Teaching Cases

Complex APC germline mutation associated metaplasia and intraepithelial neoplasia (CAM-IFN) of the gallbladder

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A R T I C L E   I N F O

Article history:
Received 10 July 2015
Accepted 10 November 2015

Keywords:
Gallbladder carcinogenesis
FAP
Complex epithelial lesion

A B S T R A C T

Preneoplastic and neoplastic changes of the gallbladder of patients with a familial adenomatous polyposis (FAP) are rare, and very little is known about their incidence in patients with an attenuated FAP. We herein report a unique case of a woman with an attenuated FAP who shows eight distinct, partially preneoplastic differentiation patterns within the gallbladder mucosa, which are: (1) regular gallbladder epithelium, (2) low grade biliary intraepithelial neoplasia, (3) papillary adenoma, (4) Paneth cell metaplasia, (5) goblet cell metaplasia, (6) pancreatic metaplasia, (7) pseudopyloric metaplasia, and (8) neuroendocrine differentiation. Moreover, this is the first case of a KRAS mutation in a gallbladder adenoma of a patient with an APC germline mutation, which is highly suggestive of an early event of malignant transformation. As a consequence of our findings, clinicians should draw special attention to the gallbladder of FAP patients, and a simultaneous protective cholecystectomy of FAP patients, which undergo colectomy and show conspicuous changes of the gallbladder mucosa, should be performed in these patients in order to eliminate the risk of a synchronous or metachronous gallbladder neoplasia.

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Introduction

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited polyposis syndrome that is caused by a mutation of the adenomatous polyposis coli (APC) gene. It comes along not only with a large number of colorectal adenomas and a highly increased risk for the development of colorectal cancer, but also with an association with extra-colorectal tumors, as it is referred to as Gardner’s syndrome. Extracolonic benign and malignant tumors and their preliminary stages occur mainly in the stomach, skin and soft tissue, but, although rare, also APC mutation associated neoplasms of the gallbladder are described [1]. Until now, only 11 cases of Gardner’s syndrome associated gallbladder adenomas, respectively, six cases of gallbladder adenocarcinomas have been reported, and nothing is known about other precancerous lesions or simultaneous gene mutations [2].

Patients with attenuated FAP do not fulfill the complete criteria for FAP, as they have less than 100 colorectal polypos. Extracolonic manifestations are rare and mainly restricted to gastric polypos, and nothing is described about epithelial alterations of the gallbladder within these patients.

Here, we report for the first time on a case of a woman with an attenuated FAP who shows eight distinct differentiation patterns of the gallbladder mucosa, which include a wide range of precancerous lesions. We found no other name in the literature for this complex metaplastic and dysplastic lesion that we named “complex APC germline mutation associated metaplasia and intraepithelial neoplasia (CAM-IFN) of the gallbladder” and that is a novel carcinogenesis model of the gallbladder, with the possibility to gain new insights into tumor biology.

Clinical history

A 53-year-old woman with a previously known attenuated FAP and a confirmed APC germline mutation (c.4788delC:p.Gln1596Argfs*54) underwent subtotal colectomy with simultaneous cholecystectomy for cholecystolithiasis. The examination of the colectomy specimen revealed multiple residuals of afore endoscopically removed colorectal adenomas with low grade intraepithelial neoplasia according to the WHO classification. The gallbladder measured 89 × 28 × 25 mm and contained multiple gallstones with a size of up to 17 mm. The gallbladder wall measured 4 mm in maximum thickness. The

http://dx.doi.org/10.1016/j.prp.2015.11.010
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specimen showed no macroscopic abnormalities, apart from a partial plane polyloid mucosa of 8 mm in diameter.

**Material and methods**

**Immunohistochemical analysis**

Serial sections of 2.5 μm thickness were obtained from formalin-fixed and paraffin-embedded tissue specimens. Immunohistochemical stainings were carried out with a Bondmax (Leica Biosystems, Wetzlar, Germany) automated slide staining system, using the Polymer Refine Detection Kit (Menarini Diagnostics, Berlin, Germany). For immunohistochemistry, we used monoclonal mouse antibodies, directed against β-Catenin (clone CAT5H10; ZytoMed Systems, Berlin, Germany; dilution 1:300), CDX2 (clone AMT28; Novocastra Laboratories Ltd, Newcastle, United Kingdom; 1:20), chromogranin A (clone LK2H10; Biologo, Kronshagen, Germany; 1:100), CD10 (clone 56C6; Novocastra; 1:10), cytokeratin 7 (CK7; clone RN7; Novocastra; 1:100), cytokeratin 20 (CK20; clone Ks20.8; NeoMarkers, Fremont, United States of America; 1:50), mucin 1 (Muc1; clone Ma695; Novocastra; 1:100), mucin 2 (Muc2; clone Ccp58; Novocastra; 1:100), mucin 5 (Muc5; clone 45M1; NeoMarkers; 1:100), p53 (clone DO-7; Novocastra; 1:100), trefoil factor 2 (TFF2; clone GE16C; LGR5 (F); 

**Table 1**

<table>
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<tr>
<th>Antigen</th>
<th>Regular gallbladder epithelium</th>
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<th>Papillary adenoma</th>
<th>Paneth cell metaplasia</th>
<th>Goblet cell metaplasia</th>
<th>Pancreatic metaplasia</th>
<th>Pseudopyloric metaplasia</th>
<th>Neuroendocrine differentiation</th>
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<td>Chromogranin A</td>
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“+” denotes positivity, “(+)” denotes weak positivity, “−” denotes negativity.
Microdissection and DNA extraction

Three morphologically peculiar areas within the gallbladder (see below in Molecular findings) were manually microdissected separately. Genomic DNA was extracted from formalin-fixed and paraffin-embedded tissue using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions.

Mutational analysis

Mutational analysis of codons 12/13, 61, 117 and 146 of the KRAS and NRAS genes, codon 600 of the BRAF gene, and mutational hotspots in exons 8 and 9 of the GNAS gene were performed by pyrosequencing on a PyroMark Q24 instrument (Qiagen). Fragments of the different genes were amplified by polymerase chain reaction.

Results

Histopathologic findings

After complete embedding of the cholecystectomy specimen, we found eight differentiation patterns within the mucosa, which were: (1) regular gallbladder epithelium, (2) low grade biliary intraepithelial neoplasia (BilIN-1), (3) papillary adenoma with intermediate grade dysplasia, (4) Paneth cell metaplasia, (5) goblet cell metaplasia, (6) pancreatic metaplasia, (7) pseudopyloric metaplasia, and (8) neuroendocrine differentiation. The light microscopic findings of the various phenotypes were confirmed by immunohistochemistry (Table 1, Fig. 1). No relevant inflammatory infiltrate, respectively, chronic inflammatory changes were observed.

Additionally, we explored three other, previously examined gallbladder specimens of FAP patients from the archive of the Institute of Pathology, University Hospital Kiel. These cases showed neither preneoplastic nor neoplastic changes of the mucosa.

Molecular findings

On the basis of the morphologic and immunohistochemical findings, we performed mutational analysis of codons 12/13, 61, 117 and 146 of the KRAS and NRAS genes, codon 600 of the BRAF gene, and mutational hotspots in exons 8 and 9 of the GNAS gene of three distinct phenotypic areas (Fig. 2). Area 1 contained the papillary adenoma with intermediate grade dysplasia and showed a gene mutation of codon 12/13 of the KRAS gene (c.34G>T; p.G12C) which results in an amino acid substitution at position 12 in KRAS-protein, from a glycine to a cysteine. Area 2 (BilIN1) and area 3 (regular

146 of the KRAS and NRAS genes, codon 600 of the BRAF gene, and mutational hotspots in exons 8 and 9 of the GNAS gene was performed separately for each phenotype. Hematoxylin and eosin, original magnifications ×5.3 (A); ×50 (B); ×100 (C); ×30 (D).
gallbladder epithelium) showed no mutation within the examined codons and exons of KRAS, NRAS, BRAF or GNAS.

**Discussion**

We herein report the first case of a complex APC germline mutation-associated metaplasia and intraepithelial neoplasia of the gallbladder in a patient with an attenuated FAP. Dysplastic changes, adenomas and carcinomas of the gallbladder in patients with an APC germline mutation are – although extremely rare – already known, but to date, nothing has been described about complex synchronous metaplastic and dysplastic changes of the gallbladder mucosa of these patients, and nothing is known about these changes in patients with attenuated FAP. In general, FAP patients are at increased risk of developing other types of cancer and mucosal alterations, such as fundic gland polyps and cancer of the stomach, as well as cancer of the small intestine [4]. However, a complex metaplasia as described here has not been found at any other organ site, e.g. stomach, small and large intestine, leading to the conjecture that the gallbladder provides a unique environment for metaplasia and dysplasia. At least 40% of the patients with FAP, who underwent cholecystectomy, are known to have epithelial dysplasia of the gallbladder [5], which might be related to a higher biliary bile acid concentration: The bile of patients with FAP is described to have a greater proportion of deoxycholic acid than the bile of patients without FAP [6]. Bile acids influence, e.g. the cellular proliferation and differentiation of colorectal epithelial cells, and may also be relevant for the development of biliary dysplasia [7].

In routine cholecystectomy specimens, metaplastic changes of the mucosa are frequent but never of this complex phenotype. In general, they seem to be associated with patient gender and age and are considered to be precursor lesions of dysplastic changes of the gallbladder mucosa [8]. In the gallbladder, adenoma and dysplasia are regarded as distinct lesions that are both associated with a highly increased risk for carcinoma development [9]. In this regard, incidental adenomas as precursor lesions for gallbladder adenocarcinoma are rare, whereas the metaplasia → dysplasia → carcinoma in situ → invasive gallbladder carcinoma sequence is much more prevalent and well-established in the concept of gallbladder cancer [10].

Several gene mutations, including mutations within the WNT-signaling pathway, KRAS, BRAF and TP53, are known to play a role in gallbladder carcinogenesis [11]. Within the WNT-signaling pathway, APC is closely connected to β-catenin. In non-neoplastic gallbladder epithelium, the β-catenin expression is solely membranous, whereas a nuclear expression comes along with β-catenin mutations, as they are known to appear in neoplastic changes. All epithelial phenotypes found in the gallbladder mucosa of our patient showed a moderate to strong membranous β-catenin expression, whereas they lacked a nuclear β-catenin expression. This observation confirms previous findings, which state that hyperplastic and dysplastic gallbladder epithelium shows a membranous β-catenin expression, whereas a nuclear or cytoplasmic accumulation is mainly reserved to gallbladder carcinomas [2,12].

Thus, the presence of a β-catenin mutation may not be present in our patient. KRAS gene mutations are known to be involved in the development of gallbladder adenoma, dysplasia and carcinoma [13]. In contrast to the minor role that adenomas play in the carcinogenesis of sporadic gallbladder carcinoma, the combination of a gallbladder adenoma with a KRAS mutation in a patient with an APC germline mutation may have further interesting implications:

1. In colorectal adenoma, an APC loss results in the initiation of an adenoma, whereas the progression to a carcinoma needs a second hit, as it is fulfilled, e.g. by a KRAS mutation [14]. As this is the first case of a KRAS mutation in a gallbladder adenoma of a patient with an APC germline mutation, this case may provide evidence that a similar sequence of events may also initiate gallbladder cancer.
2. The combination of a KRAS-mutation of a gallbladder adenoma against the background of an APC germline mutation is highly suggestive of an early event of malignant transformation. This conclusion is moreover supported by the proven LGGR overexpression within the adenoma, which is known to be associated with cancer progression in other gastrointestinal tumor entities [3,15].

**Conclusion**

We herein report on a unique case of complex metaplastic and dysplastic changes of the gallbladder and, moreover, the first case of a KRAS mutation in a gallbladder adenoma of a patient with an APC-germline mutation. The combination of our findings is highly suggestive of an early event of malignant transformation. As a consequence of our findings, clinicians should draw special attention to the gallbladder of FAP patients, and a simultaneous protective cholecystectomy of FAP patients, which undergo colectomy and show conspicuous changes of the gallbladder mucosa, should be performed in these patients in order to eliminate the risk of a synchronous or metachronous gallbladder neoplasia.

**Informed consent**

Ethical approval was obtained from the local ethical review board (D 453/10).

**Conflict of interest**

The authors declare that no conflicts of interest exist.

**Funding disclosure**

CR is supported by grants of the German Research Foundation (Ro 1173/12). All other authors have no support or funding to report.

**Acknowledgement**

The authors would like to thank Dr. Andreas Jung for the provision of the KRAS and NRAS primer sequences.

**References**