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Adenosquamous Carcinoma of the Gallbladder: A Clinicopathological, Immunohistochemical and Flow-cytometric Study of Twenty Cases

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Twenty patients (7.4%) with adenosquamous carcinoma of the gallbladder were selected from 271 surgically resected gallbladder cancers. The 20 patients were composed of 8 men and 12 women with a mean age of 66.9 years. Histologically, all twenty tumors showed an abrupt transition between the adenocarcinoma (AC) and squamous cell carcinoma (SCC) areas, and well differentiated AC was also found in the peripheral area of the tumor. A histochemical and immunohistochemical study using alcian blue, periodic acid-Schiff, cytokeratins, involculin and tissue polypeptide antigen disclosed a different nature of the two components. DNA heterogeneity between the components was detected in 5 of 7 cases by flow cytometry. The positive rate of immunostaining for proliferating cell nuclear antigen in the SCC areas (mean 20.55%) was larger than that of the AC areas (mean 11.40%) ($P=0.0029$), which indicated that the SCC areas had a greater proliferative capacity than AC areas. These results suggest that the SCC component of adenosquamous carcinoma of the gallbladder arose by a stepwise molecular progression of the pre-existing AC. Furthermore, the prognosis of adenosquamous carcinomas of the gallbladder (mean survival: 10 months) in the advanced stage (pTNM 2–4) was less favorable than those of papillary and well differentiated AC (mean survival: 99 months and 86 months) ($P<0.0001$).

Key words: Gallbladder neoplasm — Adenosquamous carcinoma — Immunohistochemistry — DNA heterogeneity

Adenosquamous carcinoma of the gallbladder is a well known, though uncommon, variant of cancer, but has been described in only a few reports.[1-3] There is controversy about the histogenesis of the squamous component of adenosquamous carcinoma in various organs. Some authors have suggested that adenosquamous carcinoma is actually squamous differentiation in an adenocarcinoma (AC),[1,4,5] while others have suggested that squamous metaplasia of the gallbladder epithelium may be closely related to the neoplastic processes of squamous cell carcinoma (SCC).[6-8]

We herein report 20 cases with adenosquamous carcinoma of the gallblasdder with mapping, histochemical, immunohistochemical, and flow-cytometric atudies, and discuss their histogenesis.

MATERIALS AND METHODS

Patients Among 271 surgically resected carcinomas of the gallbladder filed in our institution from 1973 to 1992, 25 cases were initially diagnosed as adenosquamous carcinoma. After reviewing these cases, two of them were later found to have no distinct squamous elements such as keratin pearls and/or intracellular keratinization, so they were reclassified as poorly differentiated AC. Another two showed large areas of atypical spindle cells with tiny areas of AC and SCC, and therefore they were designated as undifferentiated carcinoma, spindle cell type.[9] One case was squamous cell carcinoma focally showing some cystic spaces, but no glandular or papillary area was seen, so it was reclassified as SCC. The remaining twenty cases had distinct AC and SCC areas, and they also fulfilled the established histologic criteria (Table I).[10,11] Cases with AC containing indistinct squamoid features were excluded in this study.

Histologic study In all instances, the resected tumors were fixed in 10% formalin, and 15 cases (8.2%) were selected from 182 cases for which 5 mm stepwise tissue sections were examined, while 5 cases (5.6%) were selected from 89 cases for which representative tissue sections were examined. All the sections were stained with hematoxylin and eosin (H & E). A mucin reaction using periodic acid-Schiff (PAS) and alcian blue pH 2.5 (AB) stain was observed in 19 cases. An immunohistochemical study was also performed in 19 cases. The avidin-biotin-peroxidase complex method was applied using the Vectastain kit (Vector, Burlingame, USA).[12] The primary antibodies used in this study were AE1/AE3 (mouse monoclonal antibody, Hybritech, San Diego, USA), EAB 903 (34 beta E12), EAB 902 (34 beta H11), muscle specific antibody (HHF 35) (mouse monoclonal antibodies, Enzo Diagnostics, New York,

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Table I. Histologic Types of 271 Surgical Cases of Gallbladder Carcinoma

Histologic type	No. of cases (%)	Male/Female
Adenocarcinoma	225 (83.0)	55/170
Papillary	35 (12.9)	9/26
Well differentiated	111 (41.0)	30/81
Moderately differentiated	49 (18.1)	7/42
Poorly differentiated	29 (10.7)	9/20
Mucinous adenocarcinoma	1 (0.4)	0/1
Adenosquamous carcinoma	20 (7.4)	8/12
Squamous cell carcinoma	1 (0.4)	0/1
Undifferentiated carcinoma	13 (4.8)	3/10
Pleomorphic type	5 (1.8)	0/5
Spindle cell type	8 (3.0)	3/5
Small cell carcinoma	10 (3.7)	3/7
Composite tumor[a)]	1 (0.4)	0/1
Carcinosarcoma	1 (0.4)	1/0
Total	271 (100)	70/201

a) The composite tumor was made up of well differentiated adenocarcinoma and small cell carcinoma.

USA), CAM 5.2 (mouse monoclonal antibody, Becton Dickinson, Mountain View, USA), carcinoembryonic antigen (CEA) (mouse monoclonal antibody, Zymed Laboratories, San Francisco, USA), carbohydrate antigen 19-9 (CA 19-9) (mouse monoclonal antibody, Toray-Fuji, Tokyo), serotonin, vimentin, desmin, PC 10 (antibody against proliferating cell nuclear antigen (PCNA))[13] (mouse monoclonal antibodies, Dakopatts, Glostrup, Denmark), tissue polypeptide antigen (rabbit polyclonal antibody Sangtec Medical, Bromma, Sweden), involculin (rabbit polyclonal antibody, Biomedical Technologies Inc., Stoughton, USA), chromogranin A, α-fetoprotein (AFP), human chorionic gonadotropin (HCG, specific to β-subunit) (rabbit polyclonal antibodies, Dakopatts, somatostatin (rabbit polyclonal antibody, BioGenex), Dublin, Ireland), gastrin (rabbit polyclonal antibody, Dakocorporation, Santa Barbara, USA). After the immunostaining procedures, sections were counterstained with methylgreen, and the sections stained with EAB 903 were also counterstained with AB.

Involculin is a precursor of the cross-linked envelope protein or marginal band present in human stratum corneum, and is a specific marker for squamous differentiation in lung tumors.[14] EAB 903, generated against the human stratum corneum, recognizes the 57 and 66 kd cytokeratin proteins of the human stratum corneum, and EAB 902, generated against the cytoskeleton of a human hepatocellular carcinoma cell line (Hep3b), recognizes a 54 kd cytokeratin protein in many epithelial cell lines.[15, 16] SCC was uniformly positive with EAB 903 and largely negative with EAB 902, while AC of the gallbladder was uniformly positive with EAB 902 and negative with EAB 903.[17] CAM 5.2 is a murine molecular antibody, raised against the colon carcinoma cell line HT 29, which recognizes lower-molecular-weight cytokeratin proteins within the secretory epithelia.[18] Tissue polypeptide antigen was initially thought to be a tumor marker antigen, but was later found to be a cytoplasmic constituent of almost all human epithelia, though it was not present in the epidermis of normal cells.[19]

Measurement of PCNA staining The extent of positive immunostaining to PC 10 (PCNA) was measured using an image analysis computer system (Nexus Qube image analysis processor, Nexus Inc., Tokyo). This video-based system was able to distinguish the brown DAB reaction product from the methyl green-stained nuclei. First, the total nuclear area was determined, and then the extent of the nuclear area exceeding the manually determined staining threshold was measured. In this procedure, we only measured intensely stained nuclei. In representative AC and SCC areas of the tumors, ten fields ($20\times$objective: the final magnification on the color video monitor was $\times 800$) were examined, and the PCNA positive nuclear area was measured as a percentage of all nuclear areas.

DNA flow cytometry The flow-cytometric determination of the tumor cell DNA content was done on the paraffin-embedded tissue based on an earlier method.[20] We prepared 40-μm-thick slices from the formaldehyde-fixed and paraffin-embedded materials and deparaffinized, rehydrated, and then incubated them in trypsin 0.25% (Difco, Detroit, USA) in a citrate buffer (3 m*M* trisodium citrate, Nonidet P-40 0.1% v/v (Wako Junyaku, Osaka), 1.5 m*M* spermine tetrachloride, 0.5 m*M* Tris, pH 7.6) overnight at 37°C. After vortexing and filtration over a nylon mesh (pore size, 50 μM), approximately $1\text{–}3\times10^6$ cells were stained with propidium iodine according to a published method.[21] Cellular DNA content was measured on a FACScan flow cytometer (Becton Dickinson, Sunnyvale, USA) with a 488-nm argon ion laser. Histograms of 1×10^4 cells were recorded and analyzed with the Cell FIT Cell-Cycle Analysis software, version 2.01.2 (Becton Dickinson). The first G1/0 peak was assumed to be a diploid population and was given a DNA index of 1.0. The criterion of DNA aneuploidy was the presence of a well-defined second peak.

Statistical analysis Clinical data were available for all 20 patients. The prognoses were obtained for all 20 patients and were updated as of October 31, 1992. The cumulative survival curves of the patients with adenosquamous carcinoma and those of AC (papillary, well, moderately and poorly differentiated) were then calculated by the Kaplan-Meier method. The differences between the survival curves were measured by the generalized Wilcoxon

and logrank tests. The PCNA positivity rates between the AC and SCC areas were then calculated with the paired Student's *t* and Wilcoxon tests.

RESULTS

Clinical findings The twenty patients with adenosquamous carcinoma in this study ranged from 50 to 78 years old with a mean age of 66.9 years (Table II), while the 225 patients with AC (papillary, well, moderately, poorly differentiated, mucinous) of the gallbladder ranged from 20 to 91 yeras with a mean age of 65.4 years. The 20 patients with adenosquamous carcinoma consisted of 8 men and 12 women showing a male-to-female ratio of 2:3, while the 225 patients with AC consisted of 55 men and 170 women, showing a female predominance of 1:3.1. Only a cholecystectomy was done in ten of the twenty cases with adenosquamous carcinoma, cholecystectomy with hepatectomy was performed in eight cases, cholecystectomy and pancreatoduodenectomy were done in one case, and cholecystectomy, partial gastrectomy and colectomy were performed in one case. Eight patients had gallstones. Two tumors were mainly located in the neck of the gallbladder, five in the body, four in the fundus, and the others in the whole region of the gallbladder. Eleven tumors invaded adjacent organs such as the liver, duodenum, colon, cystic duct, peritoneum, lymph node, and omentum, while nine tumors did not show such invasion. Three of the cases belonged to pTNM stage 2, eleven were stage 3 and six were stage 4.[11]

Macroscopic findings Thirteen tumors showed a nodular configuration, while seven tumors diffusely invaded the gallbladder wall (Table II). Tumor size varied from 3.8 cm to 10.6 cm in the greatest diameter, with a mean of 6.3 cm.

Histologic findings All 20 adenosquamous carcinomas showed areas of unequivocal glandular structures and squamous epithelium. Regarding the AC areas, all tumors showed either glandular or papillary configurations. In the SCC areas, all tumors revealed keratin pearls, individual keratinization, and/or intercellular bridges. Both AC and SCC areas were intermingled, and there was an abrupt transition between the two components in all 20 tumors (Figs. 1 and 2A). In three of the 20 cases, such an abrupt transition was observed in the mucosa. Tumor giant cells were seen in four cases, while osteoclast-like giant cells were detected in only one case.[3] Lymphatic permeation, venous invasion, and perineural invasion were seen in 18, 12, and 14 cases, respectively, while both AC and SCC components were found in those invasive foci. In seven cases, intestinal metaplasia such as pyloric glands. Paneth's cells and/or endocrine cells were observed in the surrounding non-neoplastic mucosa, but no squamous metaplasia was detected in any case.

A histological mapping of the tumor was also performed on all 20 cases with adenosquamous carcinoma, referring to both the histochemical and immunohistochemical stainings (Fig. 3). As shown by Fig. 3, the SCC areas were mainly located at the deeper portion of the gallbladder wall, while well differentiated ACs were ob-

Table II. Clinical Findings of 20 Patients with Adenosquamous Carcinoma of the Gallbladder

No. cases	Age	Sex	Gallstone	Macroscopic findings	Invasion	Prognosis
1	68	F	+	Nodular	Cystic duct, Peritoneum	DOD (3 mo)
2	52	F	+	Diffuse	None	DOD (1 yr)
3	64	M	−	Diffuse	None	DOD (5 mo)
4	72	F	−	Nodular	Liver	DOD (3 mo)
5	63	F	+	Nodular	None	DOD (6 mo)
6	73	F	−	Nodular	Lymph node, Duodenum	DOD (1 mo)
7	63	F	−	Diffuse	Lymph node	DOD (11 mo)
8	78	F	+	Nodular	Lymph node, Duodenum	DOD (7 mo)
9	66	F	+	Nodular	None	DOD (6 mo)
10	78	F	−	Nodular	None	DOD (5 mo)
11	73	F	+	Diffuse	Liver, Colon, Lymph nodes	DOD (1 yr)
12	50	F	−	Nodular	Liver	DOD (6 mo)
13	64	M	−	Nodular	Liver	DOD (6 mo)
14	68	M	+	Nodular	None	AW (4 yr, 8 mo)
15	63	M	−	Diffuse	Lymph node	DOD (3 mo)
16	64	M	−	Diffuse	Liver, Colon	DOD (3 mo)
17	71	F	−	Nodular	None	DOD (1 yr, 7 mo)
18	76	M	−	Nodular	None	AW (3 mo)
19	72	M	+	Nodular	Omentum	AW (2 mo)
20	60	M	+	Diffuse	None	DOD (5 mo)

DOD: died of disease. AW: alive and well.

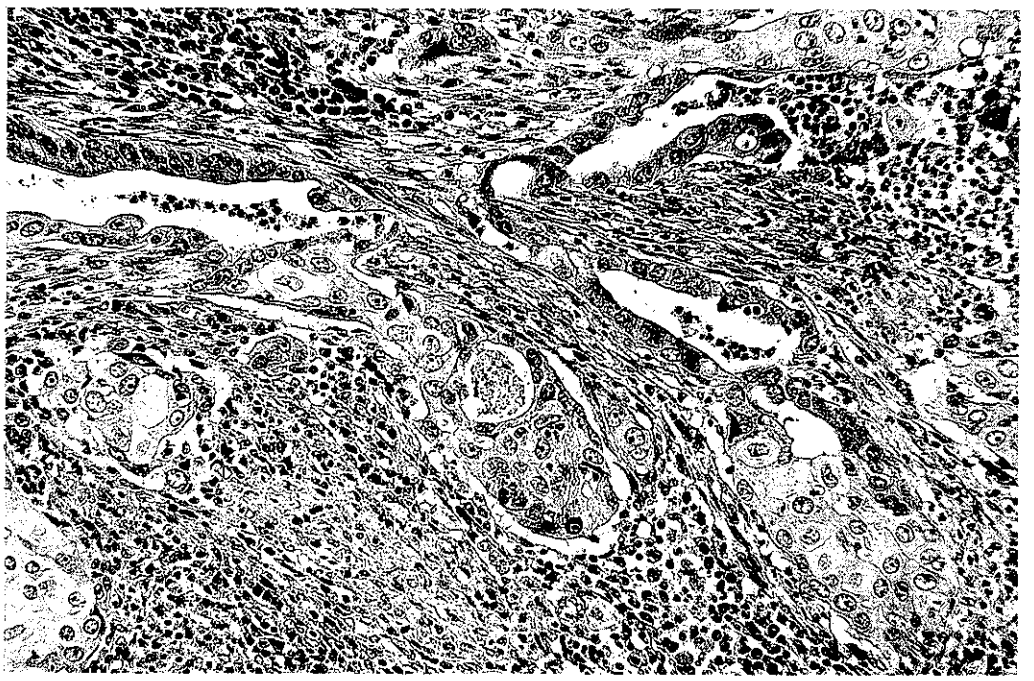

Fig. 1. Case 19. A close relationship between adenocarcinoma and squamous cell carcinoma (H & E, ×200).

served at the peripheral area of the tumor in all 20 cases (Fig. 4).

Histochemistry and immunohistochemistry The AC areas of adenosquamous carcinoma of the gallbladder were positive for AB and PAS in all 19 cases examined, while the SCC areas were negative for AB in all cases, and were positive for PAS in only four cases (Table III). The SCC areas had intracytoplasmic involculin and EAB 903 immunoreactants in 13 and 17 cases, respectively (Fig. 2, B and C). The AC areas were positive for involculin and EAB 903 in only 1 and 2 cases, respectively. The AC areas had intracytoplasmic EAB 902 and CAM 5.2 immunoreactants in 18 and 18 cases, respectively (Fig. 2, D and E). However, the SCC areas were positive for EAB 902 and CAM 5.2 in only 4 and 1 cases, respectively. Immunoreactivity to tissue polypeptide antigen was observed in 18 AC areas and 14 SCC areas. The tumor cells were diffusely positive in AC areas, but focally positive in the SCC areas (Fig. 2F). In 12 cases, individual mucin-producing cells were detected in the SCC areas by immunostaining EAB 903 with AB (Fig. 5). Immunoreactivities to HCG and neuroendocrine markers (chromogranin A, serotonin, somatostatin and gastrin) were found in 8 and 5 cases, respectively.

PCNA staining Sixteen tumors were immunoreactive to PCNA (PC 10). Of these cases, the PCNA scores in the AC areas ranged from 3.41% to 19.16%, while those in the SCC areas ranged from 5.07% to 56.60% (Table IV). In each tumor, the PCNA score in the SCC area was larger than that in the AC area. The mean PCNA score in the SCC areas (20.55%) was significantly larger than that in the AC areas (11.40%) ($P=0.0029$).

DNA flow cytometry Flow-cytometric data on cellular DNA content could be obtained in nine tumors (Table V). Of these cases, both ACs and SCCs were diploid in two cases, and either AC or SCC showed a diploid pattern in two cases. In case 4, AC showed an aneuploid pattern, while SCC was diploid. On the other hand, AC was diploid while SCC was observed to be aneuploid in Cases 7 and 19. In Cases 11 and 18, both the ACs and SCCs were aneuploid, but the DNA index of one component differed from that of the other, showing DNA heterogeneity in the same tumor.

Prognosis Sixteen of the twenty patients with adenosquamous carcinoma of the gallbladder died within one year after the initial operation, and only one patient was still alive 4 years and 8 months after the operation (Table II). In our file, the mean survivals of the 20 patients with adenosquamous carcinoma, the 27 with papillary AC, the 93 with well differentiated AC, the 48 with moderate differentiated AC, and the 29 with poorly differentiated AC in the advanced stage (pTNM stages 2–4) were 10 months,*,** 99 months,* 86 months,** 26 months, and 8 months, respectively (*,** the differences were signifi-

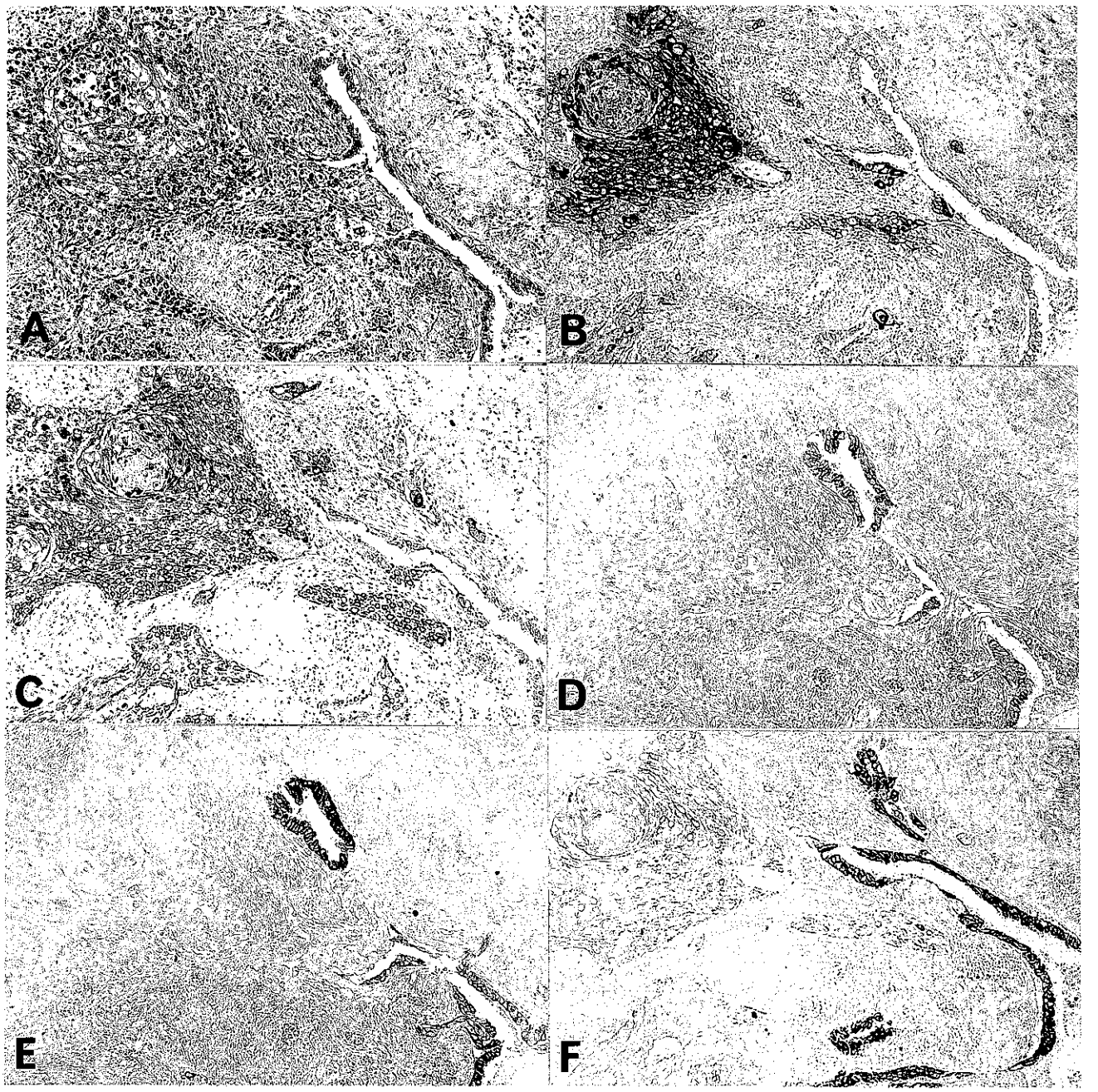

Fig. 2. Case 12. A transitional zone between adenocarcinoma (AC) and squamous cell carcinoma (SCC) areas. Each figure (A–F) is taken from the same area. The SCC area is seen on the left side, while the AC area is present on the right side (A). Immunohistochemically, the SCC is diffusely positive for involculin (B) and EAB 903 (C), while the AC is negative to both antibodies. AC is immunoreactive to EAB 902 (D) and CAM 5.2 (E), while SCC is not reactive to these antibodies. Both the AC and SCC areas are immunoreactive to tissue polypeptide antigen, but the tumor cells were diffusely positive in the AC areas, while focally positive in the SCC areas (F) (H & E, ×100 (A), ABC method, ×100 (B, D–F), ABC method and alcian blue, ×100 (C)).

cant, $P<0.0001$) (Fig. 6).[22] Furthermore, the survival of the 11 patients with adenosquamous carcinoma in pTNM stage 3 (7.7 months) was less favorable than those of patients with papillary and well differentiated AC in the same stage (53.6 and 61.5 months, respectively. $P<0.005$, $P<0.05$, respectively).

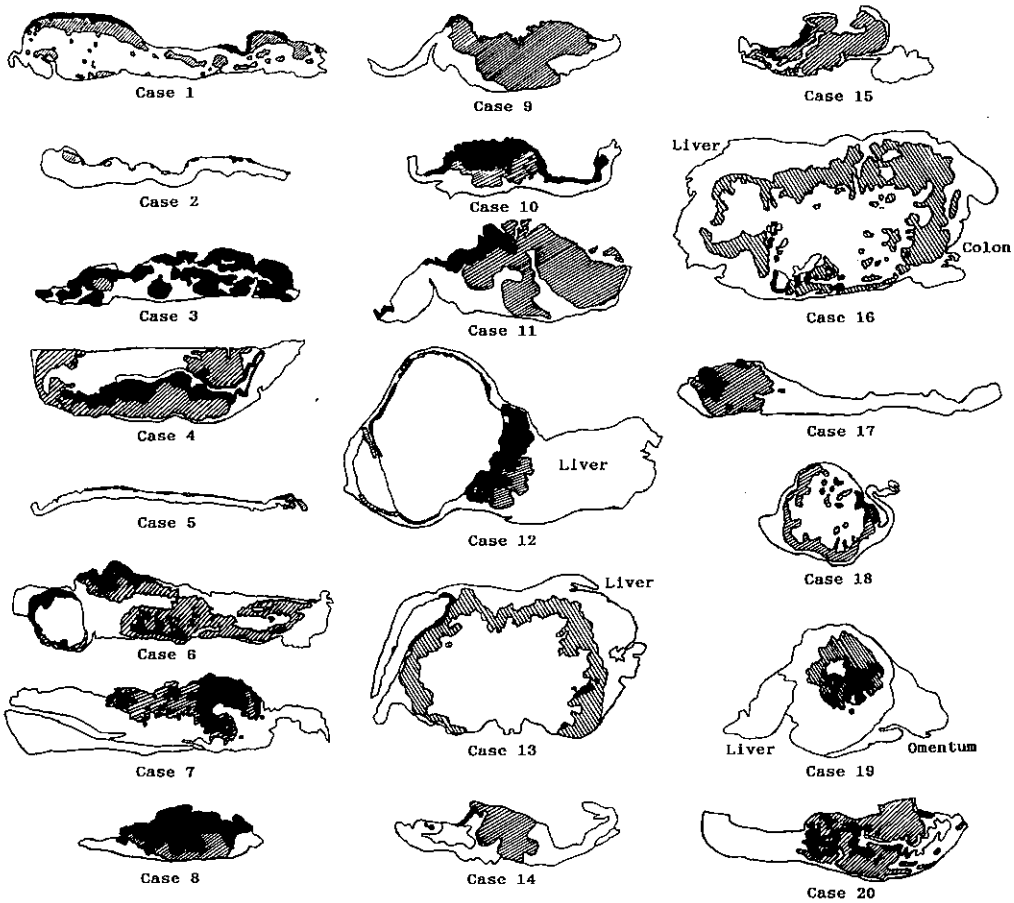

Fig. 3. Microscopic mapping results of the adenocarcinoma and squamous cell carcinoma areas from twenty cases of adenosquamous carcinoma of the gallbladder. Striped area: squamous cell carcinoma-dominant area. Black area: adenocarcinoma-dominant area.

DISCUSSION

Adenosquamous carcinoma of gallbladder has been reported to account for 1.4% to 9.6% of gallbladder carcinomas.[10, 23–26] In the present series, twenty cases (7.4%) of 271 surgically resected gallbladder carcinoma turned out to be adenosquamous carcinoma. Although, gallbladder carcinoma has been reported to occur predominantly in females,[10, 26] adenosquamous carcinoma showed a relatively low male-to-female ratio of 2:3 in the current study.

Several kinds of nomenclature have been used for carcinoma containing both AC and SCC components: adenosquamous carcinoma, adenoacanthoma, and mucoepidermoid carcinoma.[10, 27] In the uterus, adenoacanthoma means AC with benign(-appearing) squamous elements and adenosquamous carcinoma means carcinoma containing both AC and SCC components.[28, 29] Therefore, we prefer the term "adenosquamous carcinoma of the gallbladder."

Various hypotheses have been made concerning the squamous elements at sites where AC is generally found: ectopic squamous nest,[30] non-neoplastic squamous metaplasia of the epithelium,[6–8, 31] squamous metaplasia of the pre-existing AC,[1, 4, 5, 32] multipotential stem cell,[33] basal cell,[34, 35] and squamous element of adenomatous polyp.[36] In all twenty tumors, there was a close relationship between the AC and SCC areas, and both components were also seen in the metastatic and/or invasive areas. In the peripheral area of the tumor, well differentiated AC areas were seen in all twenty cases, while SCC areas were detected in the deeper site (Fig. 3). Therefore, we think that the squamous components represent the stepwise molecular progression of a pre-existing AC.

As shown by Fig. 2 and Table III, most SCC areas were immunoreactive to EAB 903 and involculin, and a

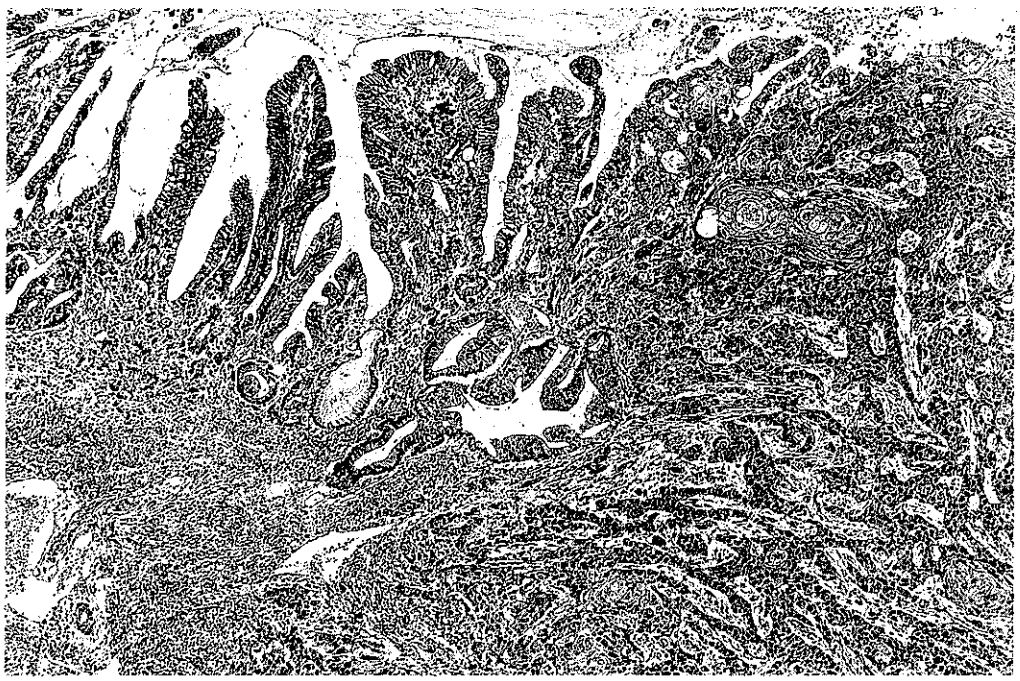

Fig. 4. Case 11. A well differentiated adenocarcinoma showing papillary features is seen in the peripheral mucosal site of the tumor (H & E, ×55).

Table III. Histochemical and Immunohistochemical Findings of Adenosquamous Carcinoma of the Gallbladder (n=19)

	AC area (%)	SCC area (%)
AB	19 (100)	12 (63)[a]
PAS	19 (100)	4 (21)
AE1/AE3	19 (100)	19 (100)
EAB 903	2 (11)	17 (90)
EAB 902	18 (95)	4 (21)
CAM 5.2	18 (95)	1 (5)
TPA	18 (95)	14 (74)
Involculin	1 (5)	13 (68)
CEA	17 (90)	10 (53)
Chromogranin A	3 (16)	0 (0)
Serotonin	1 (5)	0 (0)
Somatostatin	3 (16)	2 (11)
Gastrin	1 (5)	0 (0)
HCG	5 (26)	7 (37)

AC area: adenocarcinoma area. SCC area: squamous cell carcinoma area. AB: alcian blue. PAS: periodic acid-Schiff. EAB 903: 34 beta E12. EAB 902: 34 beta H11. TPA: tissue polypeptide antigen. CEA: carcinoembryonic antigen. HCG: human chorionic gonadotropin.
a) AB-positive cells in the SCC area.

few AC areas were positive for such markers. The AC areas were positive for AB, PAS, EAB 902 and CAM 5.2, and only a few SCC areas were immunoreactive to these markers. These findings showed that there was a histochemical and immunohistochemical contrast between the AC and SCC areas of adenosquamous carcinoma of the gallbladder.

HCG immunoreactivity is a rare event in gallbladder neoplasms, and is considered to be related to a poor prognosis.[37] We found 8 tumors (42%) positive for its antibody (Table III), while the choriocarcinoma area could not be detected in these cases. We cannot explain such high HCG-immunoreactivities. Yamamoto *et al.* reported that 14 (28.6%) of 49 cases of adenocarcinoma of the gallbladder contained endocrine cells,[38] and we found the same rate of neuroendocrine differentiation in 5 (26%) cases of adenosquamous carcinoma in the current series.

Heterogenous DNA stemlines, obtained from DNA flow cytometry, were first reported by Petersen *et al.* in five of six colorectal carcinomas based on fine-needle biopsies from different parts of single tumors.[39] Such a DNA stem line heterogeneity in the tumor was reported in 7.4 to 40% of colorectal carcinoma cases,[40,41] and in 40% of gastric carcinoma cases.[42] In the current series, flow-cytometric data could be obtained from both the AC and SCC areas in seven cases, and DNA heterogeneity between both areas was detected in five (71%) of seven cases. Such a development of intraneoplastic subpopulations is thought to result from a genetic instability of the original tumor cell clone.[42-44]

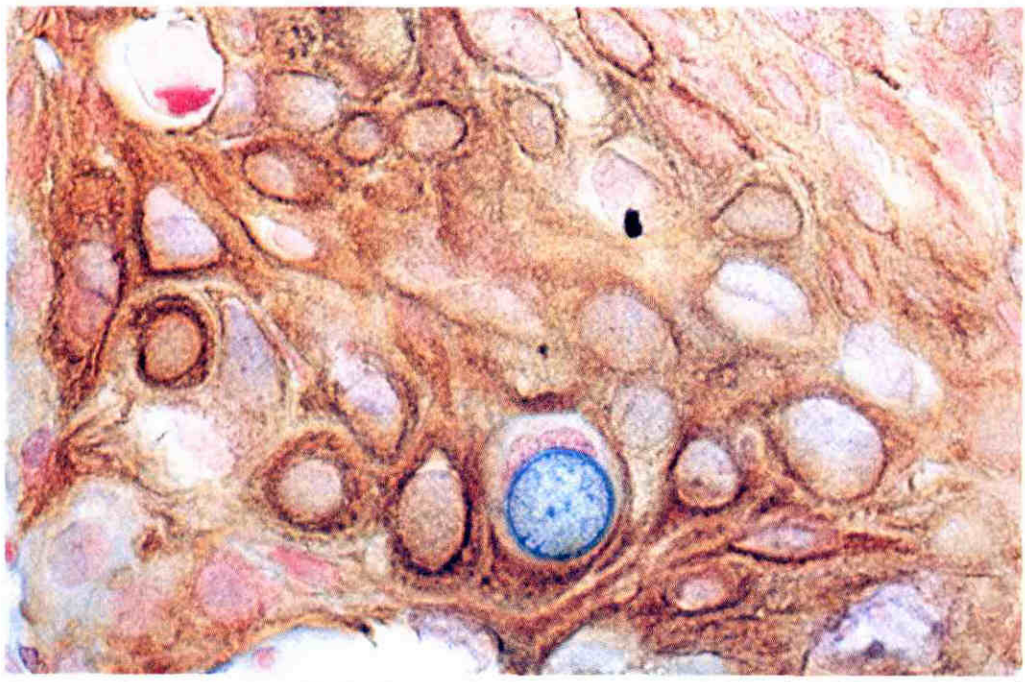

Fig. 5. Case 19. A mucin-secreting individual cell stained with AB is surrounded by the SCC component which is diffusely immunoreactive to EAB 903 (ABC method and alcian blue, ×780).

Table IV. Percentage PCNA-positive Nuclear Area in Adenosquamous Carcinoma of the Gallbladder (n=16)

Case No.	AC area	SCC area
2	8.00	9.35
4	9.71	17.51
6	12.21	19.02
7	18.91	27.33
8	15.98	22.34
10	4.73	11.60
11	10.09	11.36
12	11.00	16.99
13	19.16	30.55
14	9.62	20.99
15	9.77	14.01
16	15.47	56.60
17	3.41	5.07
18	9.21	13.28
19	8.93	34.19
20	16.16	18.53
Mean	11.40[a)]	20.55[a)]

AC area: adenocarcinoma area. SCC area: squamous cell carcinoma area.
a) $P=0.0029$ (Student's *t* test).

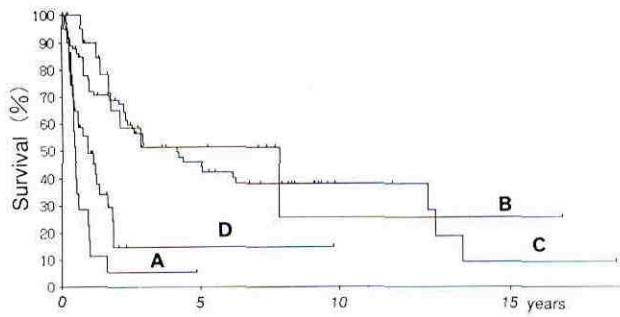

Fig. 6. The survival curves of the 20 adenosquamous carcinomas, 27 papillary carcinomas, 93 well differentiated adenocarcinomas and 48 moderately differentiated adenocarcinomas of the gallbladder in pTNM stages 2–4. A: adenosquamous carcinoma. B: papillary adenocarcinoma. C: well differentiated adenocarcinoma. D: moderately diifferentiated adenocarcinoma.

Based on clinical observations, Charbit *et al.* reported that the doubling time of SCC (mean 81.8 days) is significantly shorter than that of the AC (mean 166.3 days).[45] Iemura *et al.* established a serially transplantable human cholangiocellular carcinoma in nude mice (nuKMC-2) that showed a morphologic alteration from AC to SCC when serially transplanted in nude mice.[46] The doubling times of nuKMC-2 at the 5th and 11th passages were 9.9 and 7.4 days, respectively, which suggested that tumors with SCC components were more biologically aggressive

Table V. Flow-cytometric Study of Adenosquamous Carcinoma of the Gallbladder

Case No.	Adenocarcinoma			Squamous cell carcinoma		
	CV (G0/G1)	Ploidy pattern	DNA index	CV (G0/G1)	Ploidy pattern	DNA index
4	4.6	Aneuploid	1.46	5.2	Diploid	
6				4.0	Diploid	
7	4.1	Diploid		5.2	Aneuploid	1.22
11	4.2	Aneuploid	1.57	4.0	Aneuploid	1.76
13	4.1	Diploid				
16	7.5	Diploid		6.2	Diploid	
17	5.4	Diploid		5.1	Diploid	
18	6.5	Aneuploid	1.53	6.1	Aneuploid	1.43
19	6.2	Diploid		6.0	Aneuploid	1.47

CV (G0/G1): coefficient of variation for the reference G0/G1 peak.

than those with AC areas. PCNA was originally defined as an intranuclear polypeptide whose synthesis reaches its maximum during the S-phase of the cell cycle.[47–49] Recently, monoclonal antibody against PCNA has been employed to demonstrate the proliferative component of paraffin-embedded tissues.[13] We evaluated PCNA immunostaining in the adenosquamous carcinoma of the gallbladder, and found that the PCNA scores in the SCC areas were higher than those in the AC areas (Table IV). These results were basically the same as those obtained by Charbit *et al.* and Iemura *et al.*, and indicate that the SCC components exhibit more proliferrative behavior than the AC areas.

It has been reported that the outcome for patients with adenosquamous carcinoma in the gallbladder,[1,20] pancreas,[4,26] colon[28] and stomach[5] was poor. In a recent report, histologic type and stage of disease were shown to be correlated with the outcome in gallbladder carcinoma,[20] and we found that the survival of the patients with adenosquamous carcinoma of the gallbladder was significantly more unfavorable than that of patients with papillary and well differentiated AC in a stage-by-stage comparison (Fig. 6).

In summary, we studied 20 cases of adenosquamous carcinoma of the gallbladder. (a) Well differetiated AC was seen in the peripheral area of the tumors. (b) A close relationship was observed between AC and SCC. (c) Histochemistry, immunohistochemistry and flow cytometry showed different characteristics for the two elements. (d) AC (83%) is more common than SCC (0.4%) in the gallbladder (Table I). (e) The most progressive part of each tumor was composed of SC, while AC covered the surface of the tumor (Fig. 3). (f) The proliferative activities of the SCC areas were higher than those of the AC areas. These findings suggest that the SCC component arose through stepwise molecular progression of a pre-existing AC.

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