

Plexiform fibromyxoma of the gallbladder

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Key words

Plexiform angiomyxoid myofibroblastic tumor • Gallbladder • CKIT • GIST

Summary

We report the unusual case of a plexiform fibromyxoma, occasionally assessed in a lithiasic gallbladder. The full thickness assessment of the gallbladder wall revealed an intra-mural, well demarcated multi-nodular tumor (1 cm), consisting of a plexiform growth of spindle cells, included within a fibromyxoid stroma with a rich micro-vascular network. The tumor cells featured no nuclear atypia, nor mitotic activity. At the immunohistochemical profiling, the spindle shaped cells unequivocally featured vimen-

tin, SMA, HHF35, collagen IV, and CD34; no cells expressed CD117, PDGFRA, CD10, desmin, GFAP, EMA, and S-100. Faint STAT6 nuclear expression was observed in isolated tumor cells. The molecular profiling did not revealed any *CKIT* and *PDGFRA* genes mutations. The uncommon site of the tumor presentation and its aberrant CD34 expression both confer to the reported case a unique place among the myxoid tumors of the gastrointestinal tract.

Introduction

Myxoid non-GIST tumors of the gastrointestinal tract encompass a heterogeneous group of uncommon non-epithelial lesions¹, and the different definitions applied to such tumors (e.g. myxoma, fibromyxoma, plexiform fibromyxoma) reflect both the complex phenotype of the lesions and their controversial nosology¹. In 2007, Takahashi and colleagues defined as “plexiform angio-myxoid myofibroblastic tumor (PAMT)” a multi-nodular benign tumor, mostly arising in the gastro-duodenal district, but also occasionally described in the small and large bowel²⁻⁵.

We report the unique case of a benign myxoid, plexiform, spindle shaped cell tumor of the gallbladder wall, featuring the histological and immunohistochemical phenotype of the plexiform fibro-myxoid tumor, with aberrant expression of CD34 endothelial marker.

Clinical history

A 55-year-old Caucasian female was referred to the General Surgery Unit of the Padova teaching Hospital

for a previously established diagnosis of cholelithiasis. At the admission, no significant past medical history was recorded, and both the physical examination and laboratory tests were unremarkable. The pre-surgical abdominal ultrasound did not reveal any mural thickening. Laparoscopic cholecystectomy was performed, with no post-surgical complications.

Materials and methods

Pathology

The gross examination of the surgical specimen confirmed the pre-surgical diagnosis of cholelithiasis; the gallbladder's wall was irregularly increased in thickness, with no grossly evident nodular lesions. Multiple tissue specimens were obtained for routine histology examination, including a coronal section of the cystic duct. The tissue specimens were fixed in formalin, embedded in paraffin, and cut in 4 micrometer thick histology sections, which were stained with hematoxylin and eosin. Because of one the specimens obtained from the corpus wall showed a marginal myxoid area, the whole gall-

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bladder's wall was serially sectioned, and histologically assessed. The newly obtained tissue samples revealed a multi-nodular myxoid lesion, 1 cm large in its wider diameter. Further serial histology sections of the target lesion were obtained for additional histochemical stainings (PAS, Pas after diastase digestion, and Alcian blue), immuno-phenotyping, and molecular profiling.

Immunohistochemical profiling was automatically performed on the Bond TM Polymer Refine Detection System (Leica Microsystems, Newcastle upon Tyne, UK). The applied primary antibodies included (source and solutions): Vimentin (Novocastra labs ltd; 1:200), CD10 (Diagnostic Biosystems, Pleasanton, CA; 1:50), CD34 (Thermo Scientific; Waltham, MA 1:100), Collagen IV (Dako, Carpinteria, CA; 1:100), HHF-35 (Cell Marque, Rocklin, California; 1:20); α -smooth muscle actin (SMA, Cell Marque; 1:100); CD117 (Dako; 1:100), desmin (Dako; 1:50), GFAP (Dako; 1:800), S-100 (Histo-Line Laboratories, Milan, Italy; 1:50), AE1/AE3 (Life Technology, Milan, Italy; 1:50), STAT6 (Santa Cruz Biotechnology, Dallas, TX; 1:1000); EMA (Dako; 1:100); CD31 (Dako; 1:20), MIB1 (Dako; 1:100), PDGFR α (Santa Cruz Biotechnology; 1:200). Sections were lightly counterstained with hematoxylin. Appropriate positive and negative controls were run concurrently for all the applied antisera.

Mutational analysis

DNA profiling was performed on tissue samples obtained from the paraffin block representative of the target lesion. DNA was extracted after enrichment for neoplastic cellularity using manual micro-dissection of 10 consecutive 4- μ m FFPE sections, purified using the QIAamp DNA FFPE Tissue Kit (Qiagen), and qualified as reported elsewhere⁶. PCR products of exons 9, 11, 13, 14, and 17 of the *CKIT* gene and of exons 10, 12, 14, and 18 of the *PDGFRA* gene (primers upon request) were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter) and labelled with Big Dye Terminator v3.1 (Applied Biosystems, Monza, Italy). Agencourt CleanSEQ magnetic beads (Beckman Coulter) were used for post-labeling DNA fragment purification, and sequence analysis was performed on an Applied Biosystems 3130xl Genetic Analyzer.

Results

On the hematoxylin and eosin stain, the full thickness section of the gallbladder wall demonstrated a multi-nodular, well circumscribed, myxoid tumor (Fig. 1). The tumor cells population consisted of sparse spindle shaped cells organized in a plexiform pattern, and lying within an abundant myxoid (Alcian blue positive) matrix, which included a rich network of capillary-sized vessel. Tumor cells exhibited oval pale nuclei, and slightly eosinophilic cytoplasm. Neither nuclear atypia, nor (even typical) mitoses were observed. Very rare lymphocytes and plasma cells were dispersed within the myxoid stroma.

The tumor cells consistently expressed vimentin, α -smooth muscle actin, HHF-35, collagen IV, and CD34; no immunostain was demonstrated for CD117, desmin, GFAP, CD10, EMA, and S-100. Some isolated tumor cells showed faint STAT6 nuclear expression. MIB1 labeling index was 0%.

The molecular profiling failed in demonstrating any mutations in both the *CKIT* and *PDGFRA* genes.

The gallbladder mucosa showed multiple foci of low-grade biliary intraepithelial neoplasia (BilIN-1) co-existing with pyloric- and intestinal-type epithelial metaplasia.

Discussion

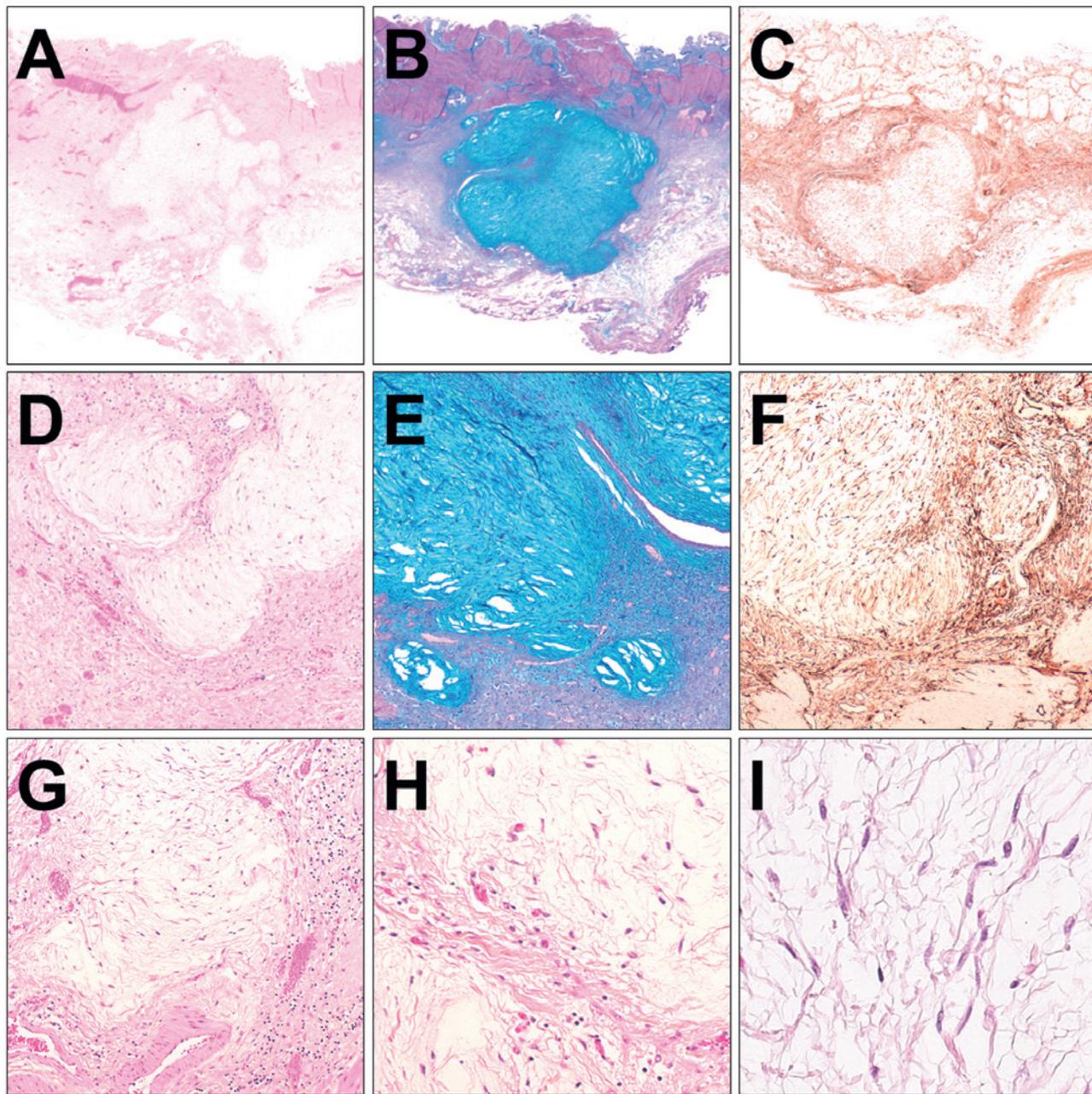
We report a peculiar case of multi-nodular tumor of the gallbladder wall, consisting of a uniform population of spindle cells, lying in an abundant myxoid matrix.

Histologically, the tumor's nodular growth pattern, its myxoid matrix, the phenotype of the neoplastic cells, and their plexiform arrangement were all consistent with the microscopic phenotype of a benign tumor originally described in the gastric wall by Takahashi and colleagues and by the same Authors defined as "plexiform angiomyxoid myofibroblastic tumors (PAMT)". Since this initial description, several gastric (and some rare intestinal) cases have been reported^{4-7,11}.

After the original "PAMT" definition, several alternative nomenclatures have been proposed, and phenotypically similar tumors have been labeled as "myofibroblastic tumor"¹⁰, and, more recently, "plexiform fibromyxoma"¹. Consistently with the original description of the gastric plexiform angiomyxoid myofibroblastic tumor (and consistently with a myofibroblastic/fibroblastic phenotype), the tumor cells consistently expressed vimentin, SMA, and HHF35; unexpectedly, however, the tumor cells also featured moderate/strong immunostain for CD34, which had been reported as negative in the original gastric cases. Among benign myxoid tumors, a SMA/CD34 positive immunophenotype has been consistently associated to superficial angiomyxoma (cutaneous myxoma); such a neoplasia, however, most frequently includes peripheral areas of increased cellularity, focal cellular atypia, and a sparse inflammatory infiltrate, which were not observed in our case¹². Moreover, this tumor is peculiarly located within the skin and is frequently characterized by the presence of abnormal epithelial structures, such as epidermoid cysts, thin strands of squamous epithelium, and small buds of basaloid cells, which have been suggested to have an etiopathogenetic role for these neoplasms. Of note, CD34 expression has been described only in a single case of gastric fibromyxoma case: this tumor, however, did not feature SMA expression¹³.

Within the gastrointestinal tract, a phenotypically benign myxoid lesion evokes a wide spectrum of differential diagnoses, particularly: spindle shaped cell myxoid GIST, leiomyoma, perineurioma, schwannoma, desmoids fibromatosis, solitary fibrous tumor, inflam-

Fig. 1. Representative histological features of the present case. The nodular and plexiform appearance of the tumor are recognizable at H&E (A) and Alcian Pas (B) stain and at immunohistochemical analysis for CD34 (C). Note the small and medium size tumor nodules dissecting the gallbladder muscular wall (H&E stain, D; Alcian Pas stain, E). (F) Tumor cells showed a moderate/strong CD34 immunoreaction. (G-I) Representative H&E stain images at different magnifications demonstrating the paucicellular tumor composed by spindled cells intermingled in a myxoid matrix. (Original magnifications 2x; 10x; 20x; and 40x)



matory fibroid polyp, and inflammatory myofibroblastic tumor⁴.

In the present case, the myxoid GIST (a relative rare variant of the GISTs' family) has been ruled out for the concurrence of a distinctive plexiform pattern, the lack of CD117/PDGFR α expression, and the absence of any *CKIT/PDGFR α* genes mutation¹⁴.

The diagnosis of inflammatory fibroid polyp, and/or inflammatory myofibroblastic tumor were basically ruled out because of the absence of any inflammatory cell

population (eosinophils, in particular). The lack of S-100 expression excluded the neurogenic origin (plexiform neurofibroma, or schwannoma) of the lesion, whereas the lack of EMA expression discharged the diagnosis of perineurioma. The inconsistent STAT6 nuclear expression was not conclusive for a case of myxoid solitary fibrous tumor¹⁵.

In summary, we describe the first case of plexiform fibromyxoma of the gallbladder, featuring aberrant CD34 expression. This benign tumor, which has been never

reported among the non-epithelial gallbladder tumors, further expands the constellation of the myxoid tumors arising in the gastrointestinal tract.

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