Experimental and Molecular Pathology 101 (2016) 150–156

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Cancer related gene alterations can be detected with next-generation sequencing analysis of bile in diffusely infiltrating type cholangiocarcinoma

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ARTICLE INFO

Article history:
Received 19 April 2016
and in revised form 25 May 2016
Accepted 22 July 2016
Available online 25 July 2016

Keywords:
Cholangiocarcinoma
Next-generation sequence analysis
Bile

ABSTRACT

Genome-wide association study in diffusely infiltrating type cholangiocarcinoma (CC) can be limited due to the difficulty of obtaining tumor tissue. We aimed to evaluate the genomic alterations of diffusely infiltrating type CC using next-generation sequencing (NGS) of bile and to compare the variations with those of mass-forming type CC. A total of 24 bile samples obtained during endoscopic retrograde cholangiopancreatography (ERCP) and 17 surgically obtained tumor tissue samples were evaluated. Buffy coat and normal tissue samples were used as controls for a somatic mutation analysis. After extraction of genomic DNA, NGS analysis was performed for 48 cancer related genes. There were 27 men and 14 women with a mean age of 65.0 ± 11.8 years. The amount of extracted genomic DNA from 3 cm^3 of bile was 66.0 ± 84.7 μg and revealed a high depth of sequencing coverage. All of the patients had genomic variations, with an average number of 19.4 ± 2.8 and 22.3 ± 3.3 alterations per patient from the bile and tumor tissue, respectively. After filtering process, damaging SNPs (8 sites for each type of CC) were predicted by analyzing tools, and their target genes showed relevant differences between the diffusely infiltrating and mass-forming type CC. Finally, in somatic mutation analysis, tumor-normal paired 14 tissue and 6 bile samples were analyzed, genomic alterations of *EGFR*, *FGFR1*, *ABL1*, *PIK3CA*, and *CDKN2A* gene were seen in the diffusely infiltrating type CC, and *TP53*, *KRAS*, *APC*, *GNA11*, *ERBB4*, *ATM*, *SMAD4*, *BRAF*, and *IDH1* were altered in the mass-forming type CC group. *STK11*, *GNAQ*, *RB1*, *KDR*, and *SMO* genes were revealed in both groups. The NGS analysis was feasible with bile sample and diffusely infiltrating type CC revealed genetic differences compared with mass-forming type CC. Genome-wide association study could be performed using bile sample in the patients with CC undergoing ERCP and a different genetic approach for accurate diagnosis, pathogenesis study, and targeted therapy will be needed in diffusely infiltrating type CC.

1. Introduction

Cholangiocarcinoma (CC) is a malignancy originating from the bile duct epithelial cells and is classified into intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC) according to the location. ICC is divided into mass-forming, periductal infiltrating, and intraductal growth type and ECC is divided into polypoid, nodular, scirrhous constricting, and diffusely infiltrating type on the basis of gross morphologic features(Bosman et al., 2010; Chung et al., 2009; Lim, 2003). Although CC is a relatively rare tumor, worldwide data shows that incidence and mortality of the CC is increasing(de Groen et al., 1999; Khan et al., 2002; Patel, 2002; Taylor-Robinson et al., 2001).

With the development of biotechnological techniques and applications, several mutations in oncogenes and tumor suppressor genes have been confirmed in many cancers by DNA sequencing(Berthiaume and Wands, 2004). DNA sequencing is a powerful method for decoding many human diseases including malignancies(Xuan et al., 2013). After the introduction of high-throughput sequencing technologies which can reduce the cost of DNA sequencing(Schuster, 2008), many studies have

Abbreviations: CC, Cholangiocarcinoma; ICC, Intrahepatic cholangiocarcinoma; ECC, Extrahepatic cholangio-arcinoma; NGS, next-generation sequencing; gDNA, Genomic DNA; SNPs, single nucleotide polymorphisms.

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http://dx.doi.org/10.1016/j.yexmp.2016.07.010
0014-4800/

aimed to determine novel markers and molecular pathways. Genomic alteration sites can be diagnostic and potential personalized treatment targets.

Although a relatively large series of cholagiocarcinoma NGS-related papers have been published recently, genetic properties of CC are still not well established. Moreover, because most previous studies performed genomic analysis using tumor tissue, it is difficult to utilize the information obtained in these studies to situations with limited tissue. On the other hand, patient bile can be easily obtained for most populations because many cases of CC undergo ERCP during the initial work-up and can also be considered in unresectable patients. Although DNA extraction from bile containing tumor cells is rarely performed, genomic analysis of bile samples may increase the usability of this technique.

Some previous cancer genetic studies have found that genomic alterations can be different according to the location of the CC and recent studies recognized that ICC and ECC represent two distinct tumor types(Cardinale et al., 2012; Churi et al., 2014; Putra et al., 2015; Ruzzenente et al., 2016). Target lesions for early diagnosis and therapeutic implication can be different regarding types of CC. Among the any types of CC, we focused on diffusely infiltrating type CC because there is limited genetic information and the diagnosis of diffusely infiltrating type CC is still difficult even though multiple diagnostic methods can be used(Van Beers, 2008). Detection of diffusely infiltrating type CC in the early stage can be difficult because of its limited detection in imaging studies and difficulty in obtaining a sufficient biopsy specimen, which may result in low diagnostic yield. Early detection of malignancy can allow for complete resection and increases the possibility of cure. Therefore, new diagnostic markers and methods for detection of diffusely infiltrating type CC at the early stage are needed. Also, screening modalities with easy, fast, low cost, convenient, and non-invasive methods should be considered.

We performed genomic analysis of bile to elucidate the effectiveness of it compared with tumor tissue, and evaluated the genomic alterations of diffusely infiltrating type CC using next-generation sequencing (NGS) to establish a protocol to analyze genomic variations related to diffusely infiltrating type CC in order to allow early detection and tailored treatment.

2. Materials and methods

2.1. Patients

We selected patients who had been diagnosed with CC at Chonbuk National University Hospital. Among eligible patients, we designed to obtain bile samples of diffusely infiltrating type CC patients and tissue samples of mass-forming type CC patients. A total of 27 bile samples were obtained during the ERCP procedures, and 17 tumor tissues, including normal tissues for control, were obtained from surgically resected specimens. Buffy coat of blood sample was also obtained as control of bile samples. Location and gross type of the CC were determined by abdominal CT imaging features. A flowchart of the study is illustrated in Fig. 1.

Of the patients, we designed sub-group analysis to compare somatic mutations between the diffusely infiltrating type CC and mass-forming type CC. Tumor-normal paired samples were needed for the process, and 14 tissue and 6 bile samples were analyzed.

This study was approved by the Institutional Review Board of Chonbuk National University Hospital, Chonbuk National University School of Medicine, Jeonju, South Korea (Institutional Review Board no. 2012-11-020).

2.2. Patient sampling procedure

2.2.1. Bile samples

For sample collection, 3–10 cm^3 of bile was aspirated through the cannula with precautions against contamination in patients with diffusely infiltrating type CC. Aspiration of bile samples was conducted before brushing cytology or tissue biopsy. Samples were collected in cryogenic vials and stored in liquid nitrogen prior to DNA extraction.

Patient bile may contain deciduous cancer cells because CC is a tumor of bile duct epithelial cells. Therefore, genomic DNA (gDNA) from all bile samples was extracted using the QIAGEN© QIAamp® DNA blood maxi kit (Cat. No. 51194), which is used to extract DNA from body fluids. For extraction of this DNA, 3 cm^3 of bile was used. All patient bile was analyzed on the basis of the manufacturer's instructions. Obtained gDNA samples were stored at −20 °C after quantification and testing.

The buffy coat, the fraction of anti-coagulated blood that contains most of the white blood cells and platelets was also sampled. Genomic extraction of the buffy coat was performed using the QIAGEN© QIAamp® DNA blood midi kit (Cat. No. 51183).

2.2.2. Tumor tissue samples

Tumor tissue and normal tissue, which was used as a control, were obtained from surgically resected specimens of mass-forming type CC patients. For gDNA extraction, we used the Zymo Research Quick-gDNA Miniprep (Cat. No. D3024). The protocol was carried out

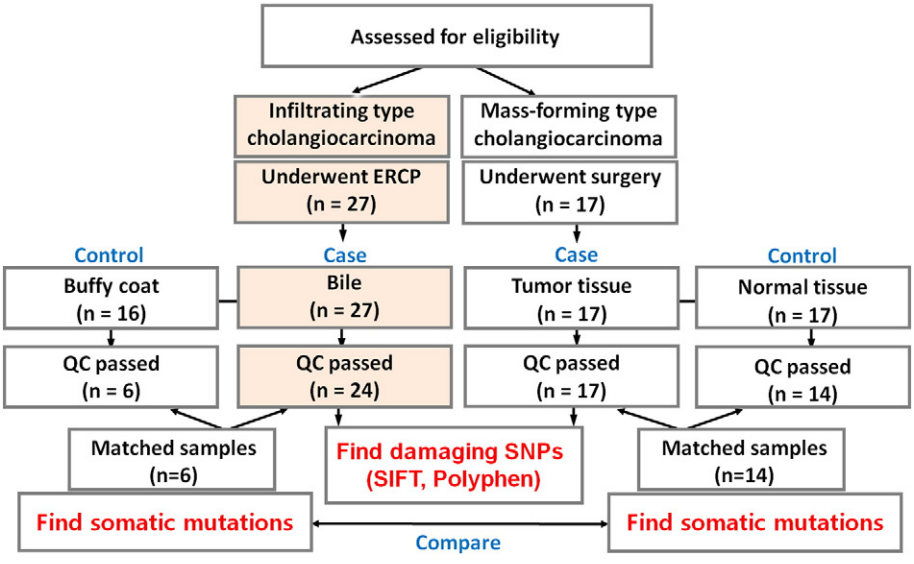

Fig. 1. Flow chart of study population and genomic analysis process.