Uterine Adenocarcinoma with Stromal Cells Containing Lipofuscin in A Cow

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ABSTRACT. A case of bovine uterine adenocarcinoma is described in an aged Holstein Cow. Neoplastic lesions were found in the uterus, pelvic lymph nodes and lungs and consisted of neoplastic glandular tissues supported by much proliferation of dense collagenous connective tissues. The neoplastic cells were characterized by microvilli with prominent filamentous core rootlets and glycocalyceal bodies in the acinar spaces. Stromal cells with lipofuscin were present near the neoplastic glands and the origin was discussed.—KEY WORDS: cow, glycocalyceal body, lipofuscin, stromal cell, uterine adenocarcinoma.


Bovine adenocarcinoma of the uterus is a frequent neoplasm and an important economic problem [16]. This neoplasm easily metastasizes to the lungs and is apt to be confused with primary carcinomas of the lung [10]. The ultrastructure of primary lung carcinomas has been investigated [7, 8, 13, 15], while uterine adenocarcinomas were observed by light microscopy [9].

Estrogen receptors and progesterone receptors may be present in human endometrial carcinoma [3, 12]. A peroxidase-antiperoxidase (PAP) immunoperoxidase method demonstrated the presence of estrogen receptors in the nucleus and cytoplasm of formalin-fixed, paraffin-embedded human endometrial epithelial cells [14]. Stromal foam cells may be found in the stroma of human adenomatous hyperplasia or adenocarcinoma [2]. They are of stromal origin [4], have stored lipids consistent with being cholesterol esters or estrogen derivatives [1] and may be indicative of estrogen action [3].

In this paper we report a bovine adenocarcinoma of the uterus, which metastasized to the lungs and lymph nodes with stromal cells containing lipofuscin. The neoplastic cells were compared with lung carcinoma cells, and the relation among the neoplasm, stromal cells and hormones was discussed by using morphological and immunohistological methods.

MATERIALS AND METHODS

An aged Holstein cow was brought to an abattoir in good condition. Only grossly involved organs were obtained for microscopy and uterus and ovaries from a normal cow were also observed. Tissues were fixed in 10% formalin, processed by standard methods and embedded in paraffin. Sections were stained with haematoxylin and eosin (HE), periodic acid-Schiff (PAS), diastase-treated PAS (D-PAS), Schmol method for lipofusins, Berlin blue, Alcian blue, Giemsa, Heidenhain's iron haematoxylin and azan. The avidin-biotin-peroxidase complex (ABC) method for the demonstration of estradiol was applied to paraffin sections by using a HistoGen kit (BioGenex Laboratories, U.S.A.). After formalin-fixation, the tissues were post-fixed in 1% osmium tetroxide and treated routinely for electron microscopy.
RESULTS

The uterus had an about 10cm diameter, hard, fibrous mass from cervix uteri to corpus uteri. Lymph nodes in the pelvic canal were mostly enlarged and there were viscous liquid, caseation and calcification on the cut surface. The lungs contained firm, multiple foci, 0.5 to 5cm in diameter. Caseous or calciferous areas were present in larger masses.

Microscopically, the neoplastic tissue was present in the myometrium and was composed of monolayer or multilayer tubular structures of variable size with abundant fibrous stroma (Fig. 1). There were a few uterine glands in the myometrium near the serosa. The neoplastic cells were large and cuboidal and had large ovoid nuclei with inconspicuous nucleoli and finely clumped chromatin. Binuclear neoplastic cells were rare and mitotic figures were occasionally seen. The cytoplasm was eosinophilic and had villi toward the lumina and there were neutrophils, desquamative tumour cells or their debris. Mucus-like material was also present in the lumina and stained positively with PAS and Alcian blue. Lymphocyte and plasma cell infiltration was found in the stroma. Similar lesions as in the uterus were observed in the lymph nodes and lungs, and showed more proliferation of the collagenous connective tissue.

Round to spindle-shaped cells were accumulated or scattered in the fibrous stroma of the lungs and lymph nodes (Figs 2, 3). These cells were located around the neoplastic cells or near the infiltrative lymphocytes and plasma cells and contained lipofuscin throughout the cytoplasm with occasional lipid droplets. The same cells were rarely seen in the uterine lesion. Lipofuscin-positive cells were also found in
the uterus and ovaries from a normal cow. The result of several stainings of such cells is presented in Table 1.

Both of the nucleus and cytoplasm of the uterine glands frequently demonstrated distinct staining with anti-estradiol serum, while the neoplastic cells demonstrated extremely weak staining of both the nucleus and cytoplasm. The cytoplasm of ovarian interstitial cells were stained positively and other lipofuscin-containing cells were negative for estrogen (Fig. 4).

Electron microscopy: The neoplastic cells had microvilli with prominent filamentous core rootlets and the acinar spaces contained innumerable rounded glycocalyceal bodies coated by a glycocalyx (Fig. 5). There were rough endoplasmic reticulum (rER) occasionally showing confronting cisternae (Fig. 6), mitochondria, free ribosomes, centrioles, a few lysosome-like granules and evenly distributed microfilaments in the cytoplasm. Junctional complexes were present between adjoining cells.

Throughout the cytoplasm of the stromal cells there were lipofuscin granules with membranous structures, some of which showed whorl-like arrangement (Fig. 7). A few lipid droplets, lysosomes, mitochondria and rER were also present in the cytoplasm. A small number of lipofuscin granules were seen in the fibroblastoid cells having moderately developed rER.

![Image](image_url)

**Table 1. Staining pattern of cytoplasmic pigments of stromal cells in the neoplastic tissues and normal genital organs**

<table>
<thead>
<tr>
<th></th>
<th>stromal cell</th>
<th>interstitial gland cell</th>
<th>hilar cell</th>
<th>stromal cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>present case</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HE</td>
<td>brown</td>
<td>brown</td>
<td>brown</td>
<td>brown</td>
</tr>
<tr>
<td>PAS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-PAS</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>lipofuscin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Berlin blue</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Giemsa</td>
<td>light blue</td>
<td>greenish blue</td>
<td>deep blue</td>
<td>deep blue</td>
</tr>
</tbody>
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+ = positively stain; - = not stain.
DISCUSSION

The neoplasm in this study was characterized by the metastasis to the lymph nodes and lungs, the predominance of dense collagenous connective tissue in the stroma and the presence of the neoplastic tissue in the myometrium. These findings were identical with those in bovine uterine adenocarcinomas, which were considered to arise from the deep uterine glands [9, 10]. Our case may be derived from uterine glands in the myometrium caused by endometriosis, be-
cause a few uterine glands were present in the myometrium near the serosa.

The ultrastructural features were glyco-
calceal bodies and penetrating microvillus core rootlets. Eosinophilic material in the acinar spaces has been observed and was considered to be cellular debris [9]. Our ultrastructural result demonstrated that the cosinophilic material was not only cellular debris but also glycocalyx, which were present around glycocalceal bodies and were stained positively with PAS.

Bovine uterine carcinomas are apt to be confused with primary carcinomas of the lung, which have been investigated ultrastructurally [8]. In one case the neoplastic cells had penetrating microvillus core rootlets and mucin granules, but glycocalceal bodies were absent. The other cases were also lacking in glycocalceal bodies and were easily distinguishable from present case because of various morphological differences. Glycocalyx may be seen in human endometrial carcinoma [11] and glycocalceal bodies may be a feature of bovine uterine adenocarcinomas. But, we examined only one case and further cases are needed to conclude it.

Human lipid cell tumour of the ovary originating from hilar cells revealed lipofusc
sin granules, abundant smooth endoplasmic reticulum (sER) and concentric membranous whorls (spironolactone bodies) derived from the sER [5, 6]. In our case the stromal cells possessed lipofuscin granules with membranous structures. Such membranous structures may be derived from sER, because some of them resembled spironolactone bodies. The ultrastructural features and resemblance of staining pattern to ovarian interstitial cells and hilar cells suggested that the stromal cells were steroidogenic cells.

Estradiol bound in vivo with the receptor protein survives the process of formalin-
fixation and paraffin-embedding and the PAP method using anti-estrogen serum can demonstrate the presence of hormone responsive cells [14]. The uterine glands in our case were stained distinctly with antiestrogen serum and the neoplastic cells stained extremely weak. This is presumably due to the decrease of receptors associated with neoplastic transformation.

Steroid hormones not bound with the receptors are easily washed away in the process preparing sections. Hormone secreting cells can barely show positive staining, when these cells contain a large amount of steroid hormone. Because the ovarian interstitial cells had numerous lipid droplets which were considered as a storage form of material of steroid hormone and were correlated with steroid hormone synthesis [6], they were stained with anti-estrogen serum. The stromal cells in our case had many lipofuscin granules and lost the ability of hormone production. Hilar cells may contain androgen.

The stromal cells were present in the uterus, lymph nodes and lungs. The cells in the latter two organs may be induced from mesenchymal cells in their organs or may infiltrate from genital organs. A type of human endometrial carcinoma is likely to be under the influence of estrogen [3]. Present study suggested the presence of reciprocal action between hormone responsive cells and hormone secreting cells in localities. Well developed connective tissue indicates chronic course of this disease and active cells in hormone secretion may be found in early lesions.

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REFERENCES


要約

リポフスチン保有間葉性細胞を伴う牛の子宮腺癌：門田耕…渡辺正信11（農林水産省家畜衛生試験場，11栃木県食肉衛生検査所栃木支所）—ホルスタイン種，乳癌用牛に子宮腺癌が認められた。腫瘍は子宮筋層，骨盤腔のリンパ節，肺にあり，豊富な結合組織性間質を伴った管状構造からなっていた。腫瘍細胞を電顕的に観察したところ，微細毛内に芯細線維があり，rootlet となって細胞質内に伸展していた。腺腔内には多数の glycocalyceal body が存在していた。また腫瘍細胞周囲の間質にはリポフスチン保有細胞があった。