Gastrointestinal Stromal Tumor with Chondroid Differentiation

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Abstract. Gastrointestinal stromal tumors are mesenchymal neoplasms of the gastrointestinal tract that represent less than 1% of all GI tumors. Here the case of such a tumor that, in addition to the classical features, also displayed chondroid differentiation is reported. Chromosomal abnormalities were also investigated.

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal neoplasms of the gastrointestinal tract, even though they represent less than 1% of all GI tumors (1, 2).

Most GI mesenchymal tumors have been previously classified into smooth muscle tumors, such as leiomyomas, leiomyoblastomas and leiomyosarcomas. However, with the introduction of immunohistochemistry techniques, it has been shown that most of them do not have features related to smooth muscle cells (3). In 1983, Mazur and Clark coined the term GIST to indicate a subgroup of mesenchymal tumor arising in the gastrointestinal tract, which could be classified neither as neurogenic nor as smooth muscle derived tumors (4).

To date, GIST are considered to originate from interstitial cells of Cajal (ICC) or their precursor cells (5) because most GIST and ICC are double-positive for KIT and CD34, and because familial and multiple GIST appear to develop from diffuse hyperplasia of ICC. The estimated incidence of GIST, based on extrapolations from tumor registry data, is about 10–20 per million people per year (6, 7).

GIST arise mainly from the stomach (60%) and the small intestine (30%). Five to ten percent of GIST are located in the colon and rectum and the remaining part into the esophagus (8).

This paper reports on a case of GIST with chondroid differentiation, in which chromosomal abnormalities were also investigated, since previous studies have shown numerical chromosome changes such as a gain of chromosome 8, mainly in malignant primary tumours (9, 10). Moreover, the behavior of three important genes involved in tumor development, HER-2/neu, EGFR and p16, are analysed.

Case History

A 79-year-old Caucasian female taking oral anticoagulant therapy for paroxystic atrial fibrillation was referred for melena, coffee ground vomiting and abdominal pain. Physical examination was unremarkable and the digital rectal examination was negative. An upper endoscopy revealed the presence of recent bleeding from a mucosal erosion coated by a clot, in the context of a large submucosal mass, lying quite near to the cardias. The remaining gastric mucosa was normal. A CT scan with contrast medium revealed extraluminal growth of the lesion (extending for about 7 centimetres, with defined and spherical margins) and excluded visible hepatic metastasis.

Due to the risk of re-bleeding the patient underwent surgery; since the lesion was close to the cardias, an upper polar resection of the stomach with a mechanical oesophageal-gastric reconstruction associated to vagotomy was carried out. The postoperative period was uneventful.

A radiographic control with hydro-soluble contrast on the sixth postoperative day and a subsequent endoscopy revealed normal, complete gastric emptying and well-consolidated anastomosis. After 8-months’ follow-up the patient enjoys good health.
Pathological findings. At the intraoperative observation, the mass appeared symmetrical, regular, well-capsulated and with defined and regular margins. Macroscopically, the resected area measured 7.5 cm on the greater curvature and 7 cm on the lesser curvature; on the external part of the fundus, a lesion measuring 7×5 cm was present. After opening of the surgical specimen, only a soft elevated lesion (1.5 cm diameter) was evident. The surgical specimen was fixed in formalin and 10 sections were then obtained from the lesion described above. Sections 4 μm-thick were stained with hematoxylin-eosin (H&E), PAS, PAS-diastase (Pas-d) and silver impregnation. Sections were also tested with monoclonal antibodies for CD117, S-100, NSE, CK7, CK20, CD34, MIB-1, AML, AMS, cromogranin, alpha 1 antitripsin and vimentin.

Histological criteria, taking into account a prognostic point of view, were tumor size and mitotic activity, as proposed by Dei Tos (11).

Immunohistochemistry. Serial sections were dewaxed in xylene and rinsed in graded alcohols. Endogenous peroxidase was blocked by incubation with peroxidase-blocking solution (DAKO ChemMate Denmark) for 15 min, followed by rinsing in tris-buffered saline (TBS). For MIB-1, CD117 (c-kit) and CD34 staining, the sections then underwent antigen retrieval by heating in a 750 W microwave oven in EDTA buffer (pH 8) for 3×5 min and washed in TBS; for desmin staining by heating in a 800 W microwave oven in citrate buffer (pH 6) for 2×5 min. Staining for S-100, actin, muscle specific actin and vimentin does not need any pre-treatment. Non-specific staining was blocked by treatment with normal goat serum (1:5) for 5 min. The immunohistochemical method involved sequential application of primary antibody to MIB-1 (monoclonal mouse anti-human Ki-67 antigen, clone MIB-1; DAKO, CA, USA), CD117 (polyclonal rabbit anti-human c-kit, CD117; DAKO), CD34 (mouse monoclonal, clone QBEnd/10; NeoMarkers, CA, USA), actin (mouse monoclonal clone HHF35; BioGenex, CA, USA), muscle-specific actin (monoclonal clone HHF33; DAKO), vimentin (mouse monoclonal; BioGenex), S-100 (rabbit anti-cow; DAKO) and desmin (monoclonal; BioGenex), a secondary biotinylated anti-mouse/rabbit antibody and streptavidin-biotin complex (Reagent kit, BioGenex). The immunoprecipitate was visualised by treatment with 3’3-diaminobenzidine (Lab Vision Corporation, USA) and counterstained with hematoxylin (DAKO). In all cases, only the positivity or negativity of the antibodies used were considered except for MIB-1, which was retained as being significant for prognostic purposes only if the stained nuclei were more than 10%.

Fluorescence in situ hybridization (FISH). Chromosomes 1, 7, 8, 9, 17, 18 and HER-2/neu, EGFR and p16 genes were investigated. Interphase nuclei derived from 3 μm sections from a formalin-fixed, paraffin-embedded tissue block containing GIST and cartilaginous area were studied. For FISH procedure the manufacturer’s instructions were strictly followed. Chromosomes were analyzed by DNA in situ hybridization with directly labelled probes (Vysis Olympus, Milan, Italy) called Chromosomes Enumeration Probes (CEP) such as: CEP1 (D1Z5) Spectrum Orange Probe α satellite (bands p11.1-q11.1), CEP 8 (D8Z2) Spectrum Orange α satellite (bands 8p11.1-q11.1) and CEP 18 (D18Z1) Spectrum Green α satellite (bands 18p11.1-q11.1). FISH for p16 gene and chromosome 7 was applied using probes (Vysis Inc., Downers Grove, IL, USA) labelling p16 region (9p21) and the respective chromosome 9. The p16 gene probe spans approximately 190 kbp and contains a number of genetic loci including D9S1749, D9S1747, p16(INK4A), p14 (ARF), D9S1748 and p15 (INK4B) while chromosome 9 was identified by a centromeric α-satellite probe, according to the manufacturer’s recommendations. The kit consists of directly labelled fluorescent DNA probes specific for p16 gene (Spectrum Orange) and for the sequence at the centromeric region of chromosome 9 (Spectrum Green).

An FDA approved kit (PathVysion HER-2 DNA Probe Kit, Vysis Inc.) was used to evaluate HER-2/neu and chromosome 17, according to the manufacturer’s recommendations. The kit consists of directly labelled fluorescent DNA probes specific for the Her-2/neu gene locus (17q11.2-q12) and a DNA probe specific for the alpha satellite DNA sequence at the centromeric region of chromosome 17 (17p11.1-q11.1). EGFR is located on chromosome 7p12 and in FISH it is investigated by a Locus Specific Identifier (LSI®) DNA probe labelled by Spectrum Orange fluorochrome (Vysis Inc.). The centromeric region of chromosome 7 (7p11.1-q11.1) was also analysed with the CEP7 labelled by Spectrum Green fluorochrome. Following hybridization, the nuclei were counterstained with the DNA-specific stain propidium iodide or with the DNA-specific stain 4,6 diamidine-2-phenylindole (DAPI). A mounting medium was used for fluorescence (Vectashield, Vector Laboratories, Inc., Burlingame, CA, USA), which preserved the fluorescence for several months.

FISH signals, visible as fluorescent spots, were counted in at least 200 non-overlapping nuclei; the scoring system was based on the percentage of nuclei with altered signals. Reference values for abnormal FISH results were based on criteria of Qian and colleagues for tissue sections, to account for the potential artifacts due to nuclear overlapping. According to these criteria an abnormal autosomal gain requires a minimum 8% of nuclei with three or more signals, whereas abnormal autosomal loss required more than 55% of nuclei with zero or one signal (12).

The number of centromere signals was counted with a Nikon Optiphot-2 microscope, equipped with a filter selective for the fluorochromes used, and representative cells were captured using a digital camera.
Results

Histologically, the tumor was characterized by proliferation of spindle cells arranged in whorls, with evident positivity for CD117 and negativity for all other antibodies employed. The diameter of the neoplasia and the number of mytoses with positivity for MIB-1 evidenced a low-risk GIST. Interestingly, in this setting chondroid differentiation was also observed, which is unusual for this type of tumor (Figure 1). All the genes analyzed (HER-2/neu, EGFR and p16) were present in two copies, as in normal tissues, so no gene alterations were found. Investigating chromosomal alterations, normal behavior was observed (disomy, 2 spots) for chromosomes 7, 8, 9, 17 and 18, while a strong aneusomy (polysomy, more than two spots) was seen for chromosome 1 in both

Figure 1. Chondroid differentiation surrounded by gastrointestinal tumor. A, H&E (×20); B, CD117 (×20); C, H&E (×40).

Figure 2. FISH analysis. It is possible to recognize the polysomy for chromosome 1 (red arrows) in both GIST (A) and chondroid (B) areas. Chromosome 8 is shown and it is present in two copies as normal (disomy, white arrows) in both GIST (C) and chondroid area (D).
spindle and chondroid cells (Figure 2). The results for FISH are summarized in Table I.

Discussion

GIST are peculiar types of neoplasms, of interest to surgeons, pathologists and biologists. Although a population-based Swedish study suggested an incidence of about 13 cases per million persons per year (6), the actual impact on the population is probably underestimated, as proposed by some autopsy studies. There is no association with geographic location, ethnicity, race, or occupation (3, 4, 13-15).

The distribution in the GI tract favours the stomach and the small intestine; the colon and rectum are affected in only about 5% of cases, while the esophageal location of GIST is rare. The even rarer mesenteric or peritoneal implants are often not diagnosed before surgery (16-18).

Literature data suggest that most cases of GIST are not diagnosed preoperatively, even though a thorough work-up is usually planned (19, 20). In our case, the patient did not undergo echoendoscopy because the risk of re-bleeding seemed too high. The surgical approach adopted aimed at removing the cause of bleeding, with a resection as close as possible to the gold standard treatment for all forms of gastric parietal tumours.

A surgical classification divides GIST in: localized resectable, localized unresectable and metastatic disease. Among the localized forms, this classification provides four prognostic categories of risk (very low, low, middle and high), based on the size of the tumour and mitotic counts (21, 22). The preoperative determination of a prognostic score is not always possible because the material obtained by echoendoscopy usually does not allow the mitotic count.

Apart from the typical GIST features, to the best of the Authors’ knowledge the associated histological appearance of chondroid differentiation of the tumour has not been described before. It remains to be established whether this association will improve or worsen the patient’s prognosis. To date, after 8 months of follow-up, no worsening of the patient’s condition has been observed.

Contrary to other studies, a polysomy for chromosome 1 was recognized in GIST, curiously in both spindle and chondroid cells. The other chromosomes analyzed (7-9, 17, 18) and the genes HER-2/neu, EGFR and p16, often involved in tumour genesis, were not altered. This is interesting in understanding the peculiarity of the case.

References


