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AUTHOR(S):
Matsumori, Tomoaki; Uza, Norimitsu; Kakiuchi, Nobuyuki; Morita, Toshihiro; Nishikawa, Yoshihiro; Shiokawa, Masahiro; Taura, Kojiro; Kodama, Yuzo; Seno, Hiroshi

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BRIEF REPORT

Whole-exome sequencing for a more accurate diagnosis of intraductal papillary neoplasms of the bile duct

Tomoaki Matsumori, Norimitsu Uza, Nobuyuki Kakiuchi, Toshihiro Morita, Yoshihiro Nishikawa, Masahiro Shiokawa, Kojiro Taura, Yuzo Kodama and Hiroshi Seno

1Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; 2Department of Pathology and Tumor Biology, Kyoto University, Kyoto, Japan; 3Department of Hepato-Biliary-Pancreatic Surgery, Graduate school of Medicine, Kyoto University, Kyoto, Japan; and 4Department of Gastroenterology, Kobe University, Kobe, Japan

*Corresponding author. Department of Gastroenterology and Hepatology, Graduate School of Medicine, Kyoto University, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto, 606-8507, Japan. Tel: +81-75-751-4302; Fax: +81-75-751-4303; Email: uzanori@kuhp.kyoto-u.ac.jp

Introduction

According to the World Health Organization (WHO, 2018), intraductal papillary neoplasms of the bile duct (IPNB) are characterized as polypoid masses in dilated bile ducts; additionally, invasive IPNB occasionally presents with a nodular surface or mass formation [1, 2]. The IPNB diagnostic criteria are, however, often ambiguous, e.g. whether IPNB is considered a type of cancer and whether it must have mucus production are debatable [3]. Therefore, establishing new IPNB diagnostic criteria based on an alternative approach, such as whole-exome sequencing, is required. Here, we present a case of IPNB with atypical features on which we performed a detailed genetic analysis to determine the genetic, morphological, and histological features of IPNB in this case.

Case presentation

A 68-year-old woman with dilatation of the right hepatic bile duct was referred to our hospital. Contrast-enhanced computed tomography images revealed a tumor in the right hepatic bile duct (Figure 1A). Endoscopic retrograde cholangiography further showed a defect with a smooth surface localized in the dilated right hepatic bile duct (Figure 1B). Additionally, intraductal ultrasoundography revealed that the tumor mass filled the dilated right hepatic bile duct and that the tumor surface showed a multinodular pattern (Figure 1C). Based on the fluoroscopic biopsy results, we suspected IPNB. Per-oral cholangioscopy (POCS) was also performed and confirmed that the tumor had a multinodular surface and moved lambently in response to water injection (Figure 1D). A POCS-guided biopsy allowed a definitive diagnosis of IPNB; horizontal spreading was not detected, and a right hepatectomy with extrahepatic bile-duct resection was performed. Histopathological examination of the resected specimen confirmed IPNB with a multinodular surface, mainly consisting of pyloric-gland-like cells with low-grade atypia (Figure 1E). The results of immunohistochemistry staining further revealed that the cells expressed MUC6, but not MUC1, MUC2, or MUC5AC; the Ki-67 labeling index was <1%. Additionally, whole-exome sequencing, in the context of tumor cells extracted from the surgically resected specimen, revealed that CTNNB1 and NFE2L2 mutations were the significant oncogenic mutations (Table 1).
KRAS is frequently identified in bile-duct cancers and IPNB, such as in the case we have reported here, which are classified as oncogenic mutations in the Catalogue of Somatic Mutations in Cancer (https://cancer.sanger.ac.uk/cosmic). Curiously, although LRIT3 has not been reported as a cancer driver, it showed the highest mutant allele frequency in our case with two-hit mutations, a nonsense mutation, and an LOH; therefore, we cannot exclude the notion that such mutations were involved in the formation of IPNB. In fact, such a hypothesis is supported by the fact that LRIT3 is a regulator of FGFR1 [10], one of the driver genes of cholangiocarcinoma. Although other mutations may have played driver roles, we could not find any reports supporting their association with the phenotype observed in this case. Therefore, large-scale genetic analysis of IPNB is further needed to completely understand the molecular pathology in IPNB and establish clear diagnostic criteria.

Discussion

In this case, the tumor filled the hepatic bile duct and caused dilatation of the peripheral bile duct. Though the tumor nearly met the WHO diagnostic criteria for IPNB, some features were atypical. Despite the absence of an invasive lesion, the surface properties exhibited a nodular pattern. Moreover, the cells constituting the tumor were primarily pyloric-gland-like cells. Furthermore, in the biliary system, pyloric-gland adenomas are often found in the context of gallbladder polyps, but of IPNB [4]. Although some whole-exome sequencing-based studies on bile-duct cancers have been reported, and KRAS and TP53 are listed as important gene variants, most analyses were performed in mixed patients with IPNB and bile-duct cancers [5]. Aoki et al. recently performed targeted capture sequencing of cancer-driver genes in the context of IPNB [3]; however IPNBSpecific gene mutations could not be identified via target gene analysis alone. In the present case, whole-exome sequencing of the tumor revealed somatic mutations in well-known cancer-driver genes such as CTNNB1 and NFE2L2; Interestingly, mutations frequently identified in bile-duct cancers and IPNB, such as KRAS and GNAS mutations, were not found (Table 1). Therefore, collectively, this IPNB case was both phenotypically and genotypically atypical, suggesting that the identified genetic mutations were potentially involved in the formation of the characteristic morphological and histological features. Furthermore, these results indicate that IPNB is heterogeneous and the current diagnostic criteria are inadequate.
Conclusions

We identified three distinct genetic alternations that may be involved in the formation of IPNB with an atypical phenotype. The accumulation of similar cases and large-scale analyses is, however, still necessary to validate the role of these genes in the pathogenesis of IPNB and to establish clear IPNB diagnostic/classification criteria.

Authors' Contributions

T.M., N.U., N.K., T.M., Y.N., M.S., K.T., and Y.K. performed the clinical examinations and experiments, and analysed the data. T.M. wrote the manuscript, and N.U., Y.K., and H.S. revised it.

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Conflict of Interest

None declared.

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Table 1. Somatic mutations revealed by whole-exome sequencing in this case-study

| Gene   | Chr | Start | Ref. C | Alternate T | Mutation type | Amino-acid change | VAF |
|--------|-----|-------|--------|-------------|---------------|-------------------|-----|
| LRIT3  | 4   | 110791232 | A | G | Missense | NM_01314057: p.M98V | 0.646 |
| MNBL1  | 8   | 30958470 | T | A | Missense | NM_000553: p.M696K | 0.416 |
| GREB1  | 2   | 11780550 | G | A | Synonymous | NM_014668: p.P1940P | 0.41 |
| PKHD1  | 14  | 56896509 | G | C | Missense | NM_146490: p.H393D | 0.397 |
| DBF4   | 7   | 87525791 | C | T | Missense | NM_002616: p.L1219F | 0.397 |
| CDHR3  | 7   | 105662725 | A | G | Missense | NM_001314057: p.M98V | 0.46 |
| AVL9   | 7   | 32609638 | C | G | Missense | NM_000553: p.M696K | 0.416 |
| WNK1   | 12  | 988975 | T | A | Synonymous | NM_014668: p.P1940P | 0.41 |
| SETD2  | 3   | 47125677 | T | A | Missense | NM_014668: p.P1940P | 0.41 |
| DCAKD  | 7   | 43112197 | C | T | Synonymous | NM_014668: p.P1940P | 0.41 |
| CTNB1  | 7   | 43126134 | C | G | Missense | NM_000553: p.M696K | 0.416 |
| ZNF544 | 19  | 58772421 | TGGTCA | – | Frameshift deletion | NM_000553: p.H393D | 0.031 |
| TRIM16 | 17  | 15532476 | T | C | Missense | NM_000553: p.M696K | 0.416 |
| SPRED1 | 15  | 38614538 | AGTTTCA | – | Frameshift deletion | NM_000553: p.H393D | 0.031 |
| CPA4   | 7   | 12994403 | C | T | Missense | NM_000553: p.H393D | 0.031 |
| ZEB1   | 10  | 31810108 | T | C | Missense | NM_000553: p.H393D | 0.031 |
| MBD1   | 18  | 47065362 | T | C | Missense | NM_000553: p.H393D | 0.031 |
| AASS   | 7   | 12173891 | T | C | Missense | NM_000553: p.H393D | 0.031 |
| RNF34  | 12  | 12185807 | CAT | – | Inframe deletion | NM_000553: p.H393D | 0.031 |
| FNDC3B | 3   | 172003752 | T | – | Frameshift deletion | NM_000553: p.H393D | 0.031 |
| FAT3   | 11  | 92533227 | C | T | Missense | NM_000553: p.H393D | 0.031 |
| ZNF3   | 22  | 29445928 | C | T | Missense | NM_000553: p.H393D | 0.031 |
| MACF1  | 1   | 39818806 | G | T | Missense | NM_000553: p.H393D | 0.031 |
| LRP1B  | 2   | 142237983 | C | T | Missense | NM_000553: p.H393D | 0.031 |
| PA2    | 5   | 108691675 | C | A | Missense | NM_000553: p.H393D | 0.031 |
| EZH1   | 17  | 40871163 | T | C | Missense | NM_000553: p.H393D | 0.031 |
| SPTAN1 | 9   | 131395509 | – | C | Frameshift insertion | NM_000553: p.H393D | 0.031 |

Genes in red: hot-spot mutations in well-known oncogenes. Chr, chromosome; Ref., reference; VAF, variant allele frequency.

Conclusions

Acknowledgements

Conflict of Interest

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