Double Cancer of Gall Bladder and Bile Duct not Associated with Anomalous Junction of the Pancreaticobiliary Duct System

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INTRODUCTION

Simultaneous double cancers in the biliary system are rare. Most are thought to be associated with pancreaticobiliary maljunction (PBM) owing to the action of the same carcinogen on the mucosa of the entire extrahepatic biliary system (1,2). With regard to biliary cancer cases without PBM, the presence of simultaneous double tumours poses the question of whether differentiation between independent primary cancers has occurred or different cancer foci have metastasised from a single tumour. From a clinical viewpoint, differentiation between these events is important since these two mechanistic origins imply different stages of disease, as well as different subsequent treatments and prognoses.

In order to track the origin of multiple cancers, altered steady state levels of p53 polypeptide or the presence or absence of mutations in K-ras can function as objective diagnostic adjuncts (3–8), since these abnormalities are due to genetic changes found frequently in a wide variety of malignancies, including biliary tract carcinomas (9,10). The patterns of immunohistochemical staining for carcinoembryonic antigen (CEA) and carbohydrate antigen (CA) 19-9 can also be used to characterize biliary tract tumours (11–13).

We have treated four cases of simultaneous double cancers of the biliary tract without PBM. In this study, we compared several characteristics of these tumours, including immunohistochemical evaluation of CEA, CA 19-9 and p53 over-expression, as well as identification of mutations in K-ras. The results reported below suggest that most double cancers of the biliary tract have multicentric development, even in the absence of PBM.

KEY WORDS: double cancer – gall bladder – bile duct – K-ras – p53

PATIENTS AND METHODS

PATIENTS

Of the 52 gall bladder and 56 bile duct cancer cases that were operated on at our hospital between 1980 and 2005, four cases of simultaneous double cancers of the biliary tract were identified. Histological diagnoses of these cases were based upon mapping examination of each entire biliary tract. Among these 108 biliary cancers, there were 12 cases with PBM; however, none of the four cases with simultaneous double cancers displayed anomalous junction of pancreaticobiliary duct system. The presence of PBM was confirmed by endoscopic retrograde cholangiopancreatography (14). Assessments of clinicopathological factors and staging were...
in accordance with the General Rules for Surgical and Pathological Studies on Cancer of Biliary Tract (15).

IMMUNOHISTOCHEMISTRY

Tissue sections from paraffin-embedded tissue were de-paraffinized and incubated individually with anti-CEA antibody (Nichirei, Tokyo, Japan), anti-CA 19-9 antibody (Japan Turner, Tokyo, Japan) or anti-p53 antibody CM1 (1:2000, Novocastra Laboratory, Newcastle, England). Pre-treatment before staining was performed in double-distilled water by heating the immersed slides in a microwave oven for 10 min. Immunohistochemical staining was performed using labelled streptavidin–biotin complexes, as described previously (16). Immunohistochemical evaluation was based on both the distribution (percentage of positive cells) and the intensity of staining, as described previously (17). The patterns of CEA and CA 19-9 immunostaining were classified into three types (apical, cytoplasmic and stromal type) in accordance with previous reports (18,19). p53 protein expression was evaluated by the intensity and distribution of immunostaining, as reported previously by the authors (16). In the present study, p53 staining was confined to the nuclei of cancer cells, but not to those of dysplastic lesions.

DNA EXTRACTION AND ANALYSIS OF K-ras MUTATION

DNA was extracted from paraffin-embedded tissue by microdissection, and DNA from dissected tissues was extracted using the DNA isolator PS kit (Wako Pure Chemical, Osaka, Japan) according to the manufacturer’s protocol. A two-step polymerase chain reaction (PCR)/restriction enzyme-based method was used to identify mutations in K-ras at codon 12. Sequences for respective sets of mismatched primers targeting codon 12 were described previously (20). For codon 12, the presence of either wild-type sequence was distinguished by fragments of 100 or 129 bp, respectively, by digestion to completion with restriction enzyme Mva I (Takara, Kyoto, Japan). DNA encoding a mutation at codon 12 of K-ras was isolated from SW 480 colon cancer cells for use as a positive control, as described previously (20).

RESULTS

CLINICOPATHOLOGICAL FINDINGS AND FOLLOW-UP

Four cases of simultaneous double cancer of the biliary tract were identified among 108 patients with biliary tract cancers (3.7%). The clinicopathological findings from these four cases are summarized in Table 1. All cases were preoperatively diagnosed as bile duct carcinomas, and no gall bladder carcinoma was detected in any case before surgery. Biliary drainage was performed for Cases 1 and 3. Pancreatoduodenectomy with regional lymphadenectomy was performed in three cases (Cases 1–3), whereas bile duct resection and cholecystectomy was performed in the fourth (Case 4). By mapping the entire biliary tract, the tumours were separated completely (Fig. 1). There were intramucosal lesions both in the gall bladder and bile duct tumours in all four cases. Dysplastic lesions around cancer lesions were seen in the gall bladder of two cases (Cases 2 and 4), but among the four cases, no hyperplastic or metaplastic lesions were seen. Although three cases (Cases 2–4) were classified with similar histological subtypes (pap and tub1), Case 1 was the only case to display different histological subtypes (tub1 and por) in double biliary cancers and to have regional lymph node metastases. Histology of these lymph node metastases identified the cells as well-differentiated tubular adenocarcinoma. The patient defined as Case 1 has survived >5 years after resection, whereas patients described as Cases 2, 3 and 4 died of recurrence within 3 years after surgery.

Serum levels of CEA and CA19-9 are shown in Table 2. Values for Case 1 and 3 were obtained after biliary drainage. A mild elevation of CEA or CA 19-9 was seen in Cases 1, 2 and 3.

IMMUNOHISTOCHEMICAL AND GENETIC FINDINGS

A representative figure depicting immunohistochemical examination of CEA, CA19-9 and p53 is shown in Fig. 2.

Table 1. Clinicopathological features of four cases of double cancers of the biliary tract

<table>
<thead>
<tr>
<th>Case</th>
<th>Year</th>
<th>Age</th>
<th>Sex</th>
<th>Tumour site</th>
<th>Tumour size (mm)</th>
<th>Gross feature</th>
<th>Histological type, depth</th>
<th>ly</th>
<th>v</th>
<th>n</th>
<th>Stage</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1987</td>
<td>77</td>
<td>F</td>
<td>BD, middle</td>
<td>15 × 12</td>
<td>Flat</td>
<td>tub1, ss</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>III</td>
<td>Alive, 77 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GB, fundus-body</td>
<td>35 × 20</td>
<td>Nodular</td>
<td>tub3, ss</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1990</td>
<td>70</td>
<td>M</td>
<td>BD, middle-upper</td>
<td>75 × 19</td>
<td>Papilllary</td>
<td>pap, fm</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>I</td>
<td>Dead, 34 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GB, body-neck</td>
<td>20 × 10</td>
<td>Flat</td>
<td>tub1, m</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1992</td>
<td>62</td>
<td>F</td>
<td>BD, middle</td>
<td>25 × 15</td>
<td>Nodular</td>
<td>tub1, se</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>III</td>
<td>Dead, 18 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GB, fundus-body</td>
<td>50 × 50</td>
<td>Flat</td>
<td>tub1, ss</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2000</td>
<td>62</td>
<td>F</td>
<td>BD, lower-upper</td>
<td>70 × 20</td>
<td>Papillary</td>
<td>tub1, ss</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>II</td>
<td>Dead, 32 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GB, fundus-body</td>
<td>10 × 10</td>
<td>Papillary</td>
<td>pap-tub1, mp</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BD, bile duct; GB, gall bladder; tub1, well-differentiated tubular adenocarcinoma; tub3, poorly differentiated tubular adenocarcinoma; pap, papillary adenocarcinoma; m, mucosal layer; mp, proper muscle layer; ss, subserosal layer; se, serosa exposed invasion; fm, fibromuscular layer; ly, lymphatic invasion; v, venous invasion; n, lymph node metastasis; *positive for #8, #12b1 and #12c; mo, months.
The identity of a K-ras mutation is shown in Fig. 3. Immuno-
histochemical and genetic findings are summarized in
Table 3. The tumours from Case 1 displayed two different
CEA patterns of immunostaining, but the same CA 19-9
pattern. In contrast, patterns of CEA and CA 19-9 immuno-
staining were distinctly different between tumour pairs
resected from Case 2, 3 or 4. Within each case, the two tumours
examined from Cases 1 and 2 showed the same pattern of p53
or K-ras, relative to each other, and the two tumours isolated
from Cases 3 and 4 showed a different pattern of p53 or K-ras,
relative to each other.

DISCUSSION

Of the biliary cancer cases in this study, 3.7% (a relatively high
frequency) involved simultaneous double cancers of the biliary
tract. On the basis of the histological type, protein staining
and gene abnormalities, the histological types of the tumours were different (tub1 and por). In contrast, double
tumours from Case 2, 3 or 4 were similar histologically, but
immunohistochemical staining patterns and K-ras abnormali-
ties were different.

There are two competing hypotheses to explain the
pathogenesis of double cancers of the biliary tract: independent
primary lesions (multicentric) or metastasis of the original
cancer. Surgeons and pathologists have established several
criteria to differentiate between primary tumours and metas-
tases according to macroscopic appearance and histological
characteristics (21,22). Many double simultaneous cancers
have been detected clinically in cases with anomalous pan-
creatobiliary ductal unions (1,2). Fujii et al. (23) reported that
62.5% of synchronous double cancers of biliary tract and 100%
of metachronous double cancers of biliary tract were asso-
ciated with PBM. Biliary cancer cases with PBM are thought
to develop multicentrically, in part owing to the influence of
pancreatic juice reflux on the mucosa of the entire biliary tract
(24). Furthermore, Fahim et al. (25) reported that intraductal
spread occurred in only ~4% of biliary tract carcinomas. Both
of these findings support the hypothesis that double carcino-
mas of the biliary tract tend to derive from different primary
lesions. In reality, however, determining whether double
cancers are metastases or independent tumours can prove
difficult. Immunohistochemical and genetic results from the
four cases of double biliary tract carcinomas presented here
suggest that, even in the absence of PBM, multicentric
neoplastic development is common in the biliary tract double
cancers than previously thought.

Genetic information from multiple neoplastic lesions pro-
vides additional criteria for differentiating between diagnoses
of metastasis or independent primary neoplasms (3–8). Using
 genetic markers, multifocal polyclonal processes have been
identified in lung (3,4) and head and neck cancers (8) (dem-
 onstrating so-called ‘field cancerization’), whereas analyses of
p53 and c-erbB-2 expression in urothelial cancers revealed that
multifocal carcinogenesis in the urothelium is generally due
to seeding or intraepithelial metastatic spread of the original
cancer cells (6,7).

With regard to double biliary cancers, only one study reports
the use of genetic assays as an adjunct of differential diagnosis
and suggests its importance for analysing LOH in bile duct
double cancers (26). Although CEA or CA 19-9 staining
patterns were informative in distinguishing double cancers,
ras or p53 are more likely to be important for differentiating
biliary tract double cancers, since CEA staining patterns have
been reported to shift from apical to cytoplasmic types, and
heterogeneous patterns of antigen localization may exist in one
tumour (19).

Histologically, biliary tract cancers that occur in cases with
PBM frequently show hyperplasia (27); nonetheless, our
cases showed no such lesions. In addition, K-ras mutation
and p53 abnormalities are more frequently detected in
cancers with PBM than in those without PBM (28,29). Although
some tumours in these four cases showed K-ras

Figure 1. Diagram of the biliary tract in Cases 1–4. Bars show the positions of
cancer lesions in each case.

Table 2. Serum tumour markers of double cancers of the biliary tract

<table>
<thead>
<tr>
<th>Case</th>
<th>CEA (ng/ml)*</th>
<th>CA19-9 (U/ml)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.7</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>6.0</td>
<td>65</td>
</tr>
<tr>
<td>3</td>
<td>2.1</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>4.6</td>
<td>23</td>
</tr>
</tbody>
</table>

*normal range: <5.0, **normal range: <37.
or p53 abnormalities, further studies with more cases will be necessary to identify the importance of genetic information in double cancers of the biliary tract without PBM.

In conclusion, in conjunction with existing pathological criteria, immunohistochemical and genetic analyses can provide valuable data for differentiating multiple cancers of the biliary tract. In the future, assays for mutations in other genes would increase the probability of identifying accurately the origins of multiple lesions of the biliary tract.

References
Double cancers of the biliary tract


