Raoofi et al., 2007; Mamom, 2008; Amin, 2009; Billis et al., 2011; Gaisa et al., 2011; Maes et al., 2011; Dudley et al., 2012). In human medicine, the prevalence squamous metaplasia and glandular metaplasia in urothelial carcinoma were reported up to 60 % and 10 % respectively (Amin, 2009). Other additional pathological features associated with urothelial carcinoma such as amyloid deposition and shadow cell differentiation had also been documented in human medicine literatures (Azzopardi and Lehner, 1966; Gupta et al., 2012; Nakamura, 2012). Immunohistochemistry using antibody to uroplakin (UP), cytokeratins 7 (CK7) and cytokeratin (CK20) is important and commonly used for diagnosis of urothelial tumors in human and some animal species (Ambrosio et al., 2001; Ramos-Vara et al., 2003; Amin, 2009; Gaisa et al., 2011; Maes et al., 2011).

In goat, metastatic urothelial carcinoma, according to the author knowledge, has never been reported in veterinary literature before. There is only one study documented about transitional cell papilloma in urinary bladder of goats (Raoofi et al., 2007).

The aim of this study is to describe clinico-pathologic features of a rare case of metastatic urothelial carcinoma with systemic secondary amyloidosis in a male pseudohermaphrodite goat.

**Case history**

Carcass of an 8-year-old mixed breed, male pseudohermaphrodite goat was submitted to veterinary diagnostic laboratory for investigation of the cause of death. The animal was born with bilateral testes but female external genitalia. It had a history of...
chronic wound at genitalia, dysuria and pyuria about two weeks prior to death. Necropsy examination was performed and tissue samples were collected in 10 % buffered formalin solution and submitted to diagnostic laboratory for histopathological and immunohistochemical examination.

Necropsy findings:
The carcass was in good nutritional stage. General appearance revealed focal area of chronic wound at vulva. The abdominal and thoracic cavities were filled with serofibrinous fluid (serofibrinous polyserositis). Area of thickening and necrosis of mucosa was found at neck of urinary bladder and upper urethra (Fig. 1). The liver showed multifocal to coalescing grayish white areas (Fig. 2). Multiple grayish white nodules were found in renal cortex and medulla adjacent to focal area of infarction at renal cortex (Fig. 3). The same types of nodules were detected at lung (Fig. 4), spleen, heart, and adrenal gland. Hemorrhagic lymphadenitis was found in inguinal lymph nodes. Other organs showed no remarkable macroscopic finding. Tentative diagnosis of lower urinary tract neoplasm and inflammation with subsequence ascending spread and metastasize to multiple organs was made.

Histopathological findings:
Multiple tumor nodules were detected in liver, kidney, lung, heart, spleen and adrenal gland. The tumor cells were polygonal epithelial cells with amphophilic cytoplasm and large round to oval vesicular nuclei and prominent nucleoli. They arranged in cords, nest, tubular or pseudoglandular patterns with necrotic cells at center (Fig. 5) and supported by large amount of fibrous tissue (desmoplasia). Mitotic figures were ranged from 3 to 6 per HPF. Thrombosis and tumor emboli were seen in kidney. Some areas of mucoid degeneration, glandular-, squamous- and chondroid metaplasia (Fig. 6) were observed. Accumulation of eosinophilic homogenous material were found in space of Disse (Fig. 7) of liver, in white pulps of spleen, in some renal glomeruli and in interstitial tissue of renal medulla and papilla. Congo red stained slides observed under polarized light revealed positive apple green color in affected areas typical of amyloid deposition. Some additional lesions were seen in liver, lung and kidney. The liver showed atrophy of hepatic cords (due to amyloid deposition), various degrees of megalocytosis of hepatocytes, bile duct proliferation and dilatation with purulent cholangitis. The lung showed focally extensive necrosuppurative and granulomatous pneumonia with presence of tremendous fungal hypha. The kidney revealed marked tubular dilatation with intraluminal hyaline casts and multifocal lymphocytic interstitial nephritis. Other organs showed no significant histopathological finding.
Immunohistochemical findings:

Immunohistochemical study using streptavidin-biotin techniques and DAB as chromogen as previously described (Ramos-Vara et al., 2003) was performed to investigate immuroreactivity to pancytokerin (AE1/AE3, Neomarker), vimentin (V9, Neomarker), cytokeratin 7 (OV-TL, Dako) and cytokeratin 20 (Ks20.8, Dako). The results were demonstrated in Table 1. Briefly, more than 80% of tumor cells were stained with strong intensity to pancytokeratin (Fig. 8). No or rare tumor cells were positive for cytokeratin 7, cytokeratin 20 and vimentin. These results indicate epithelial histogenesis of neoplastic cells. In addition, the supporting tissue or desmoplastic tissue was positively stained for vimentin.

Discussion and Conclusion

The cause of death in this animal is the result of cancer metastasis. Although the immunohistochemical study on the most specific marker for urothelium, uroplakin (UP), was not performed in this case, the diagnosis of urothelial carcinoma was relied on clinical history, macroscopic and microscopic findings including immunohistochemical results to some important intermediate filament proteins as shown in Table 1. Immunohistochemical study of cytokeratin 7 (CK 7) and cytokeratin 20 (CK 20) on urothelial tumor in goat has never been reported in the literature. In dog with urothelial carcinoma, immunohistochemical expression of CK 7 and CK 20 was 98.1% and 68.5% respectively (Ramos-Vara et al., 2003). The cancer cells in goat in this study did not expressed both CK 7 and CK 20 however a definitive conclusion could not be made according to the small sample size. The data from study in dog revealed that, urothelial carcinoma with negative CK 7/20 (-/-) profile was histologically characterized as anaplastic type (Ramos-Vara et al., 2003).

Table 1 Immunohistochemistry (IHC) results: the tumor cells were positive to pancytokeratin, negative to cytokeratin 7, cytokeratin 20 and vimentin.

<table>
<thead>
<tr>
<th>Antibodies (clone)</th>
<th>Results on tumor cells (percentage of positive cell*/ staining intensity**)</th>
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<tbody>
<tr>
<td>Pancytokeratin (AE1/AE3)</td>
<td>+++/++++</td>
</tr>
<tr>
<td>Vimentin (V9)</td>
<td>-/-</td>
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<tr>
<td>Cytokeratin 7 (OV-TL)</td>
<td>-/-</td>
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<tr>
<td>Cytokeratin 20 (Ks20.8)</td>
<td>-/-</td>
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*Percentage of positive cell: +++ = > 80% of tumor cells positive; ++ = 50-80% of tumor cells positive; + = <5% of tumor cells positive; - = all tumor cells negative

**Staining intensity: +++ = strong (dark brown color); ++ = fair (brown color); + = weak (pale brown color); - = negative (no brown color)
Fig. 1-8: Gross and microscopic findings in this animal. Fig. 1: Area of thickening and necrosis of mucosa was found at the neck of urinary bladder (white arrow), UB = intact urinary bladder. Fig. 2: Multifocal to coalescing grayish white areas seen from liver surface (white arrows), LI = intact liver parenchyma. Fig. 3: Multiple grayish white nodules were found in renal cortex and medulla (white arrows) with focal renal infarction at renal cortex (dark arrow), RP = renal pelvis. Fig. 4: Multiple grayish white nodules at lung (dark arrow). Fig. 5: The tumor cells arranged in cords and nest patterns (dark arrows). Some mitoses were seen (white arrows). Fig. 6: Area of chondroid metaplasia. Fig. 7: Eosinophilic homogenous material (amyloid) in space of Disse (dark arrows) with subsequent atrophy of hepatic cords (white arrows), H&E stained. Fig. 8: Brown color within cytoplasm of neoplastic cells with positive IHC reactivity to pancytokeratin, DAB with hematoxylin counterstain.

Urothelial carcinoma in this study was originated from the upper urethra and the neck of urinary bladder and might be responsible for lower urinary tract signs such as dysuria and pyuria observed clinically. In human, cows, sheeps, horses and dogs, the common clinical findings of lower urinary tract were urine incontinence, hematuria, oliguria and stranguria (Patterson-Kane et al., 2000; Ambrosio et al., 2001; Mamom, 2008). In fact, this goat was a male pseudohermaphrodite, had a pair of testes and exhibited male behavior, which was opposite to the ambiguous female genital tract and external genitalia presented. We postulated that, the abnormalities or inability of form a male reproductive tract, which is embryologically derived from mesonephric duct (the duct of primitive kidney), may play a part in initiation step of urothelial cancer development, in addition to prolonged exposure to toxic chemicals and possibly infection with BVP-2 as mentioned in some previous studies (Orsola et al., 2005; Roperto et al., 2005; Ambrosio et al., 2001). Urothelial carcinoma in this study metastasized to liver, kidney, lung, heart, spleen and adrenal gland. These findings correlated with the study in human cancer (Shinagare et al., 2011). The route of metastasis of urothelial cancer cells in this goat was probably lymphatic route at the beginning and followed by hematogenous route because of the presence of metastatic nodules in heart and lung.

A retrospective study on association of systemic amyloidosis with malignant diseases in human medicine revealed that, up to 7.5% of systemic amyloidosis cases were associated with carcinoma, including urothelial carcinomas (Azzopardi and Lehner, 1966). Most of the systemic amyloidosis cases in that study were classified as secondary amyloidosis. Systemic amyloidosis observed in liver, spleen and kidney of the goat in this...
study was also considered secondary due to the lack of direct association of amyloid deposition to the cancer cells. Furthermore, evidence of chronic inflammation in liver, lung, kidney and lower urinary tract might play more important role on the synthesis and deposition of amyloid protein than the cancer itself. Nevertheless, a human case of high grade urothelial carcinoma with primary amyloidosis was recently published (Gupta et al., 2012).

Some pathological features associated with urothelial carcinoma in the goat in this study such as pronoucne desmoplasia, mucoid degeneration, squamous metaplasia, chondroid metaplasia and glandular metaplasia had also been reported (Patterson-Kane et al., 2000; Ramos-Vara et al., 2003; Perez-Montiel et al., 2006; Lopez-Beltran et al., 2007; Raoof et al., 2007; Mamom, 2008; Amin, 2009; Billis et al., 2011; Gaisa et al., 2011; Maes et al., 2011; Dudley et al., 2012). The clinical significant of squamous differentiation of urothelial carcinoma is yet unclear however it seems to relate with unfavorable prognosis. On the other hand, this might be an indicator for poor response to systemic chemotherapy and radiation therapy (Lopez-Beltran et al., 2007).

In conclusion, we report clinico-pathological features of metastatic urothelial carcinoma with systemic secondary amyloidosis, glandular-, squamous- and chondroid metaplasia in a male pseudohermaphrodite goat, which is considered very rare case. The developmental abnormalities of gonads and reproductive tract, such as male pseudohermaphroditism in this animal, might be predisposing factor of urothelial cancer development. Veterinarians dealing with such condition have to be aware of the possible outcome as reported in this article.

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