Original Research

Rhabdomyosarcomatous differentiation in gastrointestinal stromal tumors after imatinib resistance: a potential diagnostic pitfall

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Abstract

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumor of the digestive tract and characterized by expression of protein-tyrosine kinase (KIT) protein. Treatment of advanced GISTs has been improved dramatically following the development of imatinib. Despite the often long-lasting clinical benefit seen in most patients treated with imatinib, many will eventually suffer disease progression. In general, progressing GISTs retain their typical morphology. In this study, we present a patient with metastatic GISTs, who received more than 16 months of treatment with imatinib and whose tumors changed their morphological and immunohistochemical characteristics after imatinib-resistance. Histological, immunohistochemical and mutational analysis was performed on the prior and post-imatinib treatment GIST samples. The imatinib-resistant tumor cells in the progressing metastases showed marked pleomorphism which proved to be rhabdomyoblastic differentiation with Desmin and Myogenin immunopositivity. However, there was no secondary mutation of *KIT*, *PDGFRA*, *KRAS* and *BRAF* genes found in the imatinib-resistant lesion, except primary *KIT* V559D mutation. To our knowledge, this case represents the few reports on this unusual type of transdifferentiation in GISTs under imatinib therapy. Awareness of this phenomenon would help to avoid diagnostic confusion when evaluating post-imatinib samples from GISTs.

Keywords: GIST, rhabdomyosarcomatous differentiation, KIT, imatinib resisitance

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Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumor of the digestive tract and characterized by expression of protein-tyrosine kinase (KIT) protein. Most GISTs have oncogenic *KIT* mutations that engender constitutive activation of this receptor tyrosine kinase, resulting in increased cell proliferation and survival and such mutations appear to play a key role in the pathogenesis of the majority of GISTs. Platelet-derived growth factor receptor alpha (PDGFRA) mutations were also found in a small subset of GISTs without *KIT* mutations.[1,2] Before the advent of imatinib, long-term outcomes of patients with unresectable or metastatic GISTs were poor because the tumors were typically resistant to conventional chemotherapy and radiation therapy. Treatment of advanced GISTs has been improved dramatically following development of imatinib, a potent small molecule inhibitor of type 3 receptor tyrosine kinases, including KIT and PDGFRA. Despite the often long-lasting clinical benefit seen in most patients treated with imatinib, many will eventually suffer disease progression. The mechanisms of acquired resistance to imatinib are heterogeneous, with most involving the emergence of secondary missense mutations in *KIT* or *PDGFRA*. Other proposed alternative resistance mechanisms include KIT/PDGFRA genomic amplification and activation of alternative oncogenes.[3,4]

Although progressing GISTs often acquire secondary *KIT* or *PDGFRA* gene mutations as a mechanism for resistance to imatinib, they generally retain previous morphology after imatinib treatment. Here, we report a case of metastatic GIST with heterologous rhabdomyosarcomatous differentiation after resistance to imatinib.

Materials and methods

Patient

A 55-year-old man diagnosed with small intestinal GIST was admitted with a two-month history of left abdominal

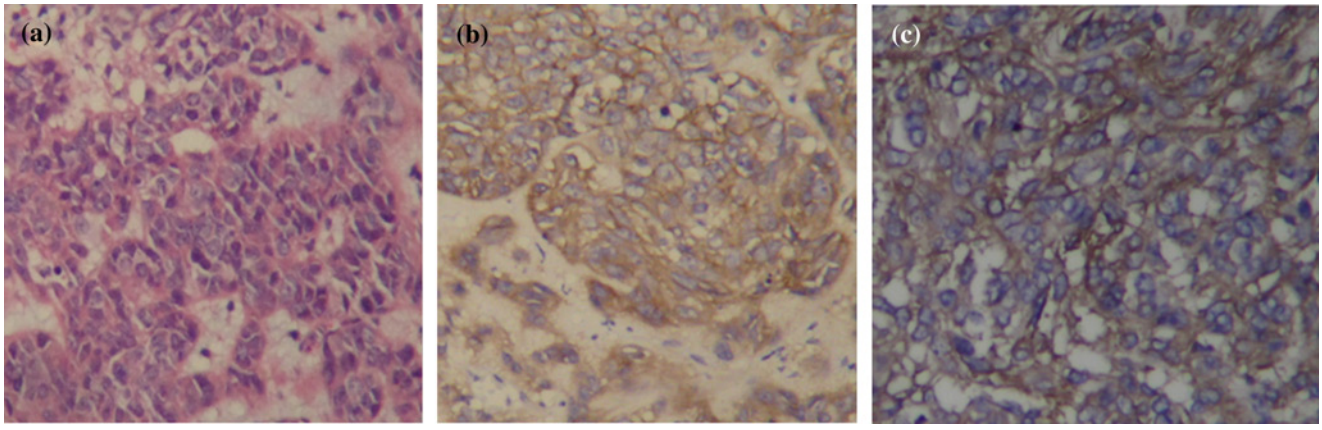

Figure 1 Primary jejunal tumor resection specimen. Typical gastrointestinal stromal tumors showing epithelioid cell morphology with palely eosinophilic cytoplasm and round nuclei (a). Strong staining for CD117 (b) and CD34 (c). (A color version of this figure is available in the online journal)

pain along with four weeks of reduced appetite. Laboratory tests including tumor markers (CEA, CA19-9, CA242 and AFP) were within normal limits. Chest and abdominal radiographs, as well as upper gastrointestinal panendoscopy and coloscopy, revealed no pathological findings. Ultrasonography showed a non-homogeneous hypoechoic mass with partial cystic areas occupying the left abdomen. A contrast-enhanced computed tomography (CT) scan confirmed the presence of a intra-abdominal mass $6 \times 6 \times 5$ cm in size with peripheral contrast enhancement. The bowel was dislocated without obvious signs of intestinal occlusion. It was thought that the tumor was primarily originated from the small intestinal. No liver or lymph node metastases were detected. The abdominal tumor was surgically removed and diagnosed as jejunal GIST with CD117 positive. The patient had not accepted adjuvant imatinib treatment because of economic reason. After primary resection, the patient presented two years later with liver metastases. A percutaneous CT-guided core needle biopsy was performed. Pathology was re-reviewed and the tumor was classified as recurrent GIST. Treatment with imatinib 400 mg daily was initiated and the patient had stable disease for 16 months until the hepatic metastases were out of control. The patient underwent surgical debulking. Sunitinib therapy was initiated after surgery and the lesions of the liver were stable again.

Histopathological analysis

Tumor specimens were fixed in formalin and embedded routinely for histological evaluation. The GIST case was evaluated for the following: tumor cell types, cytological atypia and mitotic rate (expressed as the number of mitotic figures per 50 high-power fields [HPFs]). Risk stratification was performed according to the recent National Comprehensive Cancer Network guidelines.[5] Immunohistochemical stains were performed on 4 μm sections cut from paraffin blocks, using the primary antibodies CD117, CD34, Desmin, α-smooth muscle actin (SMA) and Myogenin for the pretreatment and post-treatment tumor specimens. Immunohistochemical staining was classified as strong (+++), moderate (++), weak (+) or negative. The categorization of histopathological subtype was based on standard and widely accepted criteria.[6]

Molecular analysis

For mutational analysis, genomic DNA was extracted from fresh tissue or formalin-fixed paraffin-embedded tumor tissue sections. Mutational analysis of *KIT* exons 9, 11, 13, 14, 17 and *PDGFRA* exons 12, 14 and 18 was performed by polymerase chain reaction amplification, denaturing high-performance liquid chromatography screening and automated sequencing as previously described.[2] All polymerase chain reaction (PCR) products were screened for mutations using denaturing high-pressure liquid chromatography (DHPLC) and WAVE System 3500HT. Partially denaturing conditions for mutation detection were predicted using Navigator Software v.1.6.0. PCR products were analysed under at least two different temperatures. Simultaneously and independently from DHPLC, PCR products were screened for mutations by direct sequencing as previously reported. In addition, the tumor was also investigated for *KRAS* and *BRAF* mutations as described previously.[7,8]

Results

Histopathological features of prior treatment tumor samples

The primary jejunal tumor resection specimen was composed of epithelioid cells with palely eosinophilic to clear cytoplasm and round nuclei, arranged in sheets. Small areas of necrosis and old haemorrhages associated with focal inflammatory infiltration were seen (Figure 1a). The tumor size was 6.5 cm the mitotic count was 6/50 HPF. Based on the tumor size and mitotic activity, the tumor corresponded to a high-risk GIST in both the National Institute of Health and Armed Forces Institute of Pathology risk stratification systems.[9] The immunohistochemistry revealed strong or moderate expression of CD117 (Figure 1b) and CD34 (Figure 1c) in most of tumor cells, with limited focal SMA reactivity, while Desmin and Myogenin protein were negative. The pre-imatinib core biopsy of liver metastases also revealed epithelioid cells morphology similar to primary tumor. The mitotic count was 8/50HPF. The immunohistochemistry revealed strong or moderate expression of

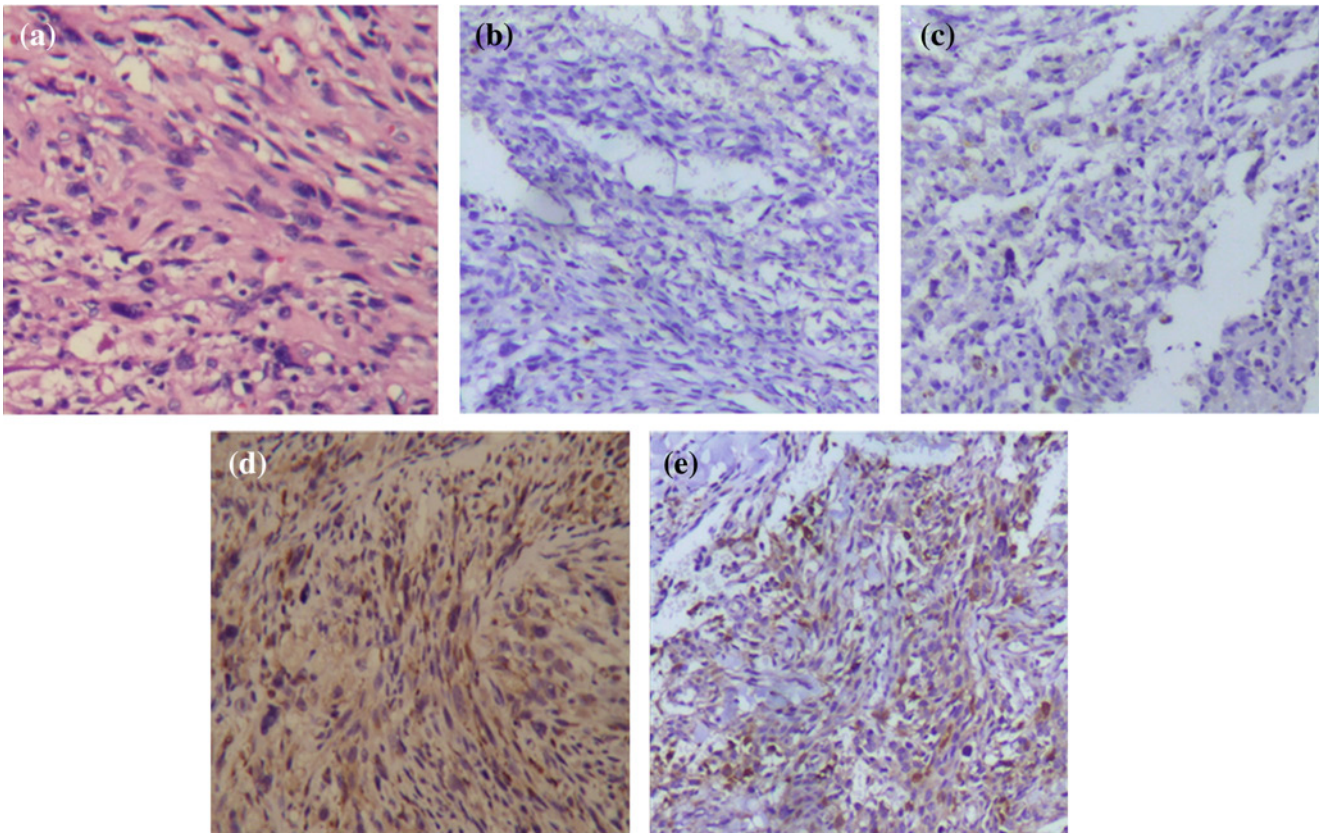

Figure 2 The imatinib-resistant tumor cells in the progressing liver metastases showed marked pleomorphism with rhabdomyoblastic differentiation (a). The rhabdomyoblastic differentiation tumor cells were completely negative for CD117 (b) and CD34 was only very weakly expressed (c). Immunostaining demonstrates strong desmin expression in a GIST metastasis with rhabdomyoblastic differentiation (d). Immunostaining shows striking nuclear Myogenin expression in a GIST metastasis with rhabdomyoblastic differentiation (e). (A color version of this figure is available in the online journal)

CD117 and CD34 in most of tumor cells, while SMA, Desmin and Myogenin protein were negative.

Histopathological features of imatinib-resistant tumor samples

The imatinib-resistant tumor cells in the progressing liver metastases showed marked pleomorphism which proved to be rhabdomyoblastic differentiation. The rhabdomyoblastic component was composed of spindle cells with round to oval nuclei, focally prominent nucleoli and amphophilic to deeply eosinophilic cytoplasm with a bipolar or tadpole configuration resembling embryonal rhabdomyosarcoma (Figure 2a). The rhabdomyoblastic differentiation tumor cells were completely negative for CD117 (Figure 2b). CD34 was only very weakly expressed (Figure 2c). They expressed strongly and diffusely Desmin (Figure 2d) and SMA. Myogenin were also expressed (Figure 2e).

Mutational analyses

Molecular analysis was performed on genomic DNA gained from the prior and post imatinib treatment GIST samples. The primary jejunal tumor resection specimen harbored a heterozygous *KIT* gene exon 11 mutation Val559Asp(V559D) (Figure 3). The recurrent metastases prior imatinib treatment revealed the same V559D without secondary mutation.

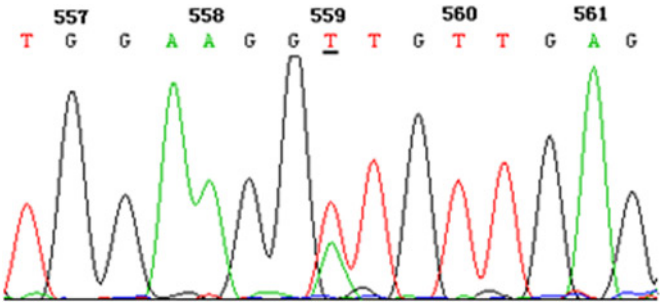

Figure 3 Molecular analysis showed *KIT* exon 11 point mutation V559D in the primary jejunal tumor. (A color version of this figure is available in the online journal)

The imatinib-resistant GIST specimen was also examined. The primary *KIT* gene exon 11 mutation V559D can still be found in the metastatic lesions. No secondary mutation was found in *KIT* exons 9, 11, 13, 14, 17, *PDGFRA* exons 12, 14, 18, *KRAS* exon 2 and *BRAF* exon 15.

Discussion

Following the discovery of constitutive KIT activation as the central oncogenic mechanism in the pathogenesis of GISTs, inhibition of the KIT signaling using diverse tyrosine kinase inhibitors, in particular, imatinib mesylate, has emerged

as an effective treatment for patients with primary unresectable and metastatic GISTs. After the initial positive response, however, most of the patients develop resistance to the drug in the later course of treatment, which is frequently associated with tumor re-growth or appearance of new, metastatic lesions. However, morphological and immunophenotypic features changes after imatinib-resistance in GISTs have rarely been reported. It is not clear if these imanitib-resistant lesions, either recurrent or metastatic, always preserve the characteristic morphological and immunophenotypic features of the original tumor. Here, we report a unique case with metastatic GISTs that underwent unusual phenotypic change following imatinib treatment.

Although imatinib-resistant GISTs often acquire secondary gene mutations as a mechanism for resistance, they generally retain previous morphology and immunophenotype after imatinib treatment. Various cytomorphological and immunophenotypic changes have been reported in some post-imatinib GISTs. In particular, tumors with spindle morphology may show a shift towards epithelioid phenotype, with or without immunophenotypic changes. In these circumstances, many tumors show variable to complete loss of KIT expression by immunohistochemistry.[10–12] Less commonly, the primary tumor and/or its metastasis may undergo metaplastic changes, trans-differentiation or heterologous differentiation (dedifferentiation).[12,13] In the current study, we describe the unusual histopathological features changes of the GIST who underwent prolonged imanitib treatment. The case showed a predominant epithelioid cell phenotype in the original tumor. However, the progressive lesions in the liver, which developed during imatinib treatment, lost their epithelioid cell character, being replaced by marked pleomorphous morphology that resembled rhabdomyoblastic differentiation. Moreover, the debulking tumor specimens taken after imatinib resistance revealed complete loss of KIT immunoreactivity but still preserved the primary *KIT* gene V559D mutation. In the progressive lesion of liver a remarkable Desmin and Myogenin immunopositivity in tumor cells was encountered, although the original GIST was completely negative for them. Rhabdomyoblastic differentiation was confirmed in the case by immunohistochemical staining for Desmin and Myogenin. Myogenin accumulates in the nucleus of differentiated cells and has been shown to be a reliable marker for skeletal muscle differentiation.[14]

Imatinib resistance has been shown to occur, on average, after two years of treatment and has been demonstrated to be associated mainly with the acquisition of secondary *KIT* kinase mutations and to occur most frequently in GISTs with primary *KIT* exon 11 mutations. Agaram *et al.* described the presence of a *BRAF* V600E mutation in addition to the primary *PDGFRA* exon 18 deletion in a imatinib-resistant GIST lesion. Thus a secondary V600E *BRAF* mutation could represent an alternative mechanism of imatinib resistance.[7] However, there was no secondary mutation of *KIT*, *PDGFRA*, *KRAS* and *BRAF* genes found in the imatinib-resistant lesion, except primary *KIT* V559D mutation. In the absence of known resistance mechanisms, an explanation for this finding could be the clonal selection of a pre-existing tumor subclone during imatinib treatment regardless of secondary mutation.[10]

Transdifferentiation of GISTs to a smooth muscle pattern phenotype under imatinib therapy is a well-known phenomenon documented in previous studies.[15] Interestingly, experimental studies have shown that blockage of KIT signaling leads to trans-differentiation of interstitial cells of Cajal to smooth muscle cells.[16] It cannot be excluded that the current case represents a similar phenomenon as a consequence of other mechanisms. Indeed, despite the central role of oncogenic KIT activation in GISTs pathogenesis, different and complex intracellular signaling events may be triggered by oncogenic KIT.[17,18] Also, there is evidence that a specific *KIT* mutation type can influence specific signal transduction pathways that are activated in that particular subset of GISTs,[19] but little is known about these alternative oncogenic mechanisms in GISTs currently. Mutational analysis of the current imatinib-resisitant samples showing rhabdomyoblastic differentiation did not find specific molecular mechanisms, which might account for this unusual line of differentiation. Nevertheless, this finding, in combination with loss of KIT expression, suggests the possibility of activation of novel pathways driven by a KIT-independent oncogenic mechanism.

Other mesenchymal tumors that should be considered in the differential diagnosis of GISTs with heterologous rhabdomyoblastic differentiation are dedifferentiated liposarcoma (DDLPS) and malignant peripheral nerve sheath tumor (MPNST). Both of these tumor types may contain heterologous elements and occur in the abdomen. Approximately 10% of DDLPS and 10–15% of MPNST show heterologous elements, most often a rhabdomyosarcomatous component. However, as rhabdomyoblastic differentiation in GISTs seems limited to those tumors that have progressed on imatinib therapy, so long as adequate clinical history is provided, then awareness of this phenomenon should lead to the correct diagnosis. Especially under these circumstances, mutational analysis of *KIT* and *PDGFRA* genes is a useful diagnostic tool to confirm the correct diagnosis.

In conclusion, we present a patient with classical GIST developing imatinib-resistant metastases, whose tumors showed a completely altered morphology and immunophenotype following imatinib treatment. Although this phenomenon is a relatively infrequent finding, awareness of this phenomenon would help to avoid a potential diagnostic pitfall. Molecular analysis might be helpful to demonstrate the link between the primary tumor and the metastasis.

Author contributions: SZ: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, and final approval of manuscript. KH: collection of data, data analysis and interpretation, final approval of manuscript. JJ: Provision of study material, collection of data, data analysis and interpretation, and final approval of manuscript. XL: Collection of data, data analysis and interpretation and final approval of manuscript. DT: Provision of study material and technology, and final approval of manuscript.

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