Relationship between metaplastic changes and occurrence of endocrin cells, lysozyme and lactoferrin in gallbladder carcinoma

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The association between metaplastic changes seen in gallbladder cancer on the one hand and the appearance of endocrine cells, lysozyme and lactoferrin on the other was studied using serial sections of 50 cases of gallbladder cancer (9/50, early stage). The sections were examined histologically and immunohistochemically for the purpose of elucidating the histogenesis of gallbladder cancer. Gastrin-secreting, somatostatin-secreting and serotonin-secreting cells in cancer lesions were observed in each of 14 cases (28%), 10 cases (20%) and 15 cases (30%) and those in the non-cancerous mucosa each in 19 cases (38%), 16 cases (32%) and 19 cases (38%). Motilin-secreting cells were not observed in cancer lesions and were observed in only 2 cases (4%) in non-cancerous mucosa. The immunohistochemical activity of lysozyme and lactoferrin were observed in 29 cases (58%) and 6 cases (12%), respectively, in the cancer lesions and in 29 cases (58%) and 4 cases (8%), respectively, in the non-cancerous mucosa. No difference in the appearance rate of metaplastic cells was noticed between advanced and early gallbladder cancer. Metaplastic changes of endocrine cells and lysozyme are closely related to the development of gallbladder cancer. It is therefore possible that the intestinal metaplasia of gallbladder mucosa is an important lesion as regards carcinogenesis.

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Regarding the histogenesis of gallbladder cancer, metaplastic changes, particularly intestinal metaplasia have been considered as important background lesions^{1,2)}. To discuss carcinogenesis not only in the gallbladder but also in the intestinal tract, it is necessary to observe the behavior of the mucosal epithelium itself and to understand the diversity of cell differentiation. Therefore, the present study is a histopathological study of resected specimens of gallbladder cancer. The cancerous and non-cancerous parts of these specimens were examined histopathologically and immunohistochemically in order to clarify the relationship between gallbladder cancer and metaplastic changes.

Materials and methods

9 cases of early gallbladder cancer, in which infiltration does not go beyond the tunica muscularis propria, and 41 cases of advanced cancer, in which infiltration goes beyond the tunica muscularis propria but remains in the gallbladder wall, were studied. 16 males and 34 females

participated in the study ; the range of age was from 39 to 84 years (mean age of 66.8 years). All resected gallbladders were immediately incised on the surface of the liver bed from the neck to the fundus. After washing, the gallbladders were fixed in a 10% buffered formalin solution, and serial sections parallel to the long axis of the gallbladder were peppered and examined under a microscope. The sections were subjected to HE staining and to periodic acid-Schiff (PAS) and alcian blue double staining.

The Grimelius method was used to detect argyrophil cells and the Fontana-Masson method was chosen for the detection of argentaffin cells. Streptoavidin-biotin staining was performed in order to study gastrin, somatostatin, motilin, glucagon, CCK, lysozyme, lactoferrin, and serotonin. Non-cancerous mucosa was defined by an area not more than 1cm from the cancer margin. The frequency of metaplastic cells was classified as follows : None (-), not stained at all ; mild (+), a small number of cells were present in part of the specimen (not more than 5-20 various intestinal metaplastic cells in a 200-magnification visual field) ; moderate (++) , metaplastic cells were clustered together in a part of the specimen (not less than 21 various intestinal metaplastic cells in a 200-magnification visual field) ; severe (+++) , metaplastic cells were clustered together in various parts of specimen.

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Fig. 1 Hormone-containing cells (arrows) in cancer of the gallbladder (Original manification, $\times 200$)
G : gastrin, SOM : somatostatin, MOT : motilin, 5HT : serotonin

Results

Gastrin-, somatostatin- and serotonin-secreting cells were observed among the endocrine cells in immunologically-stained gallbladder cancer lesions. Motilin-secreting cells were observed only in the metaplastic cells of the non-cancerous mucosa. All immunologically-stained cells observed in cancer lesion some appear in pseudopyloric gland that considered poor differentiate morphologically and they were also observed in superficial epithelium and glandular epithelium (**Fig. 1**).

Among 50 cases, gastrin-secreting cells were observed in 14 cases (28%) of cancer lesions and those were observed in 19 cases (38%) of non-cancerous mucosa. Mildly positive reactions were observed in 11 cancerous cases (22%) and in 14 non-cancerous cases (28%), moderately positive reactions were observed in 3 cancerous cases (6%) and in 4 non-cancerous cases (8%). There were no incidences of a severely positive reaction among the cancerous lesions and only 1 case (2%) in the non-cancerous mucosa showed a severely positive reaction. Among 50 cases, somatostatin-secreting cells were observed in 10 cases (20%) of cancer lesions and those were observed in 16 cases (32%) of non-cancerous mucosa. Mildly positive reaction was observed in 6 cancerous cases (12%) and in 11 non-cancerous cases (22%), moderately positive reaction was observed

in 3 cancerous cases (6%) and in 4 non-cancerous cases (8%) and severely positive reaction was observed in 1 cancerous case (2%) and in 1 non-cancerous case (2%). No cells from cancer lesions stained positive for the secretion of motilin, and only 2 cases (4%) were mildly positive in non-cancerous mucosa. Among 50 cases, serotonin-secreting cells were observed in 16 cases (32%) of cancer lesions and were observed in 19 cases (38%) of non-cancerous mucosa. Mildly positive reaction was observed in 4 cancerous cases (8%) and in 10 non-cancerous cases (20%), moderately positive reaction was observed in 7 cancerous cases (14%) and in 8 non-cancerous cases (16%) and severely positive reaction was observed in 4 cancerous cases (8%) and in 1 non-cancerous case (2%) (**Table 1**).

Lysozyme observed in 29 cases with cancer lesions and in 29 cases of non-cancerous mucosa as well. Mildly positive reaction was observed in 13 cancerous cases (26%) and in 15 non-cancerous cases (30%), moderately positive reaction was observed in 4 cancerous cases (8%) and in 7 non-cancerous cases (14%) and severely positive reaction was observed in 12 cancerous cases (24%) and in 7 non-cancerous cases (14%) (**Table 2**). Among 50 cases, lactoferrin was appeared in 6 cases (12%) of cancer lesion and in 4 cases (8%) of non-cancerous mucosa (**Table 2**).

Mildly positive reaction was observed in 5 cancerous cases (10%) and in 4 non-cancerous cases (8%), moderately

Table 1 The appearance rate of endocrine cells with gallbladder cancer

	case	gastrin secreting cells				somatostatin secreting cells			
		—	+	++	+++	—	+	++	+++
cancer lesion	50	36(72%)	11(22%)	3(6%)	0(0%)	40(80%)	6(12%)	3(6%)	1(2%)
non-cancerous mucosa	50	31(62%)	14(28%)	4(8%)	1(2%)	34(68%)	11(22%)	4(8%)	1(2%)

	case	motilin secreting cells				serotonin secreting cells			
		—	+	++	+++	—	+	++	+++
cancer lesion	50	50(100%)	0(0%)	0(0%)	0(0%)	35(70%)	4(8%)	7(14%)	4(8%)
non-cancerous mucosa	50	48(96%)	2(4%)	0(0%)	0(0%)	31(62%)	10(20%)	8(16%)	1(2%)

Table 2 The appearance rate of lysozyme and lactoferrin with gallbladder cancer

	case	lysozyme			
		—	+	++	+++
cancer lesion	50	21(42%)	13(26%)	4(8%)	12(24%)
non-cancerous mucosa	50	21(42%)	15(30%)	7(14%)	7(14%)

	case	lactoferrin			
		—	+	++	+++
cancer lesion	50	44(88%)	5(10%)	1(2%)	0(0%)
non-cancerous mucosa	50	46(92%)	4(8%)	0(0%)	0(0%)

Table 3 The appearance rate of metaplastic change by the histological type

histological type	case	non-cancerous mucosa							
		GC	PGM	Paneth	Argyo	Argenta	Lz	G cell	SOM
papillary adenoCa.	18	9	14	4	14	14	8	7	7
well diff. adenoCa.	17	7	11	2	9	9	8	6	2
mod diff. adenoCa.	10	6	8	1	5	7	5	5	6
poorly diff. adenoCa.	5	1	4	0	2	2	0	1	1
total	50	23	37	7	30	32	21	19	16

histological type	case	cancer lesion							
		GC	PGM	Paneth	Argyo	Argenta	Lz	G cell	SOM
papillary adenoCa.	18	10	9	2	11	15	13	7	3
well diff. adenoCa.	17	9	5	2	8	6	9	5	6
mod diff. adenoCa.	10	7	4	1	3	3	7	2	1
poorly diff. adenoCa.	5	2	0	0	0	0	0	0	0
total	50	28	18	5	22	24	29	14	10

GC : goblet cell, PGM : pyloric glandular metaplasia, Paneth : Paneth cell, Argyo : arbyrophill cell, Argenta : argentafin cell, Lz : lysozyme, G cell : gastrin cell, SOM : somatostatin cell

positive reaction was observed in 1 cancerous case (2%) in cancer lesion and not observed in non-cancerous mucosa. A severely positive reaction was not observed in either cancer lesions or in non-cancerous mucosa. The appearance rate of metaplastic changes, when examined by histological type, was relatively high in papillary adenocarcinoma. The appearance rate of various endocrine cells tended to be higher as the degree of differentiation increased. The appearance rate of argyrophilic cells, gastrin-secreting cells and somatostatin-secreting cells was significantly higher in well differentiated types compared with the poorly differentiated types. In the non-cancerous

mucosa of parents with papillary cancer, metaplastic changes were high in number as well. In the non-cancerous mucosa, the appearance rate of endocrine cells and lysozyme was higher than that in the poorly differentiated adenocarcinomas. This is because the degree of differentiation increases with the moderately and well differentiated types (Table 3).

It is no margin of the appearance rate of each metaplastic cells between cancer lesion and non-cancerous mucosa, studied the appearance rate of metaplastic change compared with the advancement of gallbladder cancer (Table 4).

Table 4 The appearance rate of metaplastic change by the advancement of cancer

		non-cancerous mucosa							
	case	GC	PGM	Paneth	Argyo	Argenta	Lz	G cell	SOM
early cancer	9	4	6	2	6	5	4	4	3
advanced cancer	41	19	31	5	24	27	17	15	13
total	50	23	37	7	30	32	21	19	16
		cancer lesion							
	case	GC	PGM	Paneth	Argyo	Argenta	Lz	G cell	SOM
early cancer	9	5	4	1	5	4	6	3	2
advanced cancer	41	23	14	4	23	20	23	11	8
total	50	28	18	5	28	24	29	14	10

GC : goblet cell, PGM : pyloric glandular metaplasia, Paneth : Paneth cell, Argyo : arbyrophill cell, Argenta : argentaaffin cell, Lz : lysozyme, G cell : gastrin cell, SOM : somatostatin cell

Discussion

Normal gallbladder mucosa is structured uniformly and single stratum columnar epithelium is structured comparatively simple as well. It is documented that metaplastic changes appear in various gallbladder diseases³⁾. Of the metaplastic changes that appear in the mucosal epithelium of the gallbladder, intestinal metaplasia is attracting special attention, since it is said to play a major role in the histogenesis of gallbladder cancer^{4,5)}. There is no full report about the role of metaplastic change, lysozyme, and lactoferrin in gallbladder cancer. Therefore we examined the appearance rate of endocrine cells in gallbladder cancer lesions and surrounding non-cancerous mucosa. Previous documents emphasized the existence of intestinal metaplasia; there are few reports about lysozyme, lactoferrin, and endocrine cells. In fact, intestinal epithelial cells were frequently detected in non-cancerous and cancerous tissue of the gallbladder. Therefore it is possible that these cells are included in the former lesions of gallbladder cancer.

We examined paneth cells, endocrine cells, goblet cells and lysozyme in cancer lesions and non-cancerous mucosa, considering them to be intestinal metaplasia. The appearance rate of goblet cells was in good agreement with that of the other cells. Yamamoto⁶⁾ and Nakajyou et al.⁷⁾ reported that the appearance of lysozyme and endocrine cells is the most useful indicator for metaplastic changes. Tsutsumi et al.⁸⁾ reported that gastrin and motilin secreting cells were found in peripheral non-cancerous mucosa in 3 out of 5 cases of gallbladder cancer. Iseki⁹⁾ reported that somatostatin-secreting cells were found in cancer lesions in 6 cases (16%) and in the peripheral mucosa of 8 cases (24%); gastrin-secreting cells were observed in peripheral mucosa of only 6 cases (18%) among 23 cases of gallbladder cancer.

In our study, gastrin-secreting cells (28%), somatostatin-

secreting cells (20%), and serotonin-secreting cells (30%), were observed in cancer lesions. However, no motilin-secreting cells were observed. In non-cancerous mucosa, gastrin-secreting cells (38%), somatostatin-secreting cells (32%) and serotonin-secreting cells (38%) were observed. Motilin-secreting cells were observed in 4% of the cases. These endocrine cells appeared very frequently in the cancer lesions and non-cancerous mucosa of well differentiated adenocarcinomas, while in poorly differentiated adenocarcinoma, motilin-secreting cells were observed in the non-cancerous mucosa of only 2 of the cases. These findings suggest that there is a tendency for well differentiated adenocarcinoma to develop from cases with endocrine cells. Poorly differentiated adenocarcinoma seems more likely to develop from cases without endocrine cells. In short, a strong correlation between intestinal metaplasia and endocrine cells were seen. There is a possibility of carcinogenesis in metaplastic epithelium.

In a comparison of metaplastic changes in early gallbladder cancer and advanced gallbladder cancer, no difference was found. In the non-cancerous mucosa, there was no association between metaplastic changes and the degree of differentiation, different results were obtained from cancer lesions. These results suggest that the metaplastic change is not a secondary change due to the advance of the cancer. The site of appearance of lysozyme was consistent with that of Paneth cells, with the exception of those the pseudopyloric glands. These cells have a bacteriolytic effect, which plays an important role in the local immunoresponse¹⁰⁾. In this study, lysozyme was observed in 29 cases (58%) in both cancer lesions and in non-cancerous mucosa. There was a high positive reaction in 12 cancer lesions (24%) and in 7 non-cancerous mucosa (14%).

This finding suggests carcinogenesis with pseudopyloric glands as the background. Lactoferrin was found in only 6 cases (12%) in cancer lesions and in 4 cases (8%) in

non-cancerous mucosa, suggesting that there is no association between metaplastic changes and lactoferrin. It is reported that gastrin has increased action in digestive mucosa as well as in cancer cells and somatostatin controls, which secrete various hormones^{11,12)}. In the future, we encourage studies that will survey the type and functions of many digestive endocrine cells seen in cancer lesions and peripheral non-cancerous mucosa associated with gallbladder cancer.

From these results, it is surmised that the metaplastic changes of endocrine cells and lysozyme are closely related to the development of gallbladder cancer and furthermore that such lesions are related to carcinogenesis.

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