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原　　著

Scanning Electron Microscopic Study of Gallbladder Carcinoma

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Abstract

The surface observation of gallbladder carcinoma in detail is most essential for early diagnosis in the surgical field. However, it was difficult to differentiate the bizarre surfaces of the advanced carcinomas from those caused by severe inflammation or technical loss of the mucosa, because most gallbladder carcinomas were clinically in the advanced stage. Since the SEM observation was suitable for the developmental stage of carcinoma, SEM study was performed on gallbladder carcinoma of hamsters which was induced by the insertion of beeswax pellet containing 3, 5 or 8 mg of methylcholanthrene (MC).

In 2 of 95 hamsters carcinoma was induced and detected by SEM, although the detection was impossible by other microscope examination. One was 9 months after the insertion of 3 mg of MC beeswax pellet, the another was 5 months after that of 5 mg. The folds of the surface near the cancer were much broader than that of the control. The normal cell arrangement of polygonal shapes was replaced by a pleomorphic pattern. The microvilli were bulkier and taller than those of normal cells. Variously sized and shaped, such as polypoid, verrucous and cylindrical, surface protrusions which were covered with or without the microvilli, were recognized over the gallbladder mucosa treated with MC.

Cellular component of the protrusions was not disclosed with TEM. These findings in the early stage of experimental gallbladder carcinoma were quite similar to those of the human specimens obtained surgically. Thus, the apparently normal mucosa of the human gallbladder should be examined by SEM.

Introduction

The progress of diagnostic instruments and measurements may play a very important part

Key words: Hamster, Experimental gallbladder carcinoma, Scanning electron microscopy, Precancerous lesion of the gallbladder mucosa, Polyps of the gallbladder.
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in diagnosing the carcinoma of the gallbladder[31,33,35,50,56,67].

However, few reports of early-staged carcinoma of the gallbladder have been made,[3,26,27] and most of them were detected by postoperative histological examination of specimens removed from patients with cholecystolithiasis[11,47].

Detection of carcinoma of the gallbladder at the early stage is also difficult, because the carcinoma in humans has not comparatively studied using experimental models. The malignant transformation of the gallbladder mucosa induced experimentally in hamsters by the intracholecystic implantation of beeswax pellets containing a chemical carcinogen was examined in detail by scanning electron microscopy (SEM), to examine its resemblance to carcinoma of the gallbladder in humans.

Materials and Methods

1. Induction of experimental gallbladder carcinoma:

One hundred and fifty hamsters were maintained on a commercial diet and water ad libitum and were divided into five groups. Heat-melted beeswax was homogeneously mixed with 3-methylcholanthrene (MC) where 50% of the total mixture weight constituted of beeswax. The beeswax was then prepared in pellet form.

The first group consisting of 42 male hamsters was given a beeswax pellet containing 3 mg of MC into the gallbladder operatively with the incision of the fundus under anesthesia of intraperitoneal Nembutal® and observed for as long as 13 months. The incised fundus was sutured by Dexon®. In this procedure, the cystic duct was not ligated.

The second group consisting of 34 male hamsters was given a beeswax pellet containing 5 mg of MC implanted into the gallbladder.

The third group consisting of 19 female hamsters was given a beeswax pellet containing 8 mg of MC implanted into the gallbladder.

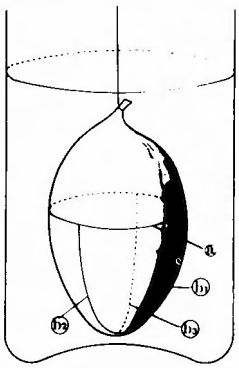
The fourth group had the incisions and some stitches at the fundus as a shame operation.

The fifth group was fed conventional rations.

2. Preparation of the specimens for SEM:

The gallbladder of hamsters was tied off at the neck and placed in a 2% glutaraldehyde solution with the entire shape preserved, because this method minimized tremendous artifacts, such as the extensive loss of the epithelium during the prefixation under the lumen of the gallbladder extended on the board. After prefixation for 4 hours in a refrigerator, the specimens were divided into 4 or 5 pieces (Fig. 1). The neck of the gallbladder was prepared for the usual histological examinations, namely hematoxylin-eosine (HE) staining and the rest were divided into 3 or 4 pieces[5].

These specimens were rinsed with 15 ml of 0.1 M phosphate buffer at least three times. After osminification, the samples were subsequently dehydrated in a graded ethanol series (60–100%), then dried in liquid carbon dioxide with the critical point drier (Hitachi, Japan). After being mounted on the tabs, the samples were coated by a thin layer of gold-parasium with an ion-coater (Eiko IB-3 model, Japan), and observed by a scanning electron microscope (Hitachi



1) The neck of the gallbladder was tied off and the entire organ was immersed in 2% glutaraldehyde solution
2) The specimen was divided along line (a)
   Neck region---for hematoxylin-eosin staining
   Body and fundus region---divided into three sections along lines (b1) (b2) (b3)
3) Rinsed with 15 ml of phosphate buffer (pH 7.2) three times

Fig. 1. Preparation of gallbladder specimen

S-430 model, Japan).

3. TEM of the specimens in the experimental model:

The sample was brought to the preparatory steps for transmitter electron microscropy (TEM, Hitachi H-600 model, Japan). After they were retrieved from the mounting tab, they was placed in methylbuthyl acetate, pure ethanol and propylene oxide in this order, and then embedded in resin (Epon 812) with whole shape preserved. The sample was trimmed carefully leaving the area with the protrusive objects and attached to the columnar Epon resin with the adhesive to make ultrathin sections of the intended site.

4. Human material:

Specimens of cases preoperatively suspected of being carcinomas of the gallbladder were obtained during cholecystectomy and examined by SEM. These included 2 cases of gallstones, 4 cases of gallbladder polyp and 3 cases of primary carcinoma of the gallbladder.

Results

1. SEM of the specimens in experimental model of gallbladder carcinoma:

About two and a half months later, a few beeswax-implanted hamsters were killed. Much of the omentum major adhered to the porta hepatis. After dissection of these adhesions, the gallbladder apparently was normal in size and other abnormalities such as serosal infiltration or hepatic tumors were not observed. On opening the gallbladder, the lumen was filled with thick gray-white mucoid material and MC-beeswax pellet. This creamy content disturbed the allowance of observation of the epithelium. The specimen was observed in almost all areas, but no abnormal findings were apparent.

The surface of the gallbladder epithelium obtained from hamsters of the control group consisted of narrow folds and was covered with pentagonal or hexagonal cells, lined regularly in pavement form.

Carcinomatous changes in the early form of the experimentally induced gallbladder carcinoma were observed in hamsters given the beeswax pellet containing 5 mg of MC after 5 months (Fig. 2). Lingual protrusions covered by the sparse and short microvilli were seen at a 4000-fold

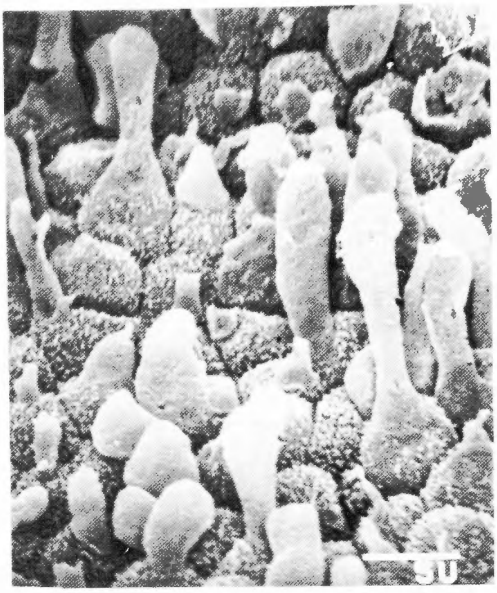


Fig. 2. Lingual protrusions covered the epithelium. (5 months after the treatment with 5 mg of 3-MC beeswax pellet)

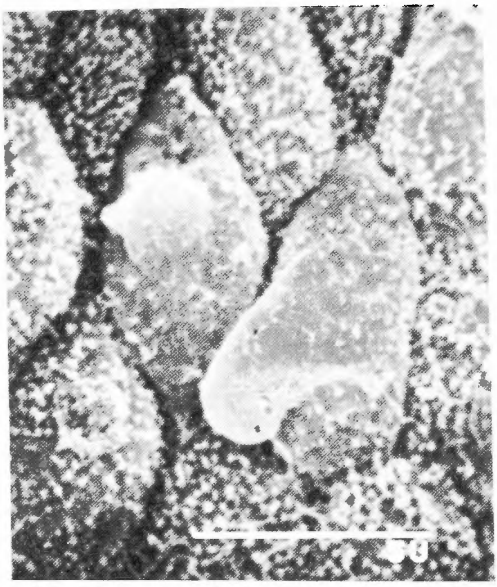


Fig. 3. The protrusions were covered with sparse microvilli. (5 months after the treatment with 5 mg of 3-MC beeswax pellet)

magnification (Fig. 3).

In the hamster killed 9 months after the insertion of a beeswax pellet containing 3 mg of MC, many more cells were covered with sparse microvilli than in the controls. Some of them had protrusions covered by longer and bulkier microvilli. The protrusion was revealed in continuity with the surface of the epithelium (Figs. 4 and 5).

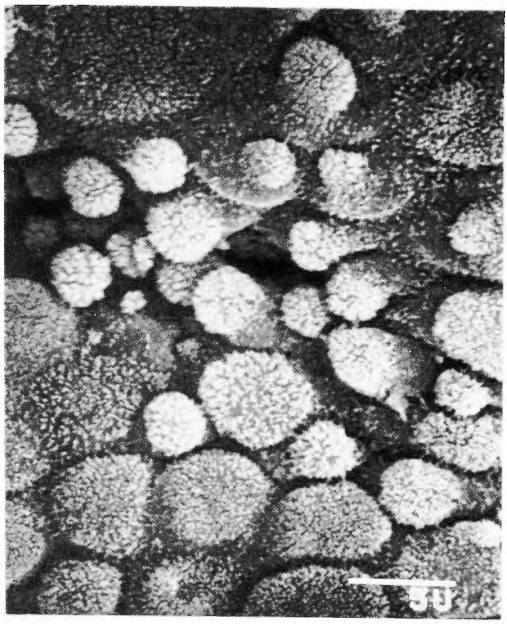


Fig. 4. The cells were covered with sparse microvilli, but the protrusive regions were covered by long and thicker microvilli. (9 months after the treatment with 3 mg of 3-MC beeswax pellet)

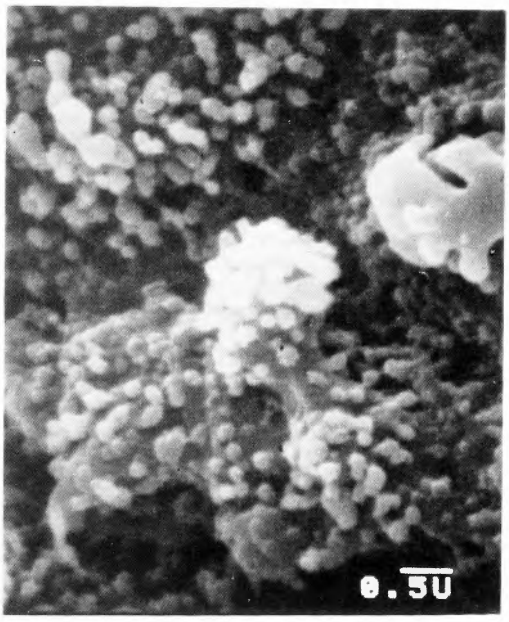


Fig. 5. High magnification of the protrusive regions. The microvilli was thicker at the top. (9 months after the treatment with 3 mg of 3-MC beeswax pellet)

Table 1 summerizes the results of experimental induction of carcinoma in hamsters.

2. TEM of the specimens in the experimental model:

These cells were cuboidal. Two small protrusions of cytoplasm extended from the surface into the lumen, and was devoid of organelles or organized structures exept nuclei (Fig. 6).

Table 1. Experimental induction of carcinoma in hamsters

Group	Number of animals	Sex	Procedures	Findings by SEM
I	42	M	Pellets with 3mg of MC	The cancerous lesions after 9 months
II	34	M	Pellets with 5mg of MC	The cancerous lesions after 5 months
III	19	F	Pellets with 8mg of MC	The cancerous lesions after 4 months
IV	32	M F	Sham operation	Atrophied gallbladder
V	23	M F	Control	Apparently normal

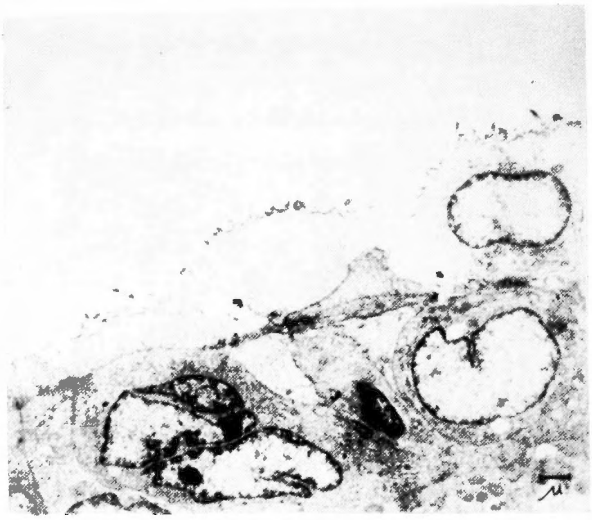


Fig. 6. TEM observation of the protrusive regions. The organella was scarcely observed. (9 months after the treatment with 3 mg of 3-MC beeswax pellet)

3. SEM of polyps and carcinoma of the human gallbladder

The specimen with polyp of the human gallbladder had a cell lines similar to that of the controls in the animals. The surface of the epithelium consisted of narrow folds and was covered with pentagonal or hexagonal cells, lined regularly in pavement form, and was covered by dense, slender and tall microvilli at a 10,000-fold magnification (Figs. 7 and 8).

The samples were obtained from a clinical case of primary carcinoma of the gallbladder. Endoscopic retrograde cholangiogram disclosed suppurative cholangitis and intrahepatic gallstones in the left hepatic duct and carcinoma of the gallbladder was suspected. HE stain of this specimen was showed a poorly differentiated adenocarcinoma that invaded to the subserosal layer. SEM revealed these areas covered by dense microvilli. Such areas with dense microvilli

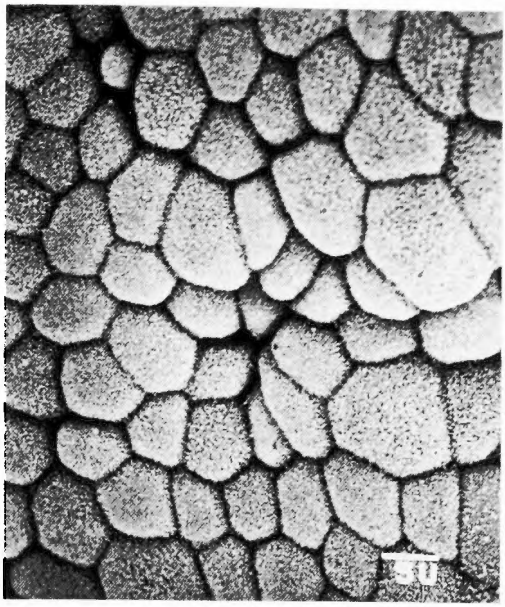


Fig. 7. The surface observation of the cholesterol polyp of the gallbladder, covered with pentagonal- or hexagonal-shaped cells.

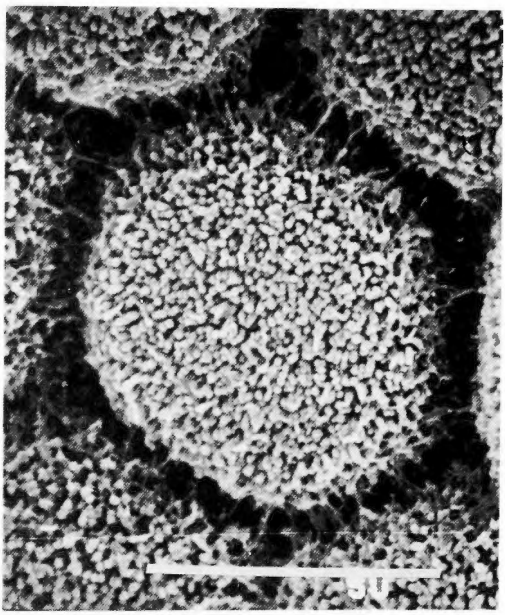


Fig. 8. High magnification of cholesterol polyp of the gallbladder covered by the dense, slender and tall microvilli.

protruded into the free lumen (Figs. 9 and 10). This finding closely resembles that of an experimentally induced carcinoma.

In another case, there was bulging at the body of the gallbladder and ordinary histological examination showed that the tumor was tubular adenocarcinoma and invaded into the muscular

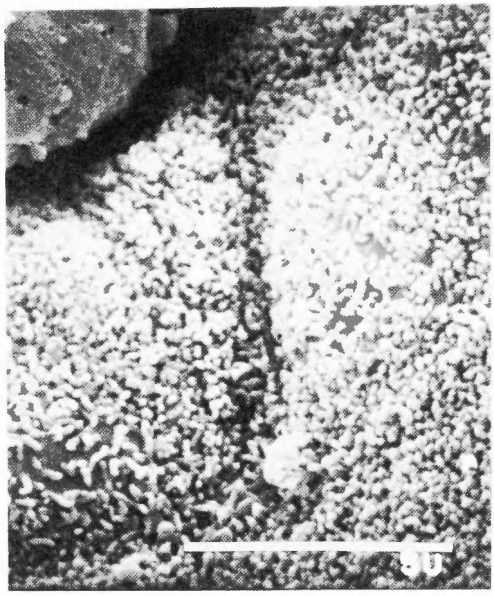


Fig. 9. The cell surfaces were covered by the dense, short microvilli (primary carcinoma of the gallbladder).

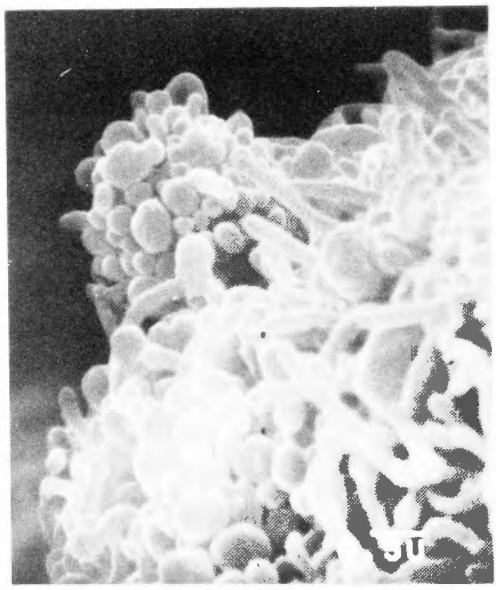


Fig. 10. The protrusive changes could be observed which covered with dense microvilli (primary carcinoma of the gallbladder).

tonic of the mucosa. By SEM observation, many ligual type protrusions were observed (Figs. 11 and 12).

The inflammatory changes of the epithelium of the gallbladder observed with SEM were inconsistent and variable with the severity associated with the inflammation (Table 2). The

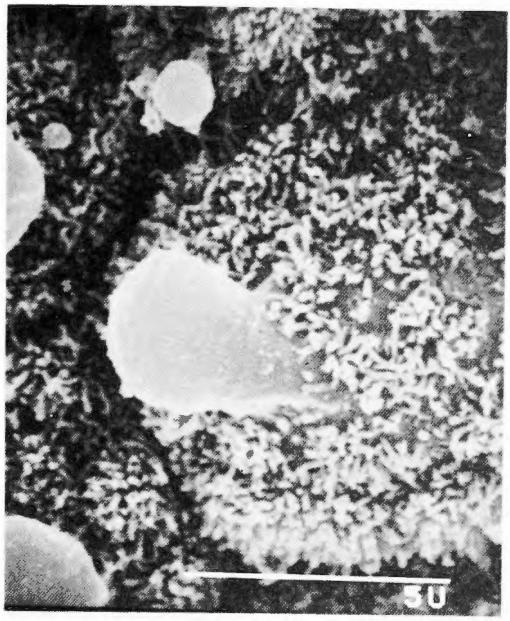


Fig. 11. Numerous lingual type protrutions could be observed (primary carcinoma of the gallbladder).

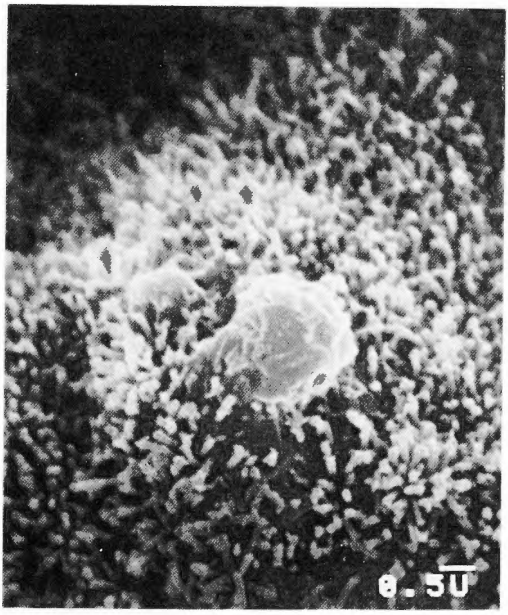


Fig. 12. High magnification of the protrusive region (primary carcinoma of the gallbladder).

most prominent features of the epithelium in cholecystitis by SEM were that the presence of broader and flatter folds, collapse and indentation of the cellular apical surface and shorter microvilli which decreased in number. Patchy desquamation and many exfoliated cells increased in proportion to the severity of the disease. In carcinoma of the gallbladder, the folds of the

Table 2. SEM findings of the gallbladder epithelia

	Normal	Cholecystitis	Carcinoma
Folds	deep, narrow repetitive pattern	much broader flatter	severely disarranged
Cell arrangements	uniform columnar epithelium	dilatation of inter-cellular spaces collapse of the apical surface	bizarre in size and shape
Microvilli	short closely packed	sparse	arranged in tuft very closely packed

epithelium were of various sizes. The epithelial cells were pleomorphic and their shapes were bizarre. Microvilli covered the carcinomatous epithelial surface and became taller and bulkier.

Discussion

Recently, malignant neoplastic diseases have replaced cardiovascular diseases as the number one cause of deaths in Japan and gallbladder carcinoma is the fifth most lethal cancer. Gallbladder carcinoma is a serious disease which is almost totally fatal since most of the patients with gallbladder carcinoma diagnosed preoperatively died whether they received a radical operation or not[11]. However, the prognosis after curative operation in gallbladder carcinoma has improved in the patients whose disease is limited within the gallbladder mucosa exclusively with no metastasis even to regional lymphnodes, although the prognosis still remains poor beyond these criteria.

Remarkable progress of diagnostic instruments and measurements, such as US[31,35], CT[33,50] and laparoscopy[10,13,30,59] may play a very important part in diagnosing carcinoma of the gallbladder[31,35,56]. However, few reports of early-staged carcinoma of the gallbladder have been made and most of them were detected by postoperative histological examinations of the specimens removed from the patients with cholecystolithiasis. Therefore, early diagnosis of gallbladder carcinoma is inevitable for a successful surgical intervention[3,26,27,47].

Early diagnosis, especially preoperative diagnosis, of gallbladder carcinoma is still difficult, because diagnostic imaging can not differentiate the thickened wall type of gallbladder carcinoma from chronic cholecystitis. Recently, in Japan, percutaneous transhepatic cholecystoscopical drainage (PTCCD) has become common for acute cholecystitis, and in some cases double-contrast cholecystography or endoscopic observation through the route of PTCCD is performed.

The surgeon must know whether a polypoid lesion is a polyp, an adenoma or an adenocarcinoma to decide the most appropriate treatment[23,39].

Most gallbladder carcinomas are associated with gallstones. Therefore gallstones have been suspected to play a very important role in the carcinogenesis of the gallbladder, that is, by irritating the gallbladder epithelium. Out of 4694 patients who had biliary tract surgery from 1982 to 1985 in our group, 94 had the gallbladder carcinoma. However, only 37 of the 68 patients who had had resective operations had curative resections. Gallstones were present in 11 of the 17 patients

with stage I carcinoma (64.7%), 8 of the 9 patients with stage II carcinoma (88.9%), 7 of the 16 patients with stage III carcinoma (43.8%), and 16 of the 51 patients with stage IV carcinoma (30.8%)[65,66]. Gallstones were diagnosed easily with the aid of diagnostic imaging techniques such as US and CT, the gallbladder carcinoma was resected concomitantly with the gallstones.

Although the radium in human gallstones hasd been suggested to play an important role in the etiology of gallbladder carcinoma, this was later denied. Induction of carcinoma of the gallbladder has been attempted by means of intracholecystic implantation of foreign bodies, but the only lesions in human gallbladder were those of 'cholecystitis glandularis proliferans' and intracholecystic foreign body implantation was never accompanied by a malignant change.

Attempts to induce gallbladder carcinoma experimentally by means of chemical substances were made in the first half of this century[12,21], but none of them were successful; intracholecystic insertion of a pellet containing 1,2,5,6-dibenzanthracene into the guinea pig gallbladder[6] and of a glass bead, a cholesterol pellet and a methylcholanthrene pellet into the guinea pig gallbladder by DESFORGES[15]. FORTNER was the first to success in inducing carcinoma of the gallbladder of cats by placing a single MC pellet in the gallbladder, followed by ligation of the cystic duct, but it took about 23–32 months before primary carcinoma of the gallbladder could be developed[18].

BAIN et al. tried experimental the production of gallbladder carcinoma in hamsters by inserting MC pellets and demonstrated the development of carcinoma in gallbladders of 12 of 19 hamsters (63%) as early as 2 to 5 months after the intracholecystic implantation of MC pellets without cystic duct ligation[7,8,9]. He stated that the guinea pig was extremely resistant to experimental production of gallbladder carcinoma. HOMBURGER summarized chemical carcinogenesis in syrian hamsters[22] and HIRANO et al. demonstrated that MC-containing beeswax pellets induced epidermoid carcinoma in the lungs of rats[20].

In 1981, SUZUKI reported the development of gallbladder carcinoma in 10 out of 18 hamsters (55%) induced by intracholecystic beeswax pellet containing 5 mg of MC and reported four microscopic types of histogenesis[51,52,53,54].

As the other methods, KOWALEWSKI[32] noticed that both diethylnitrosamine (DEN) and dimethylnitrosamine (DMN) given orally could induce hepatic tumors accompanied by neoplastic lesions in the biliary tract in rats and hamsters. The incidence of cholangiocarcinoma in hamsters receiving DMN was especially high, but localization of neoplasms in the animal gallbladder was scarcely found. Therefore, not only the presence of prolonged exposure to carcinogenic, chemical substances but also chronic, and nonspecific irritation of gallbladder mucosa may be necessary for the production of gallbladder carcinoma. Thus, he administered DEN and DMN solutions orally to hamsters receiving cholesterol pellets operatively in their gallbladders. Sixty eight percent of the DMN-treated hamsters receiving a cholesterol pellet in the gallbladder had carcinoma in the gallbladder, but no gallbladder carcinomas were found in the DEN-treated hamsters.

ICHIKAWA injected N-ethyl-N-nitro-N-nitrosoguanidine (ENNG) into the canine gallbladder by way of cholecystocutaneostomy for 180 days and succeeded in developing bile duct cancer in all 7 dogs[24]. IKURA induced gallbladder carcinoma in 21 (38%) out of 55 hamsters and intestinal

metaplasia of the gallbladder epithelium in 29 hamsters (53%) after the intracholecystic insertion of paraffin pellets containing ENNG for 6 months in 1982.

Many investigations on experimental carcinoma of the gallbladder have been reported, but, most of them have been related to cholelithiasis. In Japan, several trials have been performed by feeding a lithogenic diet or protein-free diet and carcinogens[16,44,45] and the prophylactic effect of phenobarbital was observed by MAKINO[40]. In experimental models, aging is one of the factors in incidence of polypoid hyperplasia and papilloma in gallbladder of cat and rat[59] and adenoma or adenocarcinoma in mice[68].

We chose hamsters as an experimental animal because nearly the same bile acid composition as man[58], gallbladder carcinoma is easily induced in this animal. We had first intended to determine the change in the composition of bile acid in the gallbladder bile with the stage of experimentally-induced carcinoma, but bile could not be obtained for chemical analysis; the bile flow into the animal gallbladder was disturbed by extremely atrophic changes influenced presumably by the operative maneuver. The composition of bile acid in bile duct bile of the hamsters was determined by HPLC with enzymatic detection, but the MC-containing beeswax pellet produced no changes. 3-methylcholanthrene was employed in this study to produce the early form of gallbladder carcinoma and to make a comparative study with human carcinoma, cholic acid and deoxycholic acid. The laboratory transformation of bile acids into methylcholanthrene suggests the possibility of a change from cholic to deoxycholic acid occurring in the body, and many workers will consider such a possibility in connection with the formation and degradation of cholesterol in the animal organism[28]. The relationship between the very active cancer-producing compounds and bile acid in important constituents of the human body is of special interest.

As pathology of preneoplastic lesions, the relationship between atypical hyperplasia and carcinoma of the gallbladder or carcinoma in situ have been stated as the precursor lesions of invasive gallbladder carcinoma[1,2,4,29,41,57]. Although the intestinal metaplasia-dysplasia-carcinoma sequence of the gallbladder has been demonstrated[64], no malignant change on the surface of the gallbladder mucosa has been shown by conventional histological HE staining on light microcsopic examinations in spite of numerous clinicopathological observations, because early-stage cases are very rare among the resection specimens of the gallbladder, obtained without the suspicion of cancer.

On the other hand, the multiple occurrence of precancerous change in adenoma of the colon and rectum is well recognized[48]. Change of the intestinal microvilli on SEM has been stated[59]. An ultrastructural and histochemical study of the mucous membrane adjacent to and remote from carcinoma of the colon was performed[14]. Also the relationship between the ultrastructural surface of normal and metaplastic cervical epithelia and of carcinoma in situ was demonstrated[62].

In 1964, EVETT et al. already observed the fine structure of normal mucosa in human gallbladder[17]. LATIO et al. were performed SEM and TEM observation human gallbladder epithelium[34] WILLIAMS and SMITH observed the normal and pathological human gallbladder by SEM[63]. However, the ultrafine structure in an early stage of the gallbladder mucosal change

has not been reported in human gallbladder carcinoma, because almost all the cases examined were of advanced carcinomas and primary sarcoma of the gallbladder[61]. Comparative studies between experimental carcinogenic process and polypoid lesions of human gallbladder have not been made.

In this study, tremendous artifacts, such as the extensive loss of the epithelium occurring during the prefixation could be prevented, because the gallbladder tissue was very thin. MUELLER recommended fixation of the whole gallbladder which allowed the detection of crypt-like glands and observation of distended epithelium[42]. The entire shape of the gallbladder could be preserved by tying the neck of the gallbladder and placing it into a glutaraldehyde solution. In clinical materials, the mucous layer of the specimen was previously cleaned with saline before glutaraldehyde fixation, but the mucous epithelium is preserved if the specimen is washed after the half-fixation with glutaraldehyde. Although this method may produce a slightly dirty specimen, detailed aspects can be observed by SEM sufficiently.

Our preliminary observation of the gallbladder mucosa in hamsters fed the lithogenic diet showed an increase of goblet cells in the surface of gallbladder with the production of gallstones[49]. SEM changes in the early form of the experimentally induced gallbladder carcinoma of the hamster were lingual protrusions on the epithelium covered by the sparse and short microvilli and sometimes protrusions covered by longer and bulkier microvilli, compared with narrow folds and covered with pentagonal or hexagonal cells, lined regularly in pavement-form in normal cells.

The surface of the epithelium of the human polyp of the gallbladder was covered by dense, slender and tall microvilli and these cell lines closely resembled those of the epithelium in control animals.

The most prominent inflammatory changes of the epithelium of the gallbladder observed with SEM were the presence of broader and flatter folds, the collapse and indentation of the cellular apical surface, shorter microvilli, which were decreased in number, patchy desquamation and many exfoliated cells.

In carcinoma of the gallbladder, folds of the epithelium were of various sizes. The epithelial cells were pleomorphic and their shapes were bizarre. Dense microvilli protruded into the free lumen and had many lingual type protrusions. The malignant change of the epithelium can be discriminated from the inflammation under SEM based on the folds of the epithelial surface, the shape and arrangement of cells and morphologic changes of the microvilli. The examination of biopsy specimens with SEM before conventional pathological examination has been found to be useful in making a perfect diagnosis of gallbladder carcinoma even at an early stage.

Recently, polypoid lesions were often found by US examination of the gallbladder. However, precancerous changes were not detected in adenoma in situ. In some cases treated by PTCCD, endoscopic observation was possible through the route of PTCCD and direct biopsy could be made with the forceps instrument.

The ultrastructural examination may be a helpful tool to detect early malignant change in the gallbladder biopsies.

Summary

The surface appearance is most essential for early diagnosis of gallbladder carcinoma. However, it was difficult to differentiate the bizarre surface of the advanced carcinoma from those caused by severe inflammation or technical loss of the mucosa, because most gallbladder carcinoma cases were clinically in the advanced stage. Since the SEM observation is suitable for determining the developmental stage of carcinoma, we performed SEM study of gallbladder carcinoma of hamsters induced by the insertion of beeswax pellet containing 3, 5 or 8 mg of methylcholanthrene.

Carcinoma could be detected by SEM in 2 of 95 hamsters treated, but not by other microscopic examination methods. One was 9 months after the insertion of 3 mg MC beeswax pellet, the another was 5 months after that of 5 mg. The folds of the surface near the cancer was much broader than that of the control. The normal cell arrangement of polygonal shape was replaced by a pleomorphic pattern. The microvilli were bulkier and taller than those of normal cells. Variously sized and shaped, such as polypoid, verrucous and cylindrial, surface protrusions which were covered with or without the microvilli, were recognized over the gallbladder mucosa affected with MC.

Cellular component of the protrusions was not disclosed by TEM. These findings in the early stage of experimental gallbladder carcinoma were quite similar to those of the human specimens obtained surgically.

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和文抄録

胆嚢癌の発癌初期病変の電子顕微鏡的考察

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臨床で取扱う胆嚢癌取扱規約が施行されて全国調査が進むにつれ，Stage IV の進行癌が殆んどを占め，現在でもその予後は極めて不良であることが改めて指摘されている．なかでも早期胆嚢癌は，胆石症として手術した症例に行なった病理標本の検索中に偶然に見出されるに過ぎないことが多い．胆嚢疾患の診断学に US や CT が加わり，病変の存在診断から“質的診断”へ向って前進しているとはいえ，胆嚢疾患の診断学の発達には胆嚢疾患の病理形態学的特徴が明確にされることが不可欠である．

胆嚢の形態的特徴としては粘膜筋板の欠如を指摘できるが，胆嚢における早期癌の概念は確立されていない．この早期胆嚢癌（m癌）の発見率を高めるためには，その発生形成について検討する必要がある．また，胆嚢病変を論ずる場合胆石を抜きにして論ずることはできず，また，臨床的に胆嚢結石を合併する胆嚢癌症例が多いことも勘案して，胆石症の実験モデルハムスターを用い，その胆嚢内にコレステロール石を形成させる実験を行ってきた教室の多くの経験から，ハムスターの胆嚢を用いる方法にて，実験的胆嚢癌の発生時における早期の胆嚢粘膜の微細な病変を，臨床例における胆嚢癌やポリープ症例のそれと比較し，検討し，走査電子顕微鏡（SEM）および透過電子顕微鏡にて粘膜表面の微細な構造の電子顕微鏡的考察を行った．

1）化学物質を用いる実験的胆嚢癌として，ハムスターの胆嚢内に methylcholanthrene（MC）3 mg，5 mg または 8 mg を含有する beeswax pellet を挿入し，通常飼料にて4～9カ月飼育した．ハムスターの胆嚢を一旦切開して pellet を挿入するため，その後に胆嚢の萎縮，廃用性変化は避けられず，特に長期飼育例では胆嚢が胆内に埋没し胆嚢採取時に物理的変化が加わることを避け得ないため，胆嚢が肝内に埋没しない早期に胆嚢内に腫瘍性変化を生じさせる必要があったので，MC 量を 3 mg 以上とした．

2）MC 3 mg 含有 beeswax pellet 留置群では，9カ月後に胆嚢摘出したハムスターの胆嚢に，SEM により胆嚢粘膜表面の microvilli の一部がポリープの様に突出している所見を得た．この所見は，臨床胆嚢癌にみられる microvilli が密生し，かつ一部が tufts の様に見える段階より，一歩手前の変化と考えられた．

3）MC 5 mg 含有 beeswax pellet 留置群では，5カ月後の胆嚢粘膜に乳頭腫状の隆起が多数散在しているのが見出され，その頂点付近では，細胞が顆粒状に腫大している所見を得た．これらの病変について透過電顕標本を作製して考察を加えた結果，隆起した部分には細胞成分が乏しく，発癌との関連の有無については言及できなかった．なお，MC 8 mg，4カ月群では雌雄差のためか，変化を認めなかった．

4）臨床例における胆嚢癌やポリープ症例のそれと SEM により比較，検討した結果，ポリープ症例では粘膜は規則正しく配列した五角形の円柱上皮の配列が認められるが，臨床の胆嚢癌に伴う変化としては，円柱上皮の配列が乱れ，個々の細胞の形や大きさも変化し，短かった microvilli が密生した様になり，一部には tufts 様像を呈することを観察した．したがって，ハムスターの胆嚢における同様の所見は，発癌に伴う変化であることが示唆された．

これらの所見の臨床的意義としては，臨床医が当面している最大の問題点が画像診断法の進歩によりその存在診断が容易となった小隆起病変が悪性か否かの判断基準が確立されてないことであり，隔靴掻痒の感があり，その外科的治療に苦慮させられてきたが，最近，PTCCD など体外から安全で確実に，かつ容易に胆嚢粘膜面の直接的な観察方法の進歩により，不均一粗大顆粒状の粘膜病変に注意し，胆嚢病変を生検できるようになりつつあり，それらの微小病変の SEM 観察が有用になると考えられる．