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Squamous cell carcinoma of the common bile duct: A case report with genomic profiling

Running title: Squamous cell carcinoma of the bile duct

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Abstract

Squamous cell carcinoma of the extrahepatic bile ducts is a very rare type of cancer with a virtually unknown pathogenesis. We present the case of a 66-year-old woman who underwent a pancreaticoduodenectomy with the postoperative diagnosis of squamous cell carcinoma of the intrapancreatic part of distal common bile duct. Microscopically, the entire common bile duct showed squamous metaplasia. Besides, an invasive squamous cell carcinoma was found, stage pT3pN0. A next generation sequencing assay covering 315 tumor-related genes revealed genomic alterations in seven genes: FBXW7, CREBBP, CTCF, FAT1, MAGI2, MLL2 and NOTCH1.

Introduction

Squamous cell carcinoma (SCC) of the extrahepatic bile ducts (SCC-EBD) is a rare entity. Since 1930, only seventeen cases including the case presented here have been reported. The etiology is essentially unknown and with the small number of cases a plausible pathogenesis has been difficult to establish.

Here, we present a case of SCC of the distal common bile duct (CBD) that showed complete squamous metaplasia in its entire length. Additionally, we present results from a next generation sequencing based assay covering 315 cancer-related genes (FoundationOne™), which shows genomic alterations in seven genes: FBXW7, CREBBP, CTCF, FAT1, MAGI2, MLL2 and NOTCH1.
Clinical Summary

A 66-year-old Caucasian female patient with a 15-year-old history of well-treated ulcerative colitis was referred to hospital by her general practitioner due to dark-colored urine. Blood tests showed elevated alanine aminotransferase (ALT) 125 U/L [10-70 U/L], alkaline phosphatase (AP) 1034 U/L [35-105 U/L], gamma-glutamyltransferase 986 U/L [15-115 U/L] and bilirubin 21 µmol/L [5-25 µmol/L]. A contrast-enhanced computed tomography (CT) scan revealed CBD dilation up to 25 mm with a tumor suspicious area in the distal part or possibly in the ampulla. No enlarged lymph nodes were found. Magnetic resonance cholangiopancreatography (MRCP) revealed a tumor mass in the most distal extrahepatic bile duct with intra- and extrahepatic dilation and irregular bile duct configuration (Figure 1a-b). Endoscopic ultrasound confirmed the presence of a 15 mm lesion in the distal part of the common bile duct. Based on a suspicion of an adenoma or a localized distal cholangiocarcinoma, the patient was scheduled for a laparoscopy with laparoscopic ultrasound and a subsequent pancreaticoduodenectomy (Whipple’s procedure). The postoperative course was uneventful and adjuvant chemotherapy was not advocated.

Pathological Findings

Gross examination of the surgical specimen revealed a 20 mm dilated and 2 mm thick-walled extrapancreatic CBD with a luminal surface resembling skin (Figure 1c). The lumen of the intrapancreatic CBD was covered with whitish papillary projections up to 3 mm large, while the profound changes seemed to extend beneath the muscle layer (Figure 1d). These changes were observed along the entire CBD (approximately 44 mm) including its entry into...
the ampulla of Vater (Figure 1d). The gallbladder contained no stones or tumor suspicious areas but the 3 mm thick wall appeared fibrous.

Microscopically, sections from the extrapancreatic CBD showed keratinizing squamous metaplasia (Figure 2a-b). No normal bile duct epithelium was seen. The intrapancreatic CBD also revealed metaplastic keratinizing squamous epithelium. Several areas showed transition to a well-differentiated SCC (Figure 2c-d). The tumor consisted of sheets and islands of atypical polygonal cells, some showing dyskeratosis (Figure 2e-g). Keratin pearls were present and mitoses were frequent. Invasion of the pancreas and ampulla of Vater was observed. In the tumor stroma, a mixture of different inflammatory cells were present (Figure 2d-h). No component of adenocarcinoma was found in the entire resected and fully embedded CBD. Immunohistochemically, all tumor cells strongly expressed p40 and cytokeratin 5/6 (Figure 2f), confirming the squamous phenotype. No metastases were detected in 57 retrieved lymph nodes. Sections from the gallbladder and cystic duct showed chronic inflammation but no squamous metaplasia.

Sections from one paraffin-embedded tumor tissue block were submitted to next-generation sequencing (NGS). NGS was performed with a hybrid capture–based NGS platform (FoundationOne), FDA approved for solid tumors, at a Clinical Laboratory Improvement Amendments–certified, New York State and College of American Pathologists–accredited laboratory (Foundation Medicine, Cambridge, Mass) on the Illumina HiSeq. 4000 instrument. This analysis identifies genomic alterations within 315 genes and introns of 28 genes involved in rearrangements. Genomic alterations were identified in *F-box and WD repeat domain-containing 7 (FBXW7)* (G691fs*13), *CREB binding protein (CREBBP)*

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(K13fs*34), CCCTC-binding factor (CTCF) (R129*), FAT atypical cadherin 1 (FAT1) (E2815*), membrane associated guanylate kinase, WW and PDZ domain containing 2 MAGI2 (P188L), myeloid/lymphoid or mixed-lineage leukemia 2 (MLL2) (R1757*), and notch 1 (NOTCH1) (C376* and R1984*). Intermediate microsatellite status and tumor mutational burden was also detected.

Discussion

Adenocarcinomas of the bile duct system are usually divided into intrahepatic and extrahepatic cholangiocarcinomas, with adenocarcinoma of the extrahepatic bile ducts being the by far most frequent histologic subtype\textsuperscript{5}. To date, only 17 cases of pure SCC-EBD have been reported, including this present case. Several of these reports include literature reviews\textsuperscript{2,4}.

The etiology of SCC-EBD is essentially unknown. Four main theories have been proposed: 1) derivation from ectopic squamous epithelium, 2) derivation from undifferentiated basal cells, 3) transformation of adenocarcinoma and 4) derivation from metaplastic squamous epithelium due to inflammatory irritation of the biliary mucosa\textsuperscript{4}. The development of metaplasia is usually triggered by environmental stimuli and represents the replacement or transformation of a differentiated somatic cell type to another differentiated somatic cell type in the same tissue, probably in order to better withstand the environmental irritation\textsuperscript{11}. To our knowledge, of the 17 reported SCC-EBD cases, only the present case and the one reported by Sewkani et al were associated with squamous metaplasia\textsuperscript{3}. However, two out of the other fifteen reports were based on biopsies only\textsuperscript{1,6} and in the remaining cases, it is not
clear if the specimens were fully embedded. This could explain the lack of reported squamous metaplasia. Thus, the establishment of a potential mechanism in these cases is difficult. In the current case, the extensive keratinizing squamous metaplasia was evident already at the macroscopic examination (Figure 1c-d). It is tempting to speculate whether the squamous epithelium occupying the entire common bile duct may actually represent ectopic / heterotopic squamous epithelium. However, to our knowledge, ectopic squamous epithelium in the extrahepatic bile ducts has so far not been reported in the English-language literature. Ectopic tissue in the extrahepatic bile ducts has mainly reported to be of gastric and pancreatic type.

Diffuse squamous metaplasia of the EBD is relatively rare, while focal squamous metaplasia is a sometimes recognized focally in the EBD and ampulla of Vater, particularly in patients with cancer and inflammation related to these structures. In a study of 42 extrahepatic bile ducts containing normal-appearing bile duct mucosa adjacent to either tumors or chronic inflammation, 20 cases presented with metaplastic changes, most frequently pyloric gland metaplasia. Only one case showed squamous metaplasia in addition to pyloric and intestinal metaplasia. This case was without a tumor component, representing an inflammatory stricture of the common bile duct. A similar frequency of 5% has been observed in choledochal cysts, a lesion associated with inflammation and increased risk of neoplasia. Of the 17 reported SCC cases, one was located within such a cyst. Our patient had well-treated ulcerative colitis, and no signs of hepatobiliary (inflammatory) complications associated with this disease, such as primary sclerosing cholangitis (PSC), a condition that can rarely cause squamous metaplasia. Lewis et al. examined 100 liver explants performed due to
PSC, and found focal squamous metaplasia in 3% of the cases. None of the other published cases report a history of ulcerative colitis. Neither did this current patient present with gallstones. However, microscopically, the gall bladder and EBD showed chronic inflammation. Thus, it is not unthinkable that the inflammation may have contributed to the metaplastic changes in extrahepatic bile duct.

It is well-accepted that metaplasia is a precursor to low grade dysplasia that can eventually progress to an invasive carcinoma. The most common examples are the replacement of bronchial epithelium with squamous epithelium due to smoking and squamous to intestinal metaplasia in the distal esophagus due to reflux of acidic gastric fluid. Continued stimuli and inflammation may eventually lead to lung SCC and esophageal adenocarcinoma, respectively. Thus, our case supports the proposed theory that the development of SCC in the EBDs may occur on a background of metaplastic changes.

To our knowledge, this is the first description of molecular alterations in SCC-EBD. Employing the FoundationOne NGS panel covering 315 cancer-related genes, genomic alterations were found in 7 different genes: FBXW7, CREBBP, CTCF, FAT1, MAGI2, MLL2 and NOTCH. FBXW7 is a putative tumor suppressor, controlling processes such as cell-cycle progression, cell proliferation, differentiation, DNA damage response and genomic stability through degradation oncoproteins of c-myc, cyclin E, Notch, c-Jun, Mcl-1 and mTOR. FBXW7 mutations have been reported in several cancers, and were found in 5%, 5% and 7% of advanced EBD adenocarcinomas, esophageal SCC and head and neck SCC. The prevalence in SCC-EBD is currently unknown. In 99 EBD adenocarcinomas, CREBBP rearrangement was found in one case (1%) and MLL2 alterations in four cases.
While to our knowledge alterations in the remaining four genes, \textit{NOTCH1}, \textit{FAT1}, \textit{CTCF} and \textit{MAGI2} have not been reported in EBD adenocarcinomas. In contrast, aberrations in \textit{FBXW7}, \textit{NOTCH1}, \textit{FAT1}, \textit{CREBBP} and/or \textit{MLL2} have been reported in SCC of the esophagus\textsuperscript{16,18}, lung\textsuperscript{19}, uterine cervix\textsuperscript{16} and head and neck\textsuperscript{17}. In primary esophageal SCC and in metastatic SCC of the anal canal, \textit{MLL2} was among the most frequently mutated genes\textsuperscript{17}, while in a series of 20 primary anal SCCs, \textit{FBXW7} (15\%), \textit{FAT1} (15\%) and \textit{NOTCH1} (10\%) were found most often mutated. Thus, these genes might represent driver genes of both primary and metastatic anal squamous neoplasms. Additionally, in esophageal SCC, researchers also found that \textit{NOTCH1} (18.6\%) and \textit{MLL2} (18.6\%) were frequently mutated, while also \textit{FAT1} (14.6\%), \textit{CREBBP} (7.6\%) and \textit{FBXW7} (5.6\%) were among the 15 most frequently mutated genes\textsuperscript{17}. \textit{NOTCH1} mutations are also frequent in oral SCCs\textsuperscript{15}. Using immunohistochemistry, increasing rates of NOTCH1 protein expression were found in the normal oral mucosa (20\%), oral squamous dysplasia (64.7\%) and oral SCC (84.6\%)\textsuperscript{18}, supporting that \textit{NOTCH1} mutations may play a significant role in the development of SCC at this site. In head and neck SCC, \textit{FAT1} mutations were found in approximately 30\% cases\textsuperscript{15}. The MAGI2 protein couples with vinculin, controlling expression of PTEN as well as inhibiting proliferation and migration of the cell through interaction with signaling proteins in multiple pathways. Hypermethylation of \textit{MAGI2} was found in cervical intraepithelial neoplasia (CIN) and SCC of the uterine cervix\textsuperscript{19}. In SCC of the uterine cervix, \textit{FBXW7} represented the second most frequently altered gene, and it was suggested that this might represent one of the driver mutations in this neoplasm\textsuperscript{16}. The same study found also alterations in \textit{FAT1}, \textit{MLL2}, \textit{NOTCH1} and \textit{CREBBP} in SCC of the cervix, but neither these genes nor \textit{FBXW7} were mutated in their CIN specimens\textsuperscript{16}. Taken together, these studies
indicate that some molecular features of EBD-SCC, but far from all, are shared with SCC from other organs.

The NGS analysis did not detect any alterations in the most frequently mutated genes in EBD adenocarcinomas, including TP53, KRAS, CDKN2A/B, ARID1A, ERBB2 and SMAD4. However, as our NGS analyses are based on areas with SCC, the possibility that the SCC-EBD reported here developed through transformation of an adenocarcinoma cannot be excluded entirely. As we were not able to carry out the NGS analyses separately in each of the three tissue compartments; i.e. the metaplastic and invasive compartments, it is unknown in which part of the suggested continuum the genetic changes occurred.

Of the seven gene mutations found in this present case, at present only FBXW7 alterations may have clinical importance in a therapeutic setting. Recent data indicate that tumors harboring FBXW7 could benefit from mTOR kinase inhibitors. Although SCC of the EBD is a rare entity, increased knowledge on these tumors could benefit these patients, as the disease is often advanced at the time of diagnosis with no established chemotherapy or radiation therapy regimens.

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Disclosure Statement

None declared

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Author contribution

Study conception and design: SD, KNK. Acquisition and analysis of data: SD, KNK, MBM.

Drafting of the manuscript: KNK. Critical revision: SD and MBM.

Reference List


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Figures

Figure 1. Magnetic resonance cholangiopancreatography (MRCP) (a-b) and macroscopic findings in a pancreaticoduodenectomy specimen (c-d) in a patient with squamous cell carcinoma of the distal common bile duct (CBD). (a) At MRCP, the CBD is dilated (26 mm) with an irregular wall (white arrow). (b) A mass-forming lesion identified by MRCP is shown. (c) The pancreaticoduodenectomy specimen with the luminal surface of the CBD, resembling skin to a certain extent (white arrow). (d) Cross section showing the intrapancreatic part of the CBD thick-walled with papillary projections of yellow colour (white arrow) and small foci of invasion (black arrow).
Figure 2. Microscopic findings in a pancreaticoduodenectomy specimen with squamous cell carcinoma (SCC) of the common bile duct (CBD). (a) The intrapancreatic portion of the CBD with papillary projections covered by metaplastic but well-differentiated keratinizing squamous epithelium (H&E). (b) Higher magnification of the epithelium of the CBD showing squamous metaplasia (H&E). (c) Invasive front of SCC of the CBD (H&E). (d) SCC extending into the muscular layer of the duodenum (H&E). (e) A focus of keratinizing SCC invading into the wall of the CBD, accompanied by an intense inflammatory reaction (H&E). (f) Immunohistochemically, numerous small buds of invasive SCC are appreciated (cytokeratin 5/6 immunostaining). (g) High power view of the invasive front of SCC. (h) SCC invading around a vein in the connective tissue between the CBD and pancreas (H&E).