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【 CASE REPORT 】

Synchronous Double Bile Duct Cancers with Distinct Genetic Features

Shinichi Morita[1], Takeshi Suda[1], Yoji Kishi[2,3], Toshimitsu Iwasaki[2,3], Nobuyoshi Hiraoka[4], Itsuo Nagayama[1], Takahiro Hoshi[1], Satoshi Abe[1], Kazuyoshi Yagi[1], Go Hasegawa[5], Toshihiko Ikarashi[6] and Shuji Terai[7]

Abstract:
A 69-year-old man was referred to our hospital because of appetite loss. Imaging showed a nodular tumor in the perihilar bile duct and a second flat lesion in the distal bile duct. Right hepatopancreaticoduodenectomy was performed, and the histopathological findings demonstrated that the perihilar and distal lesions were moderately and poorly differentiated adenocarcinoma, respectively, and anatomically separated. Furthermore, the resected specimens showed no pancreaticobiliary maljunction. Histological and *TP53* gene analyses in a rare case of synchronous double bile duct cancers suggest that there are various genetic pathways through which bile duct cancer develops, highlighting the complexity of its pathogenesis.

Key words: bile duct cancer, synchronous double cancer, *TP53*, polymerase chain reaction-single-strand conformation polymorphism

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Introduction

Multistep carcinogenesis has been proposed to underlie the development of tumors of various origins, including bile duct cancer (1-6). According to this theory, an overt cancer develops from a precancerous lesion of intraepithelial neoplasia (7, 8), accumulating genetic abnormalities through the transformation steps. Although the *TP53* and *KRAS* genes are known to play critical roles in the transformation process of pancreatic cancer (9, 10), a major player has not yet been identified for bile duct cancer (5, 11-14).

We herein report a rare case of synchronous double bile duct cancers, in the perihilar and distal bile ducts, without known risk factors associated, such as pancreaticobiliary maljunction (15, 16). Concomitant histological and genetic analyses of the two lesions suggested that precancerous lesions in the bile duct develop into cancer through various genetic pathways.

Case Report

A 69-year-old Japanese man presented to our hospital with loss of appetite and dark urine. He had a faint icterus of the conjunctiva without fever. The patient reported no history of other disease or history of exposure of organic solvent. On examination, no pain and tenderness in the abdomen were observed. A laboratory panel showed elevations of the following hepatobiliary enzymes: aspartate aminotransferase, 185 IU/L; alanine aminotransferase, 394 IU/L; alkaline phosphatase, 1,319 IU/L, and γ-glutamyl transferase, 1,359 IU/L. Serum total bilirubin level was elevated to 1.9 mg/dL. The tumor marker carbohydrate antigen 19-9 was also elevated to 54.8 U/L. The patient tested nega-

[1]Department of Gastroenterology and Hepatology, Uonuma Institute of Community Medicine Niigata University Hospital, Japan, [2]Division of Hepatobiliary and Pancreatic Surgery, National Cancer Center Hospital, Japan, [3]Department of Surgery, National Defense Medical College, Japan, [4]Division of Pathology and Clinical Laboratories, National Cancer Center Hospital, Japan, [5]Department of Pathology, Uonuma institute of Community Medicine Niigata University Hospital, Japan, [6]Department of Pathology, Nagaoka Central General Hospital, Japan and [7]Division of Gastroenterology and Hepatology, Graduate School of Medical and Dental Sciences, Niigata University, Japan
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Correspondence to Dr. Shinichi Morita, m0riz0u@extra.ocn.ne.jp

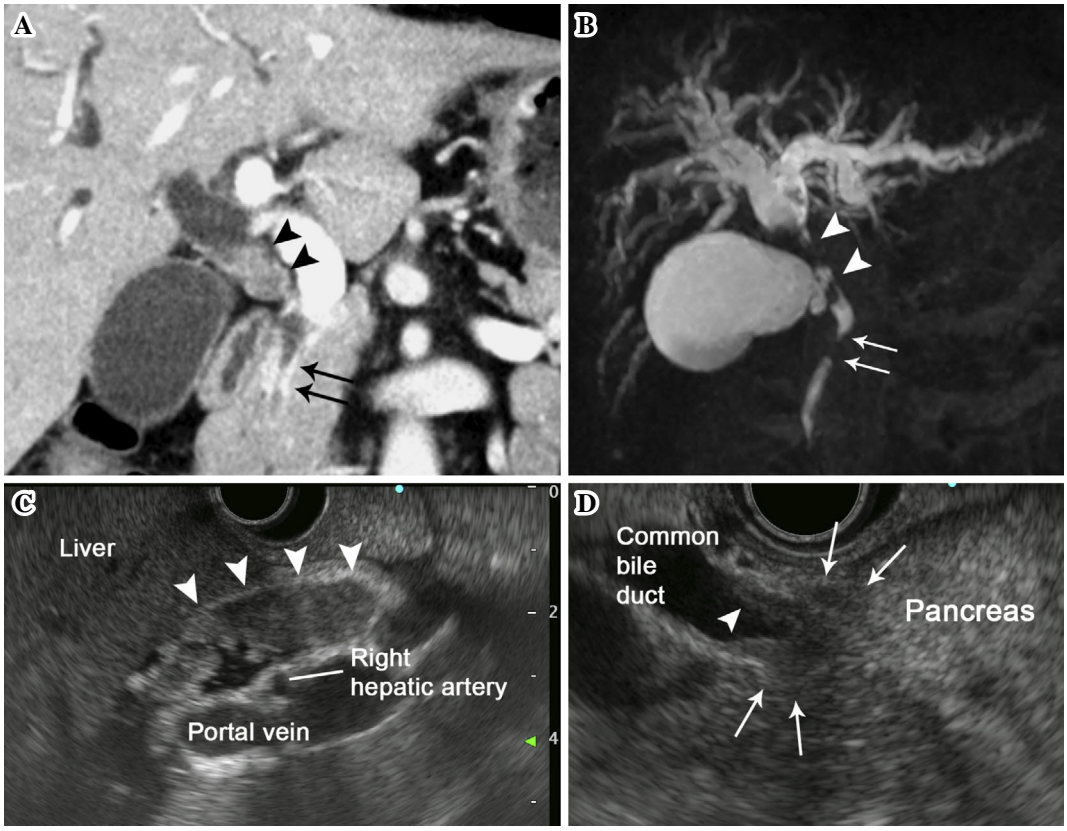

Figure 1. Imaging findings of the patient. A: Two lesions clearly visible along the bile duct in the portal phase of a dynamic computed tomography: a less-enhanced nodular lesion occupying the entire rumen at the perihilar region (arrowheads) and highly enhanced wall thickening at the distal part penetrating the pancreas (arrows). B: Magnetic resonance cholangiopancreatography showing two bile duct strictures at the distal (arrows) and perihilar (arrowheads) regions, which extend over the connection with the cystic duct and lead to both intrahepatic bile ducts and gallbladder dilatation. C and D: Endoscopic ultrasonography showing a hypo-echoic nodular tumor (arrowheads) that fills the bile duct at the vicinity of the liver hilum (C) and hypo-echoic wall thickening (arrowhead) that extends into the pancreatic parenchyma (arrowheads) at the distal bile duct (D).

tive for hepatitis B surface antigen and anti-hepatitis C virus antibody.

Subsequent contrast-enhanced computed tomography (CT) revealed a nodular tumor in the perihilar bile duct. In addition, the distal bile duct showed irregular wall thickening with arterial enhancement. The two lesions were macroscopically separated (Fig. 1A). The intrahepatic bile ducts were markedly dilated. On magnetic resonance imaging, both of the lesions exhibited similar signal intensities, with a low signal intensity on T1-weighted images and high signal intensity on T2- and diffusion-weighted images. Magnetic resonance cholangiopancreatography showed biliary strictures in the perihilar and distal bile ducts, consistent with the CT findings (Fig. 1B). These findings were further confirmed by an endoscopic ultrasonography that showed a nodular tumor in the perihilar region (Fig. 1C) and irregular wall thickening in the distal region infiltrating into the pancreatic parenchyma (Fig. 1D). Specimen biopsies revealed adenocarcinomas in each of the lesions. A biopsy that was taken from the tissues between two lesions revealed normal mucosa. After successful drainage through a transpapillary stent to improve the jaundice, a right hepatopancreaticoduodenectomy was performed following percutaneous transhepatic portal vein embolization.

The resected specimens showed no pancreaticobiliary maljunction. A perihilar tumor irregularly protruded into the lumen of the bile duct and scarcely invaded the stroma, while a flat tumor showed marked stromal invasion at the distal part of the bile duct, penetrating the pancreas (Fig. 2A, Supplementary material 1). These two tumors were separated from each other by 5 mm.

The perihilar tumor was composed of a moderately differentiated adenocarcinoma showing papillary proliferation and tubular formation (Fig. 2B), whereas the distal tumor was a poorly differentiated adenocarcinoma (Fig. 2C). There was no microscopic connection between the two lesions.

In the perihilar tumor, mild venous and perineural infiltrations could be visualized, while in the distal tumor, severe perineural infiltration and moderate venous and lymphatic infiltrations were evident. Subepithelial connections were, however, not observed between those two lesions, even at the infiltrating forefronts. Although there was invasion of the

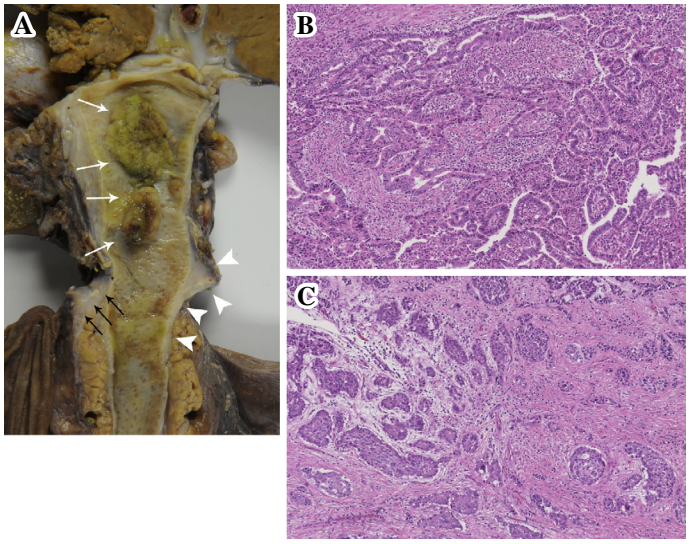

Figure 2. **Macroscopic and microscopic findings of the resected specimen. A: Formalin-fixed specimen obtained by right hepatopancreaticoduodenectomy showing nodular and wall-thickened lesions that separately occupy the perihilar (white arrows) and distal bile ducts (white arrowheads), respectively. The distal bile duct lesion involves stromal invasion (black arrows). B: Perihilar tumor composed of moderately differentiated adenocarcinoma showing papillary proliferation and tubular formation of cancer cells [Hematoxylin and Eosin (H&E) staining, magnification ×20]. C: Distal tumor of poorly differentiated adenocarcinoma consisting of cancer cells with alveolar to solid structures (H&E staining, magnification ×20).**

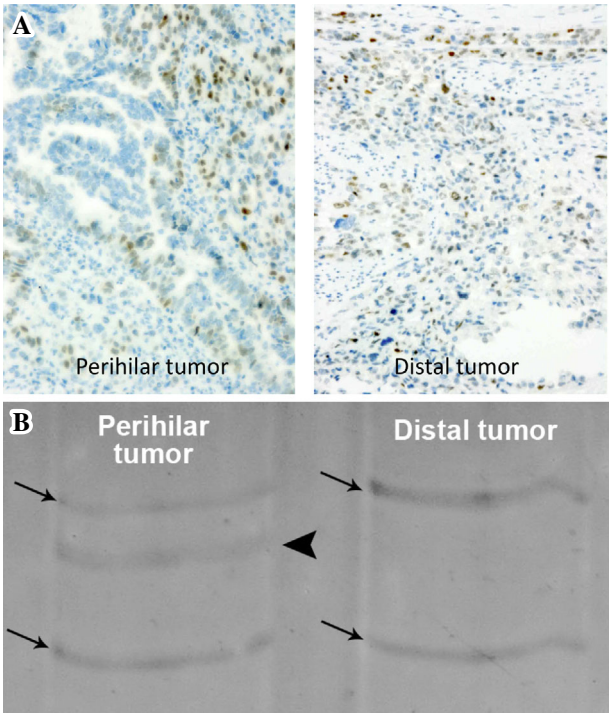

Figure 3. **Protein and gene analyses of p53. A: Immunohistochemical staining of p53 protein reveals sporadic positive signals over the cancerous tissues with similar density between the perihilar and distal lesions. B: A polymerase chain reaction-single-strand conformation polymorphism analysis, targeting exon 5 of the *TP53* gene (with the forward primer 5´-TTCCTCCTACAGTACTCC-3´ and reverse primer 5´-GCCCCAGCTCACCATCG-3´), shows an extra band (arrowhead) exclusively in the perihilar tumor. Left lane, perihilar tumor; Right lane, distal tumor.**

pancreatic subserosa by the distal tumor, the major part existed in the bile duct of the flat lesion. No metastasis was observed. Using the UICC TNM classification (8th edition) (17), the tumor of the perihilar bile duct was classified as pT2aN0M0, Stage II, and the tumor of the distal bile duct was classified as pT2N0M0, Stage IIA.

In terms of immunohistochemical staining of the p53 protein, positive signals were scattered in both lesions (Fig. 3A). Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) analyses covering exons 5-8 of the *TP53* gene resulted in an additional band only for exon 5 in the tissues from the perihilar tumor (Fig. 3B, Supplementary material 2). Sequence analyses indicated that neither of the lesions had any mutant alleles of the *KRAS* gene at codons 12 and 13 of exon 2, codons 59 and 61 of exon 3, or codons 117 and 146 of exon 4. The patient has been free from recurrent diseases for 28 months without additional treatment.

Discussion

We herein report a very rare case of synchronous double bile duct cancer without known risk factors, such as liver fluke infestation (3, 4), viral hepatitis (2), chronic cholangitis (1), pancreaticobiliary maljunction (15, 16), or exposure to 1,2-dichloropropane, which is often used in the printing industry (6). The following criteria have been proposed for the diagnosis of multiple primary malignant tumors: (1) each tumor must present a definite picture of malignancy, and (2) each tumor must be distinct; moreover, (3) the probability that one is a metastasis of the other must be ruled

Table. Reported Cases of Synchronous Double Bile Duct Cancer without Pancreaticobiliary Maljunction.

No	Reference	Age/ sex	Surgical procedure	Tumor region	Gross type	Histology	Primary or metastasis	Genetic analysis	Prognosis (months)
1	(19)	69/M	PPPD	1. Bd (middle) 2. Bd (inferior)	1. nodular 2. nodular	1. adenocarcinoma (poor) 2. adenocarcinoma (moderate)	metastasis	LOH	unknown
2	(20)	67/F	PPPD	1. Bd (middle) 2. Bd (inferior)	1. nodular 2. nodular	unknown	unknown	N/A	unknown
3	(21)	78/M	1st: EHBD resection; 2nd: PD	1. Bh 2. Bd (inferior)	1. nodular 2. papillary	1. adenocarcinoma (moderate) 2. adenocarcinoma (papillary)	unknown	N/A	31
4	(22)	67/M	PPPD	1. Bd (middle) 2. Bd (inferior)	1. nodular 2. nodular	1. squamous cell carcinoma 2. adenocarcinoma (moderate)	double primary	N/A	8
5	(11)	78/F	PPPD	1. Bd (middle) 2. Bd (inferior)	1. nodular 2. nodular	1. adenocarcinoma (well to moderate) 2. adenocarcinoma (poor, NET-like feature)	double primary	N/A	18
6	This study	69/M	RHPD	1. Bh 2. Bd (inferior)	1. nodular expanding 2. flat infiltrating	1. adenocarcinoma (moderate) 2. adenocarcinoma (poor)	double primary	PCR-SSCP (*TP53*)	28, alive

M: male, F: female, PPPD: pylorus-preserving pancreatoduodenectomy, EHBD: extra-hepatic bile duct, PD: pancreatoduodenectomy, RHPD: right hepatopancreaticoduodenectomy, Bd: distal bile duct, Bh: perihilar bile duct, NET: neuroendocrine tumor, LOH: loss of heterozygosity, N/A: not applicable, PCR-SSCP: polymerase chain reaction-single strand conformation polymorphism

out (18). In line with this, our literature search using the medical terms "double cancers" and "bile duct cancer" in PubMed identified five possible cases of synchronous double biliary cancers (Table) after exclusion of patients with gallbladder cancer and/or pancreaticobiliary maljunction (11, 19-22). In a case reported by Ogawa et al. (19), one of two lesions appeared to have metastasized from the other, as a loss of heterozygosity analysis revealed that the two lesions shared highly homologous genetic backgrounds. Bedoi et al. (20) and Sumiyoshi et al. (21) did not mention whether the relationships between two lesions were primary or metastatic lesions. Yoo et al. (22) and Nishi et al. (11) concluded that different histological differentiations demonstrate the occurrence of double primary lesions. In general, however, the tumor dedifferentiates into a different histological grade in a metastatic lesion. Therefore, variations in the histological differentiation status cannot be used to confirm the occurrence of nonmetastatic lesions. In contrast, mutant alleles generally accumulate along with dedifferentiation. This discrepancy in the accumulation of genetic errors and dedifferentiation between two lesions strongly suggests that they are independent from one another. In the present study, a mutant allele of *TP53* was exclusively detected in the perihilar tumor, which showed a higher differentiation grade than that of the distal lesion. This strongly suggests that the poorly differentiated tumor at the distal bile duct was genetically distinct and not derived from the perihilar tumor. Therefore, our case met all of the above-mentioned criteria for double primary cancers.

It has been hypothesized that many cancers, including bile duct cancer, develop from a precancerous lesion through the accumulation of various genetic errors (5, 8, 14, 23, 24). The *TP53* tumor suppressor gene is the most common target of genetic alteration, accounting for over 50% of known human tumors (25, 26). *TP53* is involved in central cellular processes, including gene transcription, DNA repair, cell cycling, genomic stability, apoptosis, and chromosomal segregation (27-29). It has, therefore, been described as the guardian of the genome (27).

However, the frequency of *TP53* alterations varies significantly among different types of human cancers. It is well known that a *TP53* mutant allele is frequently detected in pancreatic, colorectal, and breast cancers as well as in bone and soft-tissue sarcomas, whereas it is very uncommon in leukemia and gastric cancer (9, 10, 30). In bile duct cancer, the prevalence of immunohistochemical detection of p53 has been reported to range from 19-86% (31). Many factors may contribute to this broad range, such as different carcinogens within different cancer pandemic areas, antibodies used, and criteria applied (32, 33). There are several methods for detecting genetic errors-from immunohistochemical studies to whole genome sequencing, each with a specific sensitivity and specificity. A synchronous double cancer is an ideal model for reducing bias, as two cancers with different genetic errors develop in the exact same environment. Thus, the present study allows for a more correct evaluation of the underlying genetic effect. In agreement, our observations strongly indicate that a precancerous lesion of the bile duct

does not follow a specific genetic pathway but, instead, follows various towards cancer development.

As we only studied one case, it is unclear whether our findings are case-specific or if they represent a general phenomenon in bile duct cancer. Unfortunately, as most of the normal bile duct epithelium was peeled off from the resected specimen, we could not properly evaluate the presence of biliary intraepithelial neoplasia (BilIN) (7, 8), which is a precancerous lesion of bile duct cancer.

Regarding the role of p53 in bile duct cancer development, we did not sequence the entire *TP53* gene. Therefore, while it is clear that the *TP53* status differed between the two lesions, the lack of additional products from the PCR-SSCP analysis failed to indicate whether or not wild-type *TP53* was present in the poorly differentiated adenocarcinoma at the distal bile duct. Despite these limitations, it can still be inferred that diagnostic progression, through a stepwise analysis of synchronous double bile duct cancers, would enable the confirmation of the genetic background divergence in bile duct cancers.

In conclusion, within the same bile duct environment, two lesions exhibited different genetic backgrounds, strongly suggesting that cancer development in the bile duct epithelium follows various pathways. The wide variety of genetic backgrounds in bile duct cancers may explain, at least in part, why the treatment is so challenging. Our findings and further studies of similar synchronous bile duct cancers would thus aid clinicians in diagnosing and implementing appropriate treatment regimens, ultimately improving the overall prognosis of this deadly disease.

This study was reviewed and approved by the institutional Human Investigation Committee of the Uonuma Institute of Community Medicine, Niigata University Hospital (30-014). Written informed consent for the publication of this case report and accompanying images was obtained from the patient in accordance with the Declaration of Helsinki. The patient's data have been appropriately de-identified.

The authors state that they have no Conflict of Interest (COI).

References

1. Malhi H, Gores GJ. Cholangiocarcinoma: modern advances in understanding a deadly old disease. J Hepatol **45**: 856-867, 2006.
2. Zhou Y, Zhao Y, Li B, et al. Hepatitis viruses infection and risk of intrahepatic cholangiocarcinoma: evidence from a meta-analysis. BMC Cancer **12**: 289, 2012.
3. Feng M, Cheng X. Parasite-associated cancers (blood flukes/liver flukes). Adv Exp Med Biol **1018**: 193-205, 2017.
4. Kim TS, Pak JH, Kim JB, Bahk YY. Clonorchis sinensis, an oriental liver fluke, as a human biological agent of cholangiocarcinoma: a brief review. BMB Rep **49**: 590-597, 2016.
5. Jusakul A, Cutcutache I, Yong CH, et al. Whole-genome and epigenomic landscapes of etiologically distinct subtypes of cholangiocarcinoma. Cancer Discov **7**: 1116-1135, 2017.
6. Kumagai S, Sobue T, Makiuchi T, et al. Relationship between cumulative exposure to 1,2-dichloropropane and incidence risk of cholangiocarcinoma among offset printing workers. Occup Environ Med **73**: 545-552, 2016.
7. Zen Y, Aishima S, Ajioka Y, et al. Proposal of histological criteria for intraepithelial atypical/proliferative biliary epithelial lesions of the bile duct in hepatolithiasis with respect to cholangiocarcinoma: preliminary report based on interobserver agreement. Pathol Int **55**: 180-188, 2005.
8. Zen Y, Adsay NV, Bardadin K, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. Mod Pathol **20**: 701-709, 2007.
9. Hruban RH, Takaori K, Klimstra DS, et al. An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms. Am J Surg Pathol **28**: 977-987, 2004.
10. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science **321**: 1801-1806, 2008.
11. Nishi T, Sato Y, Hanaoka T, Takahashi T, Miura H, Takubo K. Synchronous double primary cancers of the extrahepatic bile duct: a case report and literature review. Int J Surg Case Rep **47**: 48-51, 2018.
12. Ito T, Sakurai-Yageta M, Goto A, Pairojkul C, Yongvanit P, Murakami Y. Genomic and transcriptional alterations of cholangiocarcinoma. J Hepatobiliary Pancreat Sci **21**: 380-387, 2014.
13. Nakamura H, Arai Y, Totoki Y, et al. Genomic spectra of biliary tract cancer. Nat Genet **47**: 1003-1010, 2015.
14. Wardell CP, Fujita M, Yamada T, et al. Genomic characterization of biliary tract cancers identifies driver genes and predisposing mutations. J Hepatol 2018.
15. Tashiro S, Imaizumi T, Ohkawa H, et al. Pancreaticobiliary maljunction: retrospective and nationwide survey in Japan. J Hepatobiliary Pancreat Surg **10**: 345-351, 2003.
16. Ohuchida J, Chijiiwa K, Hiyoshi M, Kobayashi K, Konomi H, Tanaka M. Long-term results of treatment for pancreaticobiliary maljunction without bile duct dilatation. Arch Surg **141**: 1066-1070, 2006.
17. UICC. Digestive System Tumours. In: TMN Classification of Malignant Tumors (UICC). 8th ed. M Gospodarowicz CW, Ed. Wiley-Blackwell, New York, 2017: 97-100.
18. Warren S. Multiple primary malignant tumors. Am J Cancer **16**: 1358-1414, 1932.
19. Ogawa A. Double cancers in the common bile duct: molecular genetic findings with an analysis of LOH. J Hepatobiliary pancreat Surg **8**: 374-378, 2001.
20. Bedoui R, Ajmi M, Nouira R, Dziri C. Synchronous double cancer of the common bile duct. Am J Surg **201**: e1-e2, 2011.
21. Sumiyoshi T. Synchronous double cancers of the common bile duct. World J Gastroenterol **18**: 2012.
22. Yoo Y, Mun S. Synchronous double primary squamous cell carcinoma and adenocarcinoma of the extrahepatic bile duct: a case report. J Med Case Rep **9**: 2015.
23. Nakanishi Y, Zen Y, Kondo S, Itoh T, Itatsu K, Nakanuma Y. Expression of cell cycle-related molecules in biliary premalignant lesions: biliary intraepithelial neoplasia and biliary intraductal papillary neoplasm. Hum Pathol **39**: 1153-1161, 2008.
24. Nakanuma Y, Sasaki M, Sato Y, Ren X, Ikeda H, Harada K. Multistep carcinogenesis of perihilar cholangiocarcinoma arising in the intrahepatic large bile ducts. World J Hepatol **1**: 35-42, 2009.
25. Hussain SP, Harris CC. Molecular epidemiology of human cancer: contribution of mutation spectra studies of tumor suppressor genes. Cancer Res **58**: 4023-4037, 1998.
26. Beroud C, Soussi T. The UMD-p53 database: new mutations and analysis tools. Hum Mutat **21**: 176-181, 2003.
27. Lane DP. Cancer. p53, guardian of the genome. Nature **358**: 15-16, 1992.
28. Vogelstein B, Kinzler KW. p53 function and dysfunction. Cell **70**:

523-526, 1992.
29. Lane DP, Lain S. Therapeutic exploitation of the p53 pathway. Trends Mol Med **8** (Suppl): S38-S42, 2002.
30. Wong P, Verselis SJ, Garber JE, et al. Prevalence of early onset colorectal cancer in 397 patients with classic Li-Fraumeni syndrome. Gastroenterology **130**: 73-79, 2006.
31. Khan SA, Thomas HC, Toledano MB, Cox IJ, Taylor-Robinson SD. p53 Mutations in human cholangiocarcinoma: a review. Liver Int **25**: 704-716, 2005.
32. Bertorelle R, Esposito G, Belluco C, et al. p53 gene alterations and protein accumulation in colorectal cancer. Clin Mol Pathol **49**: M85-M90, 1996.
33. Zhang H. Evaluation of four antibodies in detecting p53 protein for predicting clinicopathological and prognostic significance in colorectal adenocarcinoma. Clin Cancer Res **5**: 4126-4132, 1999.