KAWASAKI MED. J. VOL. 9 : NO. 4, 229-237 (1983)

Intestinal Nature in Gallbladder Carcinoma with Reference to its Histogenesis

Hiromichi SATO, Masato TAHARA, Masato KONO, Koichi OHMURA, Mutsue MIZUSHIMA, Jishu ITO and Kazuyoshi YOSHIOKA*

Departments of Pathology and Surgery, Division of Kawasaki Hospital, Kawasaki Medical School, Okayama 700, Japan*

Accepted for Publication on November 26, 1983

ABSTRACT. Forty advanced cases of gallbladder carcinoma were examined by the use of histochemical and immunohistological methods, in which attention was focused on intestinal metaplasia seen in and around the lesions.

Twenty-nine percent for cases of well-differentiated adenocarcinoma displayed goblet cell-type carcinoma cells within the lesions, while no similar cell was evident in cases of poorly differentiated adenocarcinoma. Ten percent for cases of gallbladder carcinoma included endocrine cells within neoplastic tissues, all of which consisted of well-differentiated adenocarcinoma and mucosal areas adjacent to which also contained similar cells. Mucosal areas in the proximity to foci of well-differentiated adenocarcinoma demonstrated a marked increase in the amount of non-sulfated acid mucin, but no such tendency was discernible around foci of poorly differentiated adenocarcinoma. Lysozyme immunoreactivity was identifiable in a high percent of well-differentiated adenocarcinoma foci and their surrounding mucosal areas, while similar reactivity was rarely present in cases of poorly differentiated adenocarcinoma.

It may be suggested from these results that an intimate relationship would exist in between intestinal metaplasia and well-differentiated adenocarcinoma occurring in the gallbladder. It is, however, to be determined whether or not intestinal metaplasia alone would act as a precancerous lesion like adenoma, dysplasia and possibly hyperplastic polyp for the histogenesis of gallbladder carcinomas.

Key words : Gallbladder carcinoma — Intestinal metaplasia — Lysozyme

Occurrence of mucous glands as well as goblet, endocrine, absorptive or Paneth cells in mucous membranes of the gallbladder would be generally accepted as of intestinal metaplasia, which not infrequently occurs in chronic cholecystitis with or without lithiasis.[1,2] Intestinal metaplasia in the stomach would be recently interpreted as being one of the precancerous lesion for the development of well-differentiated adenocarinoma[3], but the relationship between intestinal metaplasia and carcinoma in the gallbladder is not defined as yet probably because of the paucity of early intramucosal cancers and/or the poor preservation of the surrounding mucosal tissues.

佐藤博道，田原昌人，光野正人，大村晃一，水島睦枝，伊藤慈秀，吉岡一由

Based on the principle that neoplastic tissues would usually display more or less the nature of original tissues from which they are derived, we tried to approach the histogenesis of gallbladder carcinomas from the comparative study on intestinal metaplasia seen in and around the lesions by the use of histochemical and immunohistological methods.

MATERIALS AND METHODS

Forty cases of gallbladder carcinoma comprising 26 surgical and 14 autopsy cases filed in our hospital during the last 20 years were examined by the following methods : Hematoxylin-eosin, alcian blue-PAS, high iron diamine-alcian blue, Grimelius and Masson-Fontana stains for histological and histochemical study, and PAP method by the use of anti-human lysozyme rabbit serum (Dako Indust.) on paraffin sections for immunohistological study. Normal rabbit serum was used as control serum.

In 20 out of the 40 cases of gallbladder carcinoma examined in this study non-neoplastic mucosal membrances surrounding the tumors were preserved after histological processing, the metaplastic changes of which were compared with those within the neoplasms.

RESULTS

Metaplastic changes : All the 40 cases of gallbladder carcinoma examined were categorized to belong to advanced cancer in which carcinomatous invasion

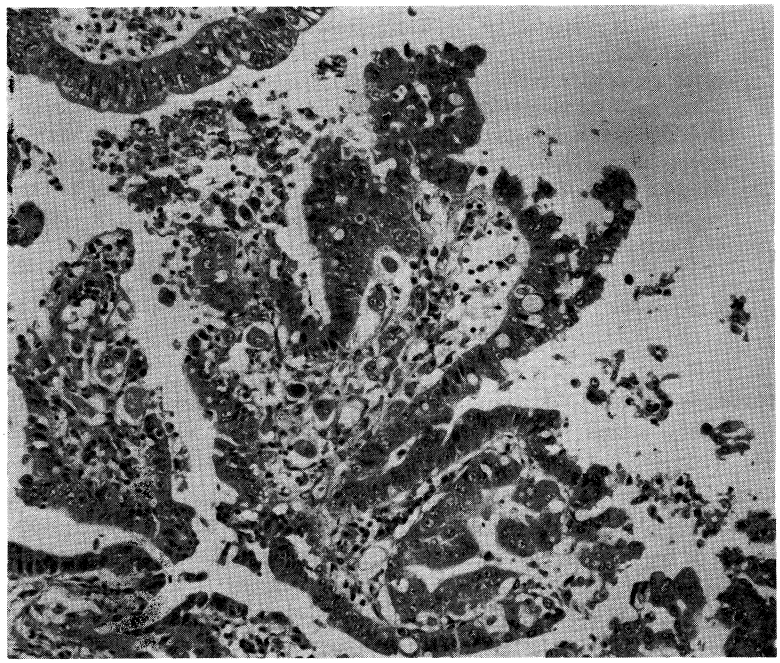

Fig. 1. Several goblet cell-type cancer cells seen in papillary adenocarcinoma (H-E stain, ×130).

extends beyond the muscle layer of the bladder wall, and they were classified as papillary adenocarcinoma in 10 cases, tubular adenocarcinoma in 11 cases, poorly differentiated adenocarcinoma in 11 cases, mucinous carcinoma in 1 case, squamous cell carcinoma in 3 cases and undifferentiated carcinoma in 4 cases. Among 21 cases of well-differentiated adenocarcinoma comprising the papillary and tubular types, cancer cells of goblet cell type were identifiable in 6 cases (28.5%) (Fig. 1), but cases of other histological type were not associated with similar goblet cells. Endocrine cells demonstrated by Grimelius stain were indentified within neoplastic tissues in 4 out of the 40 cases examined (10%); namely, 3 cases of well-differentiated adenocarcinoma and 1 case of combined undifferentiated carcinoma and carcinoid (Fig. 2). Argentaffin cells as revealed by Masson-Fontana stain were also present within neoplastic tissues in 2 out of these 4 cases. Other kinds of metaplastic changes such as mucous gland-formation or occurrence of brush-bordered cells were not evident within the neoplastic tissues.

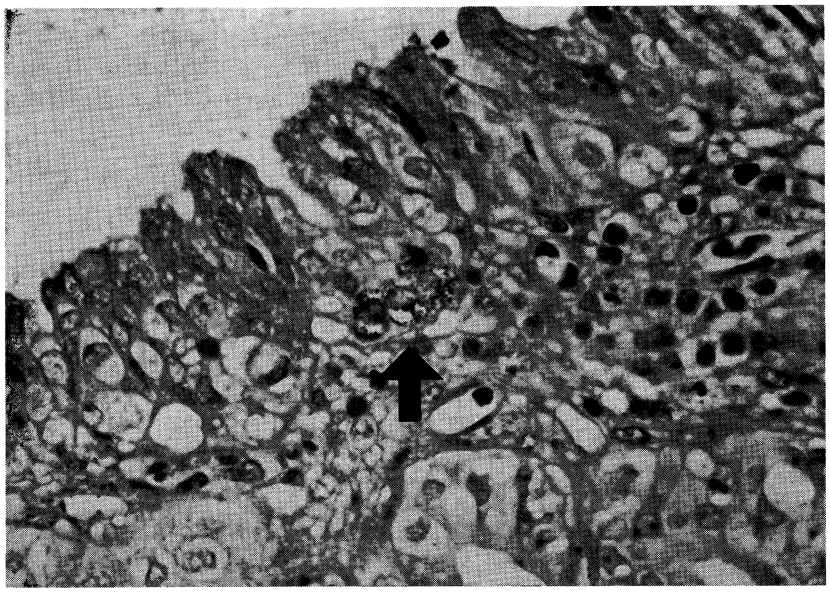

Fig. 2. A few argentaffin cells (arrow) seen in well differentiated adenocarcinoma (Masson-Fontana stain, ×650).

In 14 (70%) out of the 20 cases with non-neoplastic mucosae around the neoplasms, various types of metaplastic changes were present in those mucosal areas. The changes included mucous glands in 50%, argyrophil (endocrine) cells in 45%, goblet cells in 30%, and Paneth cells in 10%. The non-neoplastic mucosae in the cases with argyrophil cells within the neoplastic tissues were also found to include argyrophil cells as well. The overall incidence of metaplastic change in the non-neoplastic mucosae adjacent to the neoplasms, however, was found to have no significant difference in between the cases of well-differentiated adenocarcinoma (70%) and of poorly differentiated adenocarcinoma (67%) (Table 1).

Table 1. Metaplastic changes in mucosal areas adjacent to cancer foci in the gallbladder

	Presence case of metaplasia	Mucous glands	Argyrophil cells	Goblet cells	Paneth cells
Well diff. adenocarcinoma	7/10 (70%)	6 cases	5 cases	3 cases	0 case
Poorly diff. adenocarcinoma	6/ 9 (67%)	4	3	3	2
Squamous cell carcinoma	1/ 1(100%)	0	1	0	0
Total	14/20(70%)	10/20(50%)	9/20(45%)	6/20(30%)	2/20(10%)

Mucin : Various kinds of mucin were identifiable in 100% cases of well-differentiated adenocarcinoma and in 55% cases of poorly differentiated adenocarcinoma, though negative in all the cases of undifferentiated carcinoma as well as of squamous cell carcinoma. On HID-AB stain non-sulfated acid mucin appeared to predominate in the neoplastic tissues as compared with the sulfated one (Table 2). The non-neoplastic mucosae adjacent to neoplastic foci were found to contain predominantly non-sulfated acid mucin in cases of well-differentiated adenocarcinoma as in the neoplastic tissues, while the mucosae adjacent to foci of poorly differentiated adenocarcinoma did not reveal similar tendency (Table 3).

Table 2. Mucin in gallbladder cancers

	HID<AB	HID=AB	HID>AB	PAS
Well diff. adenocarcinoma	15/22 cases (68%)	6/22 cases (27%)	1/22 cases (5%)	17/22 cases (77%)
Poorly diff. adenocarcinoma	5/11 (45%)	1/11 (9%)	0/11 (0%)	5/11 (45%)
Undiff. carcinoma	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
Squamous cell carcinoma	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)

HID<AB : Cases with carcinoma tissues containing non-sulfated acid mucin more than the sulfated type.
HID=AB : Cases with carcinoma tissues containing sulfated and non-sulfated acid mucin equally.
HID>AB : Cases with carcinoma tissues containing sulfated acid mucin more than the non-sulfated type.

Table 3. Mucin in mucosal areas adjacent to cancer foci of the gallbladder

	HID<AB	HID=AB	HID>AB	PAS
Well diff. adenocarcinoma	7/10 cases (70%)	1/10 cases (10%)	2/10 cases (20%)	9/10 cases (90%)
Poorly diff. adenocarcinoma	4/9 (44%)	2/9 (22%)	3/9 (33%)	9/9 (100%)
Squamous cell carcinoma	0/1 (0%)	0/1 (0%)	1/1 (100%)	1/1 (100%)

Lysozyme : Immunoreactive lysozyme as revealed by PAP method was detected in 12 out of the 40 cases examined (30%). The positive reaction was

present in 70% of the patients with papillary adenocarcinoma, in 36% of the patients with tubular adenocarcinoma and in 9% of the patients with poorly differentiated adenocarcinoma, while absent in the patients with other types of bladder carcinoma (Table 4). The intracellular localization of the lysozyme immunoreactivity appeared to be restricted mostly to apical portions facing luminal spaces within neoplastic tissues, but on occasion it was evenly distributed throughout the cytoplasm (Fig. 3). Non-neoplastic mucosal areas adjacent to the neoplasms disclosed similar lysozyme activity in 5 out of 9 patients with the well-differentiated adenocarcinoma (56%), but they did so in only 1 of 9 patients with poorly differentiated adenocarcinoma (11%). The lysosome immunoreactivity appeared to be located mainly in metaplastic mucous gland epithelia and/or in surface lining epithelia (Table 5).

Table 4. Lysozyme within foci of gallbladder carcinomas

	Positive cases
Total	12/40 (30%)
Papillary adenocarcinoma	7/10 (70%)
Tubular adenocarcinoma	4/11 (36%)
Poorly diff. adenocarcinoma	1/11 (9%)
Undifferentiated carcinoma	0/ 4 (0%)
Squamous cell carcinoma	0/ 3 (0%)
Mucinous carcinoma	0/ 1 (0%)

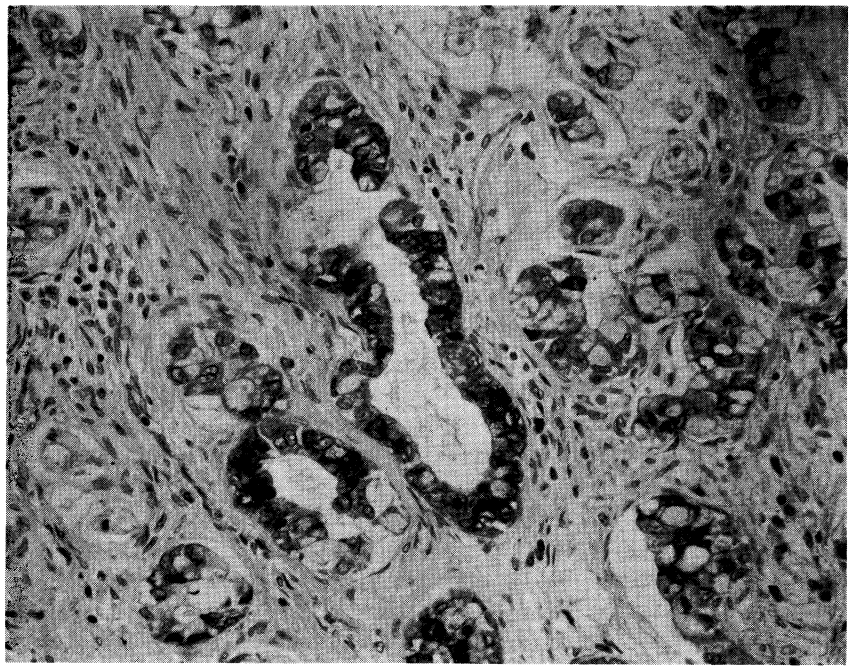

Fig. 3. Lysozyme immunoreactivity in the cytoplasm of cancer cells forming tubular adenocarcinoma (PAP method, ×260).

Table 5. Lysozyme in mucosal areas adjacent to cancer foci in the gallbladder

	Positive cases	Mucous glands	Surface epithelium	Paneth cells
Well diff. adenocarcinoma	5/9 (56%)	4	3	–
Poorly diff. adenocarcinoma	1/9 (11%)	1	1	1
Squamous cell carcinoma	1/1 (100%)	–	1	–
Total	7/19 (37%)			

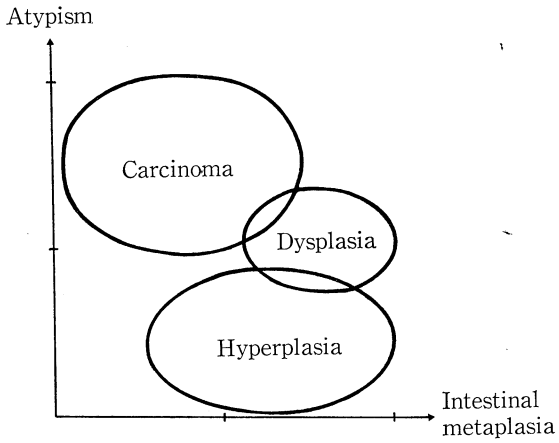

Fig. 4. Carcinoma, dysplasia and hyperplasia of the gallbladder in relation to cellular atypism and intestinal metaplasia

DISCUSSION

The histogenesis of gallbladder carcinoma has been discussed from some aspects ; namely, those of chronic stimulation by gall stones, malignant transformation from benign adenoma or mucosal dysplasia and of chronic inflammation with or without reflux of pancreatic juice.[4]

The frequency of the association of gall stones with gallbladder carcinomas ranges from 65 to 95% in English literatures while somewhat low in Japanese literatures ranging from 40 to 70%.[5] Ample evidence suggesting this association from either epidemiological[6] or experimental[7] study seems to have been accumulated, and herein gall stones would be considered to play a role for the promotion of cancers in the bladder.

Sawyer[8] described 4 cases with malignant change out of 29 papillomas in the gallbladder. Araki and Tahara[9] reported from the review of Japanese literatures that 15% of benign gallbladder tumors underwent malignant transformation. Kozuka et al.[10] considered, on the other hand, that most of the gallbladder carcinoma would arise from adenomas. Black et al.[11,12] and Albores-Saavedra[13] found a frequent occurrence of mucosal dysplasia or atypical mucosal hyperplasia among American or Mexican Indians who are both well

known to be predisposed for gallbladder cancer and they speculated that such lesions would be precancerous. In comparison with gallbladder carcinoma, however, either benign tumor including adenoma or mucosal dysplasia in the bladder appears extremely rare in occurrence. We have experienced 5 cases of mucosal dysplasia of the gallbladder, but 3 of them had been associated with intramucosal carcinoma, the other one with advanced cancer and only the rest one without associated change.[14,15] We are now speculating that mucosal dysplasia of the gallbladder would be readily transformable to carcinoma and rapidly replaced by the malignant overgrowth.

Intimate relationship between anomalous arrangement of pancreatico-biliary ducts and gallbladder carcinoma was noticed recently,[16] and association of choledochal cyst with gallbladder carcinoma was also substantiated.[17] These reports suggest that reflux of pancreatic juice into the gallbladder would result in a repeated irritation against its mucosae finally leading to the formation of cancer.

Evidence has been increasing recently that intestinal metaplasia would play an important role for the histogenesis of gastric carcinoma.[3] In the gallbladder, however, the relationship of intestinal metaplasia to neoplastic changes remains unsolved. We found that the prevalence of intestinal metaplasia in mucosal areas adjacent to the well-differentiated adenocarcinoma foci was almost similar to that in the poorly differentiated adenocarcinoma. Nevertheless, foci of the well-differentiated adenocarcinoma themselves appeared to disclose the intestinal nature more clearly than those of the poorly or undifferentiated carcinoma as suggested from the following aspects.

1. Twenty nine percent of the well-differentiated adenocarcinoma cases included goblet cell type carcinoma cells, while no such cell was identifiable in the poorly differentiated adenocarcinoma cases.
2. Mucosal areas adjacent to the well-differentiated adenocarcinoma foci demonstrated a marked increase of non-sulfated acid mucin, but no such tendency was present near the poorly differentiated adenocarcinoma foci. Since we found that non-sulfated acid mucin tended to become predominant in lining epithelia of the bladder as intestinal metaplasia progressed,[18] it may be likely that the mucin composition in mucosal areas next to the well-differentiated adenocarcinoma foci resembles that of the intestinal mucosa.
3. Ten percent of gallbladder carcinoma cases examined included endocrine cells within neoplastic tissues and all of the positive cases consisted of the well-differentiated adenocarcinoma, the surrounding mucosal areas of which also contained similar endocrine cells. Because of the absence of any endocrine cell in the normal bladder mucosa, these cells should be interpreted to have been newly formed for some reason, either metaplastic[19] or neoplastic. We reported an intimate association of metaplastic endocrine cells with mucosal hyperplasia in the gallbladder of chronic cholecystitis,[20] and Tsutsumi et al.[21] detected immunohistologically the presence of gastrin within these endocrine cells, which was known to have potential of stimulation for cell growth. For this reason it could be possible that these metaplastic endocrine cells promote mucosal hyperplasia, which would be followed by mucosal dysplasia or carcinoma.
4. Immunoreactivity of lysozyme was identified in a high percentage of the

well-differentiated adenocarcinoma foci and in metaplastic mucous glands or surface lining epithelia in the surrounding mucosal areas. However, similar immunoreactivity was rarely identifiable in the poorly differentiated adenocarcinoma foci and the surrounding mucosae. Although the lysozyme activity is absent in the normal mucosa of the gallbladder, it was reported to occur in metaplastic mucous glands or Paneth cells seen in chronic cholecystitis.[21] Therefore it may be reasonable to assume that the lysozyme activity would have been newly acquired through intestinal metaplasia. Immunoreactivity of lysozyme within neoplastic tissues was also reported in gastric[22] and jejunal[23] carcinomas.

It was previously indicated that some gallbladder carcinomas were extensively associated with intestinal metaplasia and that the metaplasia would be suggested to play as a role for precancerous change.[24–27] The present study also demonstrated that foci of the well-differentiated adenocarcinoma included various kinds of changes usually identifiable in those of intestinal metaplasia in the gallbladder, suggesting an intimate relationship between them. In addition we previously reported the presence of mucosal dysplasia of the gallbladder which possessed various kinds of the intestinal nature.[14,15] When the dysplasia would play truly as a precancerous lesion for gallbladder carcinomas, it may be reasonable to assume that carcinomas originating from the dysplasia would frequently disclose similar intestinal nature in it. Recently we are proposing "hyperplastic polyp" as a new entity in the gallbladder, which could be a new candidate for precancerous lesions in the bladder.[28] Lesions of what we call hyperplastic polyps also are associated with a considerable degree of intestinal metaplasia in them, though somewhat less extensive than in the mucosal dysplasia. The relationship of carcinoma, dysplasia and hyperplasia in the gallbladder to grades of their cellular atypism and associated intestinal metaplasia may be depicted as in Fig. 4. It is to be determined as for the stomach or other digestive organs whether or not intestinal metaplasia alone would act as a precancerous lesion for the histogenesis of gallbladder carcinomas.

REFERENCES

1) Nicholson, G.W. : Heteromorphoses (metaplasia) of the alimentary tract. J. Pathol. Bacteriol. **26** : 399–417, 1923
2) Järvi, O. and Lauren, P. : Intestinal metaplasia in the mucosa of the gallbladder and common bile duct. Ann. Med. Exp. Fenn. **45** : 213–223, 1967
3) Matsukura, N., Suzuki, K., Kawachi, T., Aoyagi, M., Sugimura, T., Kitaoka, H., Numajiri, H., Shirota, A., Itabashi, M. and Hirota, T. : Distribution of marker enzymes and mucin in intestinal metaplasia in human stomach and relation of complete and incomplete types of intestinal metaplasia to minute gastric carcinomas. J.N.C.I. **65** : 231–240, 1980
4) Jesseph, J.E., Nyhus, L.M., Fletcher, T.L. and Harkins, H.N. : Carcinoma of the gallbladder : Clinical and experimental considerations. Bull. Soc. Internat. Chir. **3** : 164–173, 1958
5) Kameda, H., Ishihara, F., Shibata, K., Tsukie, E., Takagi, I., Hihara, M. and Yamaaki, T. : Cholelithiasis and carcinoma of the gallbladder. Biliary tract & Pancreas **2** : 1621–1626, 1981 (in Japanese)
6) Hart, J., Modan, B. and Shani, M. : Cholelithiasis in the etiology of gallbladder neoplasms. Lancet **5** : 1151–1153, 1971
7) Kowalewski, K. and Todd, E.F. : Carcinoma of the gallbladder induced in hamsters by insertion of cholesterol pellets and feeding dimethylnitrosamine. Proc. Soc. Exp. Biol. Med. **136** : 483–486, 1971

8) Sawyer, K.C. : The unrecognized significance of papillomas, polyps, and adenomas of the gallbladder. Am. J. Surg. **120** : 570–578, 1970
9) Araki, O. and Tahara, E. : A case report of early cancer arised from papillary adenoma of gallbladder ; Statistical observation of benign tumors of gallbladder in Japan. Jpn. J. Cancer Clin. **21** : 220–229, 1975 (in Japanese)
10) Kozuka, S., Tsubone, M., Yasui, A. and Hachisuka, K. : Relation of adenoma to carcinoma in the gallbladder. Cancer **50** : 2226–2234, 1982
11) Black, W.C., Key, C.R., Carmany, T.B. and Herman, D. : Carcinoma of the gallbladder in a population of southwestern American Indians. Cancer **39** : 1267–1279, 1977
12) Black, W.C. : The morphogenesis of gallbladder carcinoma, Progress in surgical pathology, vol. II, ed. by Fenoglio, C.M. and Wolff, M. Masson Publishing USA Inc. 1980, pp. 207–223
13) Albores-Saavedra, J., Alcantra-Vazqurz, A., Cruz-Ortiz, H. and Herrera-Goepfert, R. : The precursor lesions of invasive gallbladder carcinoma. Hyperplasia, atypical hyperplasia and carcinoma in situ. Cancer **45** : 919-927, 1980
14) Sato, H., Ohmura, K., Mizushima, M., Ito, J. and Yoshioka, K. : Dysplasia of the gallbladder mucosa with special reference to well differentiated adenocarcinoma. Biliary tract & Pancreas **4** : 393–399, 1983 (in Japanese)
15) Sato, H., Ohmura, K., Mizushima, M., Ito, J. and Yoshioka, K. : A case of dysplasia of the gallbladder demonstrating disaccharidase activity. Pathol. Clin. Med. **1** : 631–636, 1983 (in Japanese)
16) Hanyu, F., Ohhashi, M. and Ohi, I. : Clinical aspect of biliary duct carcinoma associated with anomalous arrangement of pancreatico-biliary duct. Biliary tract & Pancreas **2** : 1637–1644, 1981 (in Japanese)
17) Kuroda, S., Wada, H. and Atomi, H. : Association of carcinoma with congenital dilatation of the bile duct. Biliary tract & Pancreas **2** : 1627–1635, 1981 (in Japanese)
18) Ohmura, K. : Disaccharidase and related intestinal metaplasia in the mucous membrane of lithiasic gallbladders. Jpn. J. Gastroenterol. **80** : 1772–1781, 1983 (in Japanese)
19) Delaquerriere, L., Tremblay, G. and Riopelle, J. : Argentaffine cells in chronic cholecystitis. Arch Pathol. **74** : 142–151, 1962
20) Sato, H., Ohmura, K., Mizushima, M., Ito, J. and Yoshioka, K. : Metaplastic endocrine cells in various diseases of the gallbladder. Jpn. J. Gastroenterol. **79** : 2106–2111, 1982 (in Japanese)
21) Tsutsumi, Y., Nagura, H., Osamura, Y., Watanabe, K. and Yanaihara, N. : Immunohistochemical studies of metaplasia in the mucosa of the gallbladder (English translation). Dig. Organ Immunol. **9** : 78–84, 1982 (in Japanese)
22) Tahara, E., Ito, H., Nakagami, K., Shimamoto, F., Yamamoto, M. and Sumi, K. : Scirrhous argyrophil cell carcinoma of the stomach with multiple production of polypeptide hormones, amine, CEA, lysozyme, and HCG. Cancer **49** : 1904–1915, 1982
23) Reitamo, S., Reitamo, J., Konttinen, T., Collan, Y. and Sipponen, P. : Lysozyme in neoplastic Paneth cells of a jejunal adenocarcinoma. Acta Patho. Microbiol. Scand. A **89** : 165–168, 1981
24) Azadeh, B. and Parai, S.K. : Argentaffin cells, intestinal metaplasia and antral metaplasia in carcinoma of the gallbladder. Histopathology. **4** : 653–659, 1980
25) Laitio, M. and Häkkinen, I. : Intestinal type carcinoma of gallbladder. A histochemical and immunologic study. Cancer **36** : 1668–1674, 1975
26) Matsumine, T., Kubota, Y., Yamaoka, I., Sasaki, H., Aoki, M. and Seto, K. : A histopathological study of the gallbladder carcinoma-with special reference to intestinal metaplasia (duodenalization) of the cholecystic mucosa. J. Jpn. Pract. Surg. Soc. **39** : 927–934, 1979 (in Japanese)
27) Hirai, S. : Clinicopathological study of metaplasia in resected gallbladder–as a background of histogenesis of gallbladder cancer. Jpn. J. Gastroenterol. Surg. **13** : 35–44, 1980 (in Japanese)
28) Sato, H., Mizushima, M., Ito, J. and Doi, K. : Hyperplastic polyp of the gallbladder : A candidate for precancerous lesions. : submitted for publication