CDX2 and MUC2 Protein Expression in Extrahepatic Bile Duct Carcinoma

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Abstract

Although CDX2-mediated intestinal metaplasia and its association with gastric and esophageal carcinoma have been well described, its function in extrahepatic bile duct (EBD) carcinoma remains unclear. CDX2 and MUC2 expression were examined in 193 EBD carcinomas, and observed in 37.3% and 42.0%, respectively. Both CDX2 and MUC2 were observed in 27.4%. CDX2 (P < .001) and MUC2 (P < .001) were correlated with histologic subtypes and present, respectively, in all intestinal-type adenocarcinomas and mucinous carcinomas, 12 (71%) and 13 (76%) of 17 papillary carcinomas, 2 (40%) and 2 (40%) of 5 adenosquamous carcinomas, and 28.4% and 33.5% of adenocarcinomas, not otherwise specified. CDX2 was observed more frequently in tumors with papillary growth (P = .03) and no vascular invasion (P = .04), whereas MUC2 was more common in cases with low stage (P = .01) and no vascular invasion (P < .001). Patients with CDX2+/MUC2+ tumors had significantly better overall survival in univariate but not multivariate analysis than patients with other tumors (P < .05). Expression of CDX2 and MUC2 supports that intestinal differentiation is present in specific subtypes of EBD carcinomas, and their expression status correlates with patients’ overall survival.

Extrahepatic bile duct (EBD) carcinoma is an uncommon neoplasm with a dismal prognosis.¹² Although several molecular mechanisms, such as activation of K-ras and β-catenin oncoproteins and inactivation of p53, p16, APC, and DPC4 tumor suppressor genes³⁸ have been implicated in some EBD carcinomas, the pathogenesis of EBD carcinomas is still poorly understood.

CDX2 is a member of the caudal-related homeobox gene family and has an important role in mammalian early intestinal development and maintenance of intestinal epithelia through its regulation of intestine-specific gene transcription.⁹-¹² Normally, CDX2 is expressed in small intestinal and colonic epithelia and is not presented in gastric epithelium.⁹-¹³ However, CDX2 expression is observed in intestinal metaplasia of the esophagus and stomach and in intestinal-type gastric adenocarcinomas.¹³-¹⁹ In gastric carcinogenesis, intestinal metaplasia is a well-known component of the stepwise series of mucosal changes, especially in intestinal-type adenocarcinomas. In intestinal metaplasia also has been reported to be present in 20% of the nonmalignant mucosa adjacent to biliary dysplasia or EBD carcinomas. In the biliary tract, CDX2 and intestinal metaplasia have been observed in intraductal papillary mucinous neoplasms of the liver. In the past, CDX2 studies were limited to small numbers of EBD carcinomas and there was no study on MUC2.
expression in EBD carcinomas. In the present study, we studied CDX2 and MUC2 expression in a large number of EBD carcinomas and compared the CDX2 and MUC2 expression results with various clinicopathologic factors, including survival.

Materials and Methods

Case Selection

We searched the surgical pathology database of Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea, for cases from January 1991 to December 2000, and identified 222 consecutive, surgically resected EBD carcinomas. Of these, paraffin blocks and medical records were available for 193 cases that were included in this study. Only carcinomas with the epicenter in the EBD were examined. Carcinomas with the epicenter in the ampulla of Vater or pancreas and those with obvious precancerous epithelial changes in the ampulla of Vater or pancreas were excluded. Carcinomas arising in the gallbladder or intrahepatic bile duct with extension to the EBD also were excluded. Data obtained from reviewing medical records were patient’s age, sex, surgical procedure, survival time, and survival status.

Gross examination obtained from pathology reports included tumor location, size, and growth pattern. H&E-stained slides for all cases were evaluated, and histologic types were classified using the 6th edition of the American Joint Committee on Cancer staging manual.30 One representative slide and matched paraffin-embedded block of each tumor were selected for use in tissue microarray (TMA) construction. The designated area for each tumor block was punched with a 3-mm-diameter tissue cylinder and transferred to a recipient TMA block.

Immunohistochemical Studies and Assessment

Immunohistochemical staining was carried out on TMA sections using the avidin-biotin method and a commercially available kit (Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA). Deparaffinized sections were treated with methanol containing 3% hydrogen peroxide for 10 minutes after conducting antigen retrieval using a microwave oven at 95°C for 5 minutes. After washing with phosphate-buffered saline, blocking serum was applied for 10 minutes followed by a peroxidase-marked streptavidin for an additional 10 minutes. The reaction was visualized by using 3,3′-diaminobenzidine tetrahydrochloride. The nuclei were counterstained with hematoxylin.

Colon cancer specimens containing normal colonic tissues were used as positive control samples for MUC2 and CDX2. Negative control samples were composed of identically treated histologic sections with omission of additional primary antibodies. More than 5% of nuclear staining for CDX2 and cytoplasmic staining for MUC2 were regarded as positive.

Statistical Analysis

Statistical analyses were performed using SPSS (SPSS, Chicago, IL) and R (http://www.r-project.org). Associations between categorical variables were examined by using the Pearson χ2 and Fisher exact tests. Survival rates were calculated by the Kaplan-Meier method, and statistical significance was examined by the log-rank test and the Cox proportional hazards regression model. A P value of less than .05 was considered statistically significant.

Results

Clinicopathologic Characteristics of Cases

The ages of the patients ranged from 30 to 84 years (mean, 60.4 years; median, 62.0 years). Of the patients, 136 were men and 57 were women. The tumors showed an infiltrative growth pattern in 152 cases, a papillary pattern in 25, and a nodular pattern in 16. Tumor sizes ranged from 0.4 to 6 cm (mean, 2.5 cm). Of the 193 cases, 39 were T1 tumors, 61 were T2, 83 were T3, and 10 were T4. Hepatic and duodenal invasion were present in 11 and 10 cases, respectively, and invasion into the pancreas was observed in 82 cases. Perineural and vascular invasion were present in 131 and 43 cancers, respectively. Lymph node metastasis occurred in 63. The histologic type of EBD carcinomas included the following: adenocarcinoma, not otherwise specified, 155; papillary carcinoma, 17; intestinal-type adenocarcinoma, 8; mucinous carcinoma, 5; adenosquamous carcinoma, 5; and clear cell carcinoma, signet-ring cell carcinoma, and sarcomatoid carcinoma, 1 each.

Types of surgery included pylorus-preserving pancreaticoduodenectomy (n = 70), standard pancreaticoduodenectomy (Whipple operation, n = 55), bile duct resection (n = 33), hepatic lobectomy with bile duct resection (n = 28), pancreaticoduodenectomy with extended hepatic lobectomy (n = 4), pylorus-preserving pancreaticoduodenectomy with bile duct resection (n = 2), and standard pancreaticoduodenectomy (Whipple operation) with bile duct resection (n = 1). The length of follow-up ranged from 1 to 128 months (mean, 35.8 months; median, 31.0 months).

CDX2 and MUC2 Protein Expression in Biliary Dysplasia

Eleven cases of biliary dysplasia adjacent to CDX2+ and MUC2+ EBD carcinomas were examined using conventional sections. Of these cases, 7 were positive for CDX2 and 6 for
MUC2. Three were positive for both CDX2 and MUC2. CDX2+ dysplasia tended to show diffuse and patchy staining, whereas the staining for MUC2 was focal or limited to single cells Image II.

CDX2 Protein Expression in EBD Carcinomas

CDX2 was observed in 72 (37.3%) of 193 tumors Image II. A comparison between CDX2 expression and clinicopathologic variables is summarized in Table 1. CDX2 was observed more frequently in cases with a papillary growth pattern (15/25 [60%]) than in those with an infiltrative (53/152 [34.9%]) or a nodular (4/16 [25%]) pattern (P = .03; χ² test). CDX2 was present in 100% of the intestinal-type adenocarcinomas (n = 8) and mucinous carcinomas (n = 5), 71% of papillary carcinomas (12/17), 40% of adenosquamous carcinomas (2/5), and 28.4% of adenocarcinomas, not otherwise specified (44/155; P < .001). CDX2 was observed in 1 signet-ring cell carcinoma, whereas 1 each clear cell and sarcomatoid carcinoma lacked CDX2. CDX2 was seen in 41.3% of cancers without vascular invasion (62/150) and in 23% of those with vascular invasion (10/43; P = .04). There was no statistically significant correlation between the CDX2 and other clinicopathologic factors.

MUC2 Protein Expression in EBD Carcinomas

MUC2 was observed in 81 (42.0%) of 193 tumors (Image 1). A comparison between MUC2 expression and clinicopathologic variables is summarized in Table 1. MUC2 was observed in 100% of intestinal-type adenocarcinomas (n = 8) and mucinous carcinomas (n = 5), 76% of papillary carcinomas (13/17),...
40% of adenosquamous carcinomas (2/5), and 33.5% of adenocarcinomas, not otherwise specified (52/155; \( P < .001; \chi^2 \) test). MUC2 was present in 1 signet-ring cell carcinoma, whereas each clear cell and sarcomatoid carcinoma were not stained for MUC2. MUC2 was present in 48.7% without vascular invasion (73/150) and in 19% of those with vascular invasion (8/43; \( P < .001 \)). It was present in 66% of stage IA tumors (21/32), 40% of stage IB (17/42), 43% of stage IIA (22/51), 35% of stage IIB (20/57), and 10% in stage III (1/10; \( P = .01 \)). There was no statistically significant correlation between the status of MUC2 expression and other clinicopathologic factors.

**Correlation Between CDX2 and MUC2 in EBD Carcinomas**

Of 193 EBD carcinomas, 53 (27.5%) showed both CDX2 and MUC2 expression, whereas 93 (48.1%) were negative for both markers. The correlation in the status of CDX2 and MUC2 expression is summarized in Table 2. MUC2 was observed in 53 (74%) of 72 EBD carcinomas with CDX2 expression, whereas it was observed in 28 (23.1%) of 121 without CDX2. There was a significant correlation between the status of CDX2 and MUC2 proteins (\( P < .001; \chi^2 \) test).

**Patient Survival Based on CDX2 and MUC2 Expression**

For patients whose tumors were CDX2+, 1-, 3-, and 5-year survival rates were 81.7%, 57.4%, and 46.8%, respectively, and were 78.7%, 42.6%, and 28.2%, respectively, for patients whose EBD carcinomas lacked CDX2. The median duration of survival for patients whose tumors were CDX2+ was 42 months, whereas that for patients with CDX2– EBD carcinomas was 29 months. Although the \( P \) value was near the cutoff value, there was no statistical significance for the survival based on only CDX2 protein expression.

The respective 1-, 3-, and 5-year survival rates were 86.1%, 55.3%, and 43.4% for patients whose tumors were MUC2+ and 75.2%, 43.0%, and 28.7% for patients with MUC2– EBD carcinomas. The median duration of
survival for patients with MUC2+ tumors was 42 months, and that for patients with MUC2– tumors was 29 months. There also was no statistical significance for the duration of survival based on MUC2 protein expression.

Statistical significance was not found for CDX2 and MUC2 protein expression in the 193 cases, although their P values were small. When the median survival time was compared for combined CDX2 and MUC2 status, the median survival time for patients with carcinomas positive for both CDX2 and MUC2 (63 months) was significantly better than for those with carcinomas that were negative for both CDX2 and MUC2, negative for CDX2 and positive for MUC2, and positive for CDX2 and negative for MUC2. Figure 3.

Univariate Analysis of Other Clinicopathologic Factors

The median survival times for patients with papillary and infiltrative EBD carcinomas were 53 and 28 months, respectively. The median survival time for patients with a nodular...
pattern was not obtained because more than half of patients (9 of 16 cases) were alive after 5 years of follow-up. When the tumor was confined to the bile duct, the median survival time also was not obtained. When EBD carcinomas invaded beyond the bile duct and into the liver or the pancreas, the median survival times were 29 months and 25 months, respectively ($P < .001$; log-rank test). The median survival times for other clinicopathologic factors are summarized in Table 3.

Other factors that correlated with survival were patients’ age ($P = .02$), tumor growth pattern ($P = .006$), depth of invasion ($P < .001$), lymph node metastasis ($P < .001$), and pancreas ($P = .03$), duodenal ($P = .004$), and vascular ($P < .002$) invasion. The median duration of survival for patients whose tumors were positive at the surgical resection margin was 20 months, and that for patients with negative margins was 39 months. The median survival times for patients with and without perineural invasion were 29 and 51 months, respectively. Sex, tumor size, hepatic invasion, and type of surgery were not statistically significant factors.

**Multivariate Analysis of Prognosis**

The prognostic significance based on combined CDX2 and MUC2 expression, along with other prognostically significant clinicopathologic variables, was analyzed further by using the Cox proportional hazards model. Multivariate analysis revealed that combined CDX2 and MUC2 expression was not a significant prognostic factor among the other variables. Age of patients, depth of invasion, and lymph node metastasis were independent prognostic factors.

**Figure 1** Survival according to CDX2 staining. The median survival times for patients whose tumors were CDX2– and CDX2+ were 29 months and 42 months, respectively ($P = .054$; log-rank test).

**Figure 2** Survival according to MUC2 staining. The median survival times for patients whose tumors were MUC2– and MUC2+ were 29 months and 42 months, respectively ($P = .087$; log-rank test).

**Figure 3** Survival based on expression of CDX2 and/or MUC2 by tumors. There was a significant survival difference for patients with CDX2+/MUC2+ tumors (median survival, 63 months) vs CDX2–/MUC2– tumors (median survival, 32 months; $P = .022$; log-rank test); CDX2+/MUC2+ vs CDX2–/MUC2+ tumors (median survival, 23 months; $P = .026$); and CDX2+/MUC2+ vs CDX2+/MUC2– tumors (median survival, 20 months; $P = .033$). However, there was no survival difference for patients with CDX2–/MUC2+ vs CDX2+/MUC2– tumors, CDX2+/MUC2– vs CDX2–/MUC2– tumors, and CDX2+/MUC2+ vs CDX2–/MUC2– tumors.
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Discussion

Although only a few studies of CDX2 expression in EBD carcinomas have been performed, there has been inconsistency about the frequency of CDX2 expression, which has ranged from 9% to 48%.\textsuperscript{13,18,19} These studies examined a limited number of cases; therefore, we performed a large-scale study of EBD carcinomas to more accurately evaluate the frequency of CDX2 expression. We observed CDX2 in 37.3% of EBD carcinomas, which is similar to the findings in smaller studies by Moskaluk et al\textsuperscript{13} (11/23 cases [48%]) and Werling et al\textsuperscript{19} (4/16 cases [25%]).

CDX2 is not present in normal biliary epithelial cells\textsuperscript{13,29}; however, CDX2 was observed in scattered cells of biliary dysplasia. CDX2 was highest in invasive carcinomas with intestinal differentiation, both intestinal-type adenocarcinomas and mucinous carcinomas, and it also was observed in 71% of papillary carcinomas (12/17), 40% of adenosquamous carcinomas.
(2/5), and 28.3% of adenocarcinomas, not otherwise specified (44/155). Intestinal metaplasia of the stomach often precedes gastric carcinomas. A recent study of a CDX2 transgenic mouse model demonstrated that long-term intestinal metaplasia led to gastric adenocarcinoma. In the EBD, intestinal metaplasia presents within the nonmalignant mucosa adjacent to dysplasia or EBD carcinoma. In the present study, CDX2-mediated intestinal metaplasia was observed not only within invasive carcinomas but also around the dysplastic biliary epithelium, which suggests that CDX2-mediated intestinal metaplasia might be a component of the intestinal pathway sequence in biliary carcinogenesis.

Only a few studies have reported MUC2 expression in cholangiocarcinomas, and MUC2 was not observed in conventional intrahepatic cholangiocarcinoma. In our study, however, MUC2 was observed in 42.0% of EBD carcinomas. This discrepancy might be due to the limited number of previous studies or the absence of intestinal metaplasia in intrahepatic cholangiocarcinoma. As with CDX2, MUC2 positivity was highest in carcinomas with intestinal differentiation. It was observed in 100% of intestinal-type adenocarcinomas (8/8) and mucinous carcinomas (5/5). MUC2 also was observed in 76% of papillary carcinomas (13/17), 40% of adenosquamous carcinomas (2/5), and 33.5% of adenocarcinomas, not otherwise specified (52/155).

The presence of CDX2 was correlated with the findings for MUC2 in EBD carcinomas. Both CDX2 and MUC2 were observed in 27.5% of all EBD carcinomas. MUC2 was observed in 74% of cases with CDX2 (53/72) and in 23.1% of cases without CDX2 expression (28/121). These results were compatible with the observations of a previous study of mucinous intrahepatic cholangiocarcinoma. Recently, it was shown that CDX2 regulates MUC2 expression by binding to the promoter region of MUC2. A study on MUC2-deficient mice demonstrated that invasive carcinomas arose from adenomas in small intestine. Although tumors with either CDX2 or MUC2 positivity were not associated with significantly better survival ($P = .054$ and $P = .087$, respectively), the tumors positive for both CDX2 and MUC2 were associated with significantly better survival than tumors that were CDX2−/MUC2−, CDX2−/MUC2+, or CDX2+/MUC2−. Because the tumors with positivity for only 1 marker showed borderline $P$ values of .054 and .087, which are near the predetermined significance level of .05, further study is needed with more cases for more concrete conclusions about survival differences according to CDX2 and MUC2 expression. In a few previous reports on intrahepatic cholangiocarcinoma, patients whose tumors were MUC2+ had a more favorable outcome.

CDX2 and MUC2 immunostaining results were examined in 193 cases of EBD carcinoma using TMA sections. CDX2 and MUC2 were observed in 37.3% and 42.0% of EBD carcinomas, respectively. Both CDX2 and MUC2 were observed more commonly in tumors with intestinal differentiation and also were observed in areas of biliary dysplasia adjacent to carcinomas. There was a significant correlation between CDX2 and MUC2 expression. Although patients with either CDX2+ or MUC2+ tumors did not have significantly better survival, patients whose EBD carcinomas were both CDX2+ and MUC2+ had better survival than those whose tumors lacked CDX2 and MUC2. For more concrete conclusions about CDX2 or MUC2 expression and survival, further studies with more cases are needed.

References


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