Morphologic shift associated with aberrant cytokeratin expression in a GIST patient after tyrosine kinase inhibitors therapy. A case report with a brief review of the literature

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ARTICLE INFO

Accepted 3 November 2015

Keywords:
GISTs
Imatinib-resistance
Aberrant cytokeratin expression
Therapy-induced changes
Immunephenotype

ABSTRACT

After an initial benefit from tyrosine kinase inhibitors (TKI), most gastrointestinal stromal tumors (GISTs) eventually develop disease progression or secondary resistance. An altered tumor (immune)phenotype with anaplasia and morphological changes secondary to therapy have occasionally been described in the literature.

We present a 52-year old patient, diagnosed with high risk, spindle-cell, GIST (CD117 positive, Pankeratin negative) in 2003, showing a *c-Kit* exon 11 mutation. After TKI therapy, he developed drug resistance and disease progression. Pathological assessment of the last surgical specimen showed a pure epithelioid/clear cell histology, without evidence of cellular anaplasia. Tumor cells were CD117 positive, DOG1 positive but also E-cadherin positive and Pankeratin positive, whereas molecular analysis confirmed the presence of the *c-Kit* exon 11 mutation, with no additional mutations. We describe an unusual case of GIST showing peculiar (immuno)phenotypic changes under therapy, different from the vast majority of therapy-driven changes, which include marked cellular pleomorphisms and KIT immunonegativity. Possible molecular explanations to understand these phenomena and a brief review of the literature are also addressed.

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal (GI) tract, with an estimated incidence of 11 to 19.6 per million population [1]. They are typically composed of spindle/epithelioid cells and are thought to originate from the interstitial cells of Cajal (ICC), which function as the pacemaker of the GI tract. Because GISTs have a wide spectrum of clinical presentation, as well as a variable biological behavior, ranging from completely benign to highly aggressive tumors, various risk stratification systems for the assessment of their malignant potential have been developed, the most widely used of which are those by Joensuu [2] and those by Miettinen and Lasota [3]. The discovery of activating mutations in the receptor tyrosine kinases KIT [4] and platelet-derived growth factor receptor-α (PDGFRA) [5], occurring in the vast majority of GISTs, dramatically changes diagnosis, treatment and prognosis for GISTs. Between 10 and 15% of GISTs lack KIT and PDGFRA mutations (the so called "wild-type" GISTs) and carry different genetic alterations, such as BRAF V600E substitution, RAS mutations and defects in the succinate dehydrogenase (SDH) complex of respiratory chain complex II [1]. The standard treatment for localized, low-risk GISTs is a complete surgical excision, without dissection of regional lymph nodes, whereas adjuvant treatment with TKIs, such as Imatinib mesylate, is recommended in metastatic disease or as neoadjuvant treatment if complete surgery is not feasible [6]. After an initial benefit from Imatinib, most patients eventually develop disease progression or

Abbreviations: GIST, gastrointestinal stromal tumors; GI, gastrointestinal; ICC, interstitial cells of Cajal; NIH, National Institute of Health; PDGFRA, platelet-derived growth factor receptor-α.

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Table 1
Morphological, immunohistochemical and molecular findings reported in literature in GISTs before (A) and (B) after therapy.

Reference no	Age / Gender	Location	Tumor size (cm)	Morphology	Immunohistochemical markers	Genotype
Ref. [8]–(IA)	37/M	Stomach	N/A	Spindle cells	CD117+, CD34+, SMA+ (focal), S100– Desmin–, Keratins–	KIT exon 11 del WK 557–558
(IB)		Intrabdominal	16	Large epithelioid cells	CD117–, CD34–,SMA–, S100–, Desmin–, Keratins–	KIT exon 11 del WK 557–558
(IIA)	73/M	Rectum	7,5	Spindle cells	CD117+, CD34+, SMA+ (focal), S100+, Desmin–, Keratins–	KIT exon 11 K558N
(IIB)		Liver and peritoneum	8,5	Large epithelioid cells (eosinophilic cytoplasm)	CD117–, CD34+, SMA+ (focal), S100–, Desmin+, Keratins–	KIT exon 11 K558N
Ref. [9]–(IA)	70/M	Stomach	9	Spindle cells	CD117+, CD34+, SMA–, S100–, Desmin–	KIT exon 11 del WK 557–558
(IB)	70/M	Intrabdominal	11,5	Spindle + anaplastic epitheliod cells + heterologous elements	CD117–, CD34–SMA–, S100–, Desmin–	KIT exon 11 del WK 557–558
Ref. [10]–(IB)	66/M	Sigmoid wall Intrabdominal	5 4	Epithelioid cells Epithelioid cells	CD117+/– CD117–	IA) KIT exon 9 ins AY502–503 IB) NMF
IIB)	50/M	Stomach	12,3	Epithelioid + giant cells	CD117+	IB) KIT exon 17 N822K IIA) KIT exon 11 del KPMYEVQWK550–558 IIB) KIT exon 13 V654A
IIIB)	67/F	Stomach Liver Intrabdomial	35 2,7 3,0	Epitheliod cells(cytoplasmic inclusions) Epitheliod cells (cytoplasmic inclusions + atypia)	CD117–	IIIA) KIT exon 11 del WKV557–559 IIIB) NMF IIIB) NMF IIIB) NMF
IVB)	54/M	Intrabdominal	2,5	Epitheliod cells (cytoplasmic inclusions + atypia)	CD117+	IVA) KIT exon 11 del WKV557–559 IVB) KIT exon 17 D820Y
VB)	74/M	Stomach	13	Epithelioid cells	CD117+	VA) KIT exon 13 K642E VB) NMF
VIB)	63/M	Ileal serosa	1	Epithelioid cells	CD117+	VIA) KIT exon 13 K642E VIB) KIT exon 17 D816H
VIIB)	60/M	Liver metastases Sigmoid wall	2.1–2.8 14	Epithelioid cells	CD117+ CD117+	VIIA) NMF VIIB) NMF VIIB) NMF
VIIIB)	65/M	Perigastric Liver metastases	2.4–3.5 19.8	Epithelioid cells Epithelioid cells Epithelioid cells Epithelioid cells with mild atypia	CD117+ CD117+	VIIIA) NMF VIIIB) NMF VIIIB) NMF
Ref. [11]–2 cases IA IB	62/F	Intrabdominal Intrabdominal	20 13	Spindle cells Spindle + anaplastic epithelioid cells	CD117+, CD34+, SMA+ (focal), S100– Desmin–, Pankeratin (KL–1)– CD117–, DOG-1–, PDGFRA–, CD34–, SMA–, S100–, Desmin–, Pankeratin (KL-1)+, vimentin+, CK7–, CK 20–, 34bE-12	not available NMF
Ref.12–IA	60/M	Small bowel	7.5	Spindle cells	CD117+, CD34+, Desmin–, Keratins–	IA) KIT exon 11 V559G KIT exon 13 V654A
IB		Liver, peritoneal metastases	N/A	Epithelioid pleomorphic cells	CD117–, CD34–, Desmin–, Keratins –	IB) KIT exon 11 V559G KIT exon 13 V654A
IIA	65/M	Colon	25	Spindle cells	CD117+, CD34+, Desmin–, Keratins –	IIA) KIT exon 11 29bp del KIT exon 13 V643Sfs6
IIB		Peritoneal metastases	N/A	Epithelioid pleomorphic cells	CD117–, CD34–, Desmin–, Keratins–	IIB) IIA) KIT exon 11 29bp del KIT exon 13 V643Sfs6

secondary resistance, due to acquired mutations in KIT or PDGFRA, occurring almost exclusively in the same gene and allele as the primary oncogenic driver mutation [1]. Multiple surgeries combined with sequential regimens of TKIs (including imatinib resumption) may significantly improve patients' survival in advanced GISTs [7].

An altered tumor phenotype secondary to therapy has occasionally been described in the literature (Table 1; [8–12]), showing frequent loss of CD117 (Kit) expression, coupled with morphological changes in neoplastic cells. Such morphological changes are an important finding because they represent potential diagnostic pitfalls, with relevant implications on different protocols of therapy.

We present an interesting GIST case, showing peculiar morphologic changes and resistance phenomena after TKI therapy, with a brief review of similar cases reported in the literature and a hint at possible molecular explanations to understand these phenomena.

Clinical history

In August 2003, a 52-year old man was referred to our Institute for surgical resection of a major (15 cm) ileal mass and multiple smaller peritoneal nodules. Subsequently, the patient underwent five surgeries for recurrences, three lines of therapy (Imatinib, 400 mg/day and 800 mg/day; Sunitinib, 50 mg 4 weeks on, 2 weeks off, later reduced to 37.5 mg/day weekly, due to intolerance; Nilotinib, 800 mg/day). After the last surgery, Imatinib (800 mg/day) was resumed and continued for 8 months with radiological stable disease. However, in August 2011, due to the deterioration of the patient's general condition, anemia, neutropenia and sub-occlusive symptoms, Imatinib was stopped and the patient was treated only with palliative care until death, which occurred on November 2011, 8 years after primary diagnosis.

Materials and methods

Histopathology and immunohistochemistry

Surgical specimens were sampled according to current protocols. Formalin-fixed, paraffin-embedded tissue samples were obtained, and 4-μm sections were stained with hematoxylin and eosin. 2.5-μm sections were cut and immunohistochemical analysis was performed in an automated system (Benchmark-XT, Ventana, Tucson, AZ, US). The following primary antibodies were used: CD117 (pathway c-kit, clone 9.7, pre-diluted; Ventana, Tucson, AZ, US), DOG-1 (monoclonal antibody, clone SP31; prediluted; CellMarque, Rocklin, CA, USA), CD34 (monoclonal antibody, clone QBEND/10; 1:10 dilution; Diagnostic BioSystems, Pleasanton, CA, US), Pankeratin (CkAE1/AE3/pCk26, pre-diluted, Ventana, Tucson, AZ, US), smooth muscle actin (SMA) (monoclonal antibody, clone 1A4, 1:400 dilution; DAKO, Glostrup, Denmark), S100 (polyclonal antibody, 1:2000 dilution; DAKO), Desmin (monoclonal antibody, clone DE-R-11, prediluted, Roche Diagnostics, Mannheim, Germany). Color was developed with 3.3′-diaminobenzidine (DAB) and slides were counterstained with Meyer's hematoxylin. Appropriate positive and negative controls were concurrently done.

Sections from the last relapse only were stained, in addition, for PDGFRA(polyclonal antibody, 1:100 dilution, Spring Bioscience, Pleasanton,CA, US), E-cadherin (monoclonal antibody, clone 36, pre-diluted, Ventana, Tucson, AZ, US).

Molecular analysis

Tumor samples were enriched with neoplastic component by manual microdissection to ensure a neoplastic component higher than 70%. Three consecutive unstained 10-μm thick FFPE sections of each specimen were scraped in a 1.5 ml tube using the hematoxylin and eosin stained slide as guide. DNA was isolated from formalin-fixed, paraffin-embedded tumor tissues with the QIAmp DNA mini-kit (Qiagen, Milan, Italy), according to the manufacturer's instructions.

The DNA underwent a polymerase chain reaction (PCR) treatment with primer pairs specific for KIT exons 9, 11, 13 and 17 and PDGFRA exons 12, 14 and 18. The amplified products were then sequenced with a BigDye Terminator v1.1 Cycle Sequencing Kit and fluorescent capillary electrophoresis (ABI PRISM 310 genetic analyses; Applied Biosystems, Foster City, CA, USA) with specific forward and reverse primers. Sequencing results were compared with the NCBI GeneBank KIT (Accession number X06182) and PDGFRA (Accession number M21574) gene mRNA sequences. In addition, tumor samples from the last relapse were investigated for genetic mutations on *BRAF*, *HRAS*, *NRAS*, *KRAS* genes.

Results

Pathological evaluation of the first (pre-treatment) resection showed a neoplastic proliferation with spindle cell morphology, arranged in a fascicular pattern, in the absence of necrosis and pleomorphism (Fig. 1A and B). The mitotic activity was 10 mitoses per 5 mm^2. Neoplastic spindle cells were found to be CD117, DOG-1 and CD34 positive (Fig. 1C–E, respectively), Desmin and Pankeratin negative (Fig. 1F), whereas smooth muscle actin and S100 were focally positive. The patient was diagnosed with GIST (now reclassified, in accordance with the recently proposed criteria, as high risk GIST) [13]. A *c-kit* exon 11 mutation (p.Val560Asp) was identified supporting this diagnosis.

The molecular analysis of the second relapse confirmed the presence of *c-kit* exon 11 mutation, while in some of the largest nodules removed, during the third and fourth surgeries, a second *c-Kit* exon 13 mutation (p.Val654Ala) – known to be associated with Imatinib resistance – was identified in addition to the *c-Kit* exon 11 mutation [14,15]. Histologic response to therapy was evaluated and graded on the basis of macroscopic and microscopic percentage of necrosis and fibrosis, as reported elsewhere [15]. All recurrent specimens showed a minimal response (0–10% response) to tyrosine kinase inhibitors.

The pathological examination of the mass removed during the last surgery showed a quite different cytological morphology compared to previous lesions. In fact, it demonstrated a pure epithelioid/clear cell histology, without evidence of cellular anaplasia (Fig. 2A). Immunohistochemical analyses showed that tumor cells were CD117 positive (Fig. 2B), CD34 positive (not shown) and DOG1 positive (not shown), but also E-cadherin positive and Pankeratin (CkAE1/AE3/pCk26) positive (Fig. 2C and D respectively), the latter showing a faint and patchy positivity. Molecular analysis confirmed the presence of the *c-Kit* exon 11 mutation, with no additional mutations in the *c-Kit*, *PDGFRA*, *BRAF*, *HRAS*, *NRAS*, *KRAS* genes.

Discussion

This patient's clinical history is paradigmatic of resistance phenomena that may develop in GIST patients. There are several possible mechanisms for the development of acquired resistance to Imatinib mesylate, including an increased gene copy number or secondary mutations to the receptor. In our patient, after the second recurrence, an additional *c-Kit* exon 13 mutation (Val654Ala) associated with Imatinib resistance was detected. This is the most frequent secondary mutation in patients with a primary c-Kit exon 11 mutation and who eventually progress during Imatinib [15] or Sunitinib [10] treatment.

GIST surgical samples after Imatinib treatment have shown variable morphological alterations as either evidence of histological response (hyalinization, necrosis, hemorrhages) or as a consequence of chronic tyrosine kinase inhibitors therapy. In the literature, diverse cytoarchitectural and immunophenotypic changes have been reported in post-Imatinib GISTs. Tumors with spindle morphology may show a shift toward a pure epithelioid – usually anaplastic/pleomorphic – phenotype, with or without immunophenotypic changes: in particular, many tumors show complete loss of KIT expression by immunohistochemistry (Table 1; [9–12]). Less commonly, the primary tumor and/or its metastasis may undergo heterologous differentiation [9]. Keratin positivity can be seen in primitive GISTs with antibodies reacting with keratins 8/18 but keratins 7, 13, 14, 17, 19, and 20 are not present.

Therefore, antibody cocktails, such as AE1/AE3, would usually give negative results [16]. In our case, after three lines of therapy, a pure epithelioid/clear cell morphology emerged, as demonstrated

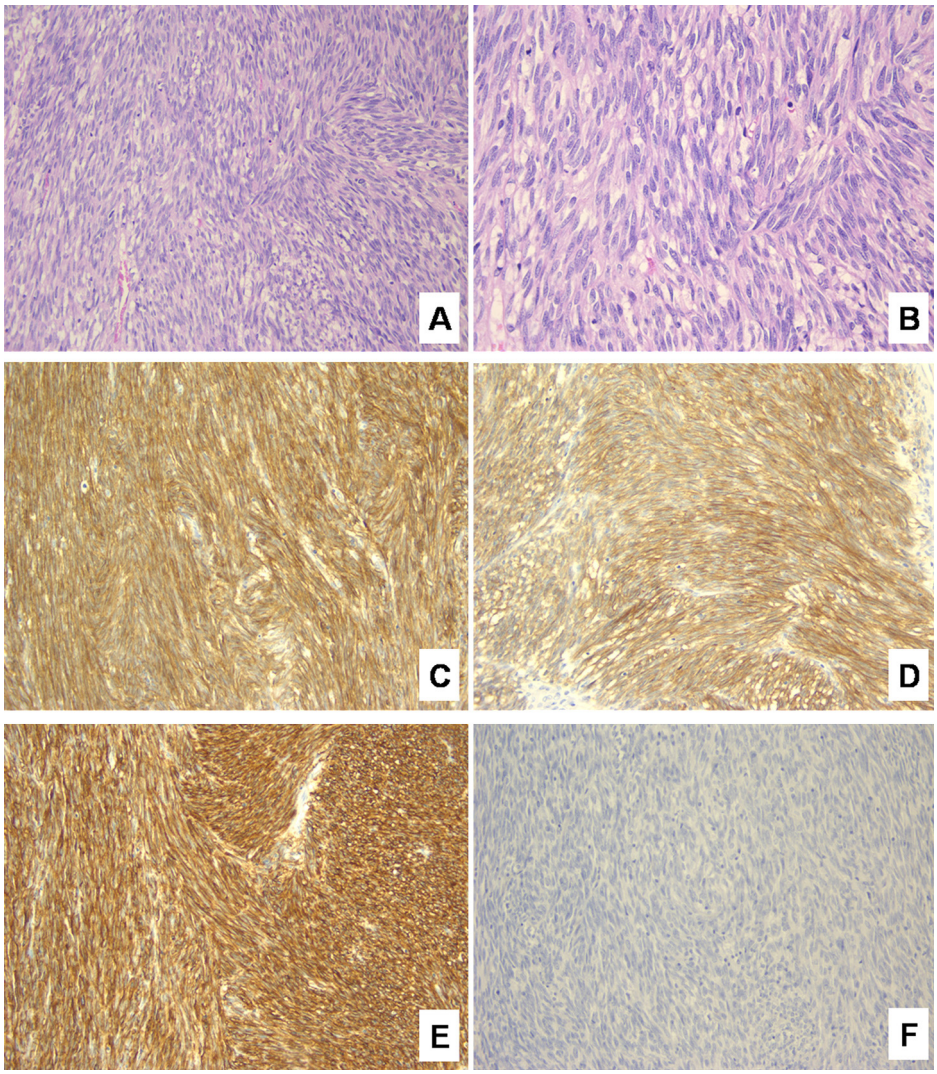

Fig. 1. Morphologic and immunohistochemical findings from the pre-treatment resection specimen. (A) and (B) Histological assessment of the first (pre-treatment) resection showed a neoplastic proliferation with a bland spindle cell morphology, arranged in a fascicular pattern, without necrosis and pleomorphism. (C) Neoplastic cells dysplayed a positive immunostaining for CD117, (D) DOG-1 and (E) CD34. (F) Immunohistochemical staining for Pankeratins (CkAE1/AE3/pCk26) was negative.

by the acquisition of the "epithelial" phenotype (Pankeratin and E-cadherin positive) with retained KIT expression.

A smooth muscle phenotype in GISTs after Imatinib has already been demonstrated: experimental studies have shown that blockage of KIT signaling leads to transdifferentiation of ICC to smooth muscle cells [17]. It cannot be excluded that the present case represents a similar phenomenon, toward epithelial rather than smooth muscle morphologic shift, as a consequence of other mechanisms. Despite the central role of oncogenic KIT activation in GIST pathogenesis, different and complex intracellular signaling events may underlie such morphologic changes.

The histology of recurrences shows only limited signs of classic therapeutic response, such as hyalinization, necrosis and hemorrhages, thus the morphological shift may be a "reactive" process rather than a regressive one. Notably, it is quite probable that the appearance of pure epitheloid/clear-cell morphology is a therapy-driven result, as an effect of cellular selection from a preexisting clone, even if this hypothesis should be confirmed by further observations.

Finally, these immunophenotypic changes could represent a diagnostic pitfall for pathologists. As suggested by Antonescu et al. [12] and Pauwels et al. [8], the possibility of a collision tumor (GIST + carcinoma/sarcoma), a desmoid-type fibromatosis, as well as a carcinomatous or melanoma-like proliferation (in case of epithelioid differentiation) should be considered in the differential diagnosis. An appropriate panel of immunohistochemical markers, associated with mutational analysis when needed, is usually sufficient to rule out such alternative diagnostic hypotheses.

In conclusion, we described an unusual case of GIST showing peculiar (immuno)phenotypic changes under therapy. The relevance of these changes relies on the fact that this case acquired a pure epithelioid phenotype, without anaplasia and with retention of KIT expression, in contrast with the vast majority of therapy-driven changes, which include marked cellular pleomorphisms and KIT immunonegativity, beside epithelioid morphology. Awareness of this phenomenon should prevent diagnostic dilemmas by pathologists. Finally, such peculiar morphologic changes need to be elucidated in further studies, as they may possibly impact on GIST therapy.

Consent

Written informed consent was obtained from the patient for publication of this Case Report and any accompanying images.

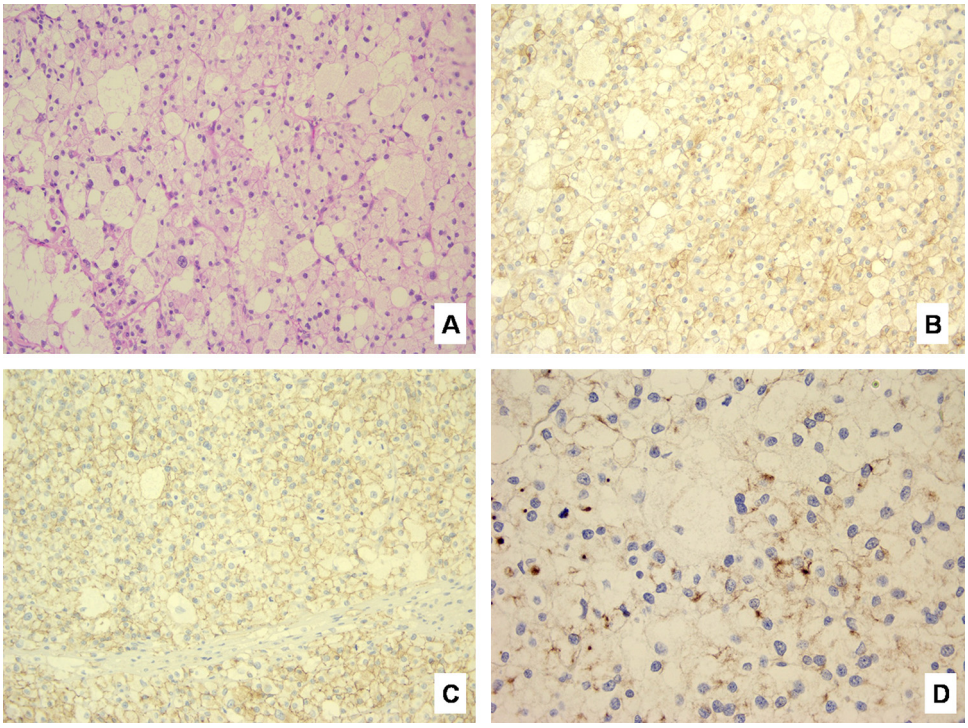

Fig. 2. Morphologic and immunohistochemical findings from the last resection specimen.
(A) The last resection specimen showed a pure epithelioid/clear cell histology, without evidence of cellular anaplasia. (B) Immunohistochemical analyses demonstrated that tumor cells were CD117 positive, (C) but also E-cadherin positive and (D) Pankeratin (CkAE1/AE3/pCk26) positive (patchy and faint).

Competing interests

The authors declare that they have no competing interests.

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