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ORIGINAL ARTICLE

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Rare KIT (CD117) expression in multiple myeloma abrogates the usefulness of imatinib mesylate treatment

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Abstract *Background:* Imatinib mesylate blocks the tyrosine kinase activity of KIT (CD117) and is an effective treatment for gastrointestinal stromal tumors. In multiple myeloma, KIT expression has been detected by flow cytometry in about 33% of specimens, but no previous immunohistochemical assessment has yet been made of the expression pattern of KIT. *Materials and methods:* We performed immunohistochemical analyses of 100 patients, including 72 with multiple myeloma (MM), 8 with lymphoplasmacytic lymphoma (LPL), 10 with monoclonal gammopathy of undetermined significance (MGUS) and 10 with reactive plasmocytosis. One KIT-positive MM was sequenced using polymerase chain reaction analysis. *Results:* In MM, only 2 cases (2.8%) were KIT positive. The great majority of the cases (97, 2%) did not express the KIT receptor tyrosine kinase. No mutation of the c-kit gene was detected. *Conclusions:* KIT expression is a rare event in MM and not detectable in MGUS and LPL. Therefore, treatment with imatinib is unlikely to be effective in these patients.

Keywords KIT (CD117) · Multiple myeloma · Imatinib mesylate

Introduction

Imatinib mesylate (Glivec) is a specific inhibitor of tyrosine kinases, such as Abl, KIT (CD117) and platelet-derived growth factor receptor (PDGF-R). Several studies have shown the effectiveness of imatinib mesylate as a molecular target treatment in chronic myelogenous leukemia [9] via blockade of bcr-abl activity, in gastrointestinal stromal tumors (GIST) [4, 15] via KIT inhibition and in dermatofibrosarcoma protuberans [14] and hypereosinophilic syndrome [3] via interference with PDGF-R activation. This has raised hopes that other malignancies resulting from oncogenic activation of these kinases may benefit from molecular target therapy.

Multiple myeloma (MM) is a malignant plasma cell neoplasia, characterized by osteolytic lesions, marrow plasmocytosis and monoclonal gammopathy [7]. Currently, the therapy for MM includes conventional low-dose chemotherapy or high-dose chemotherapy with autologous or allogeneic stem cell transplantation. Although the tumor burden can be reduced, a complete and sustained remission of disease is rare, and most of the patients suffer from relapsing disease [16]. Therefore, novel therapies are urgently needed. Because flow cytometry has revealed KIT expression in 33% of MM (range 26–43%) [1, 5, 10, 11, 12], aberrant KIT expression in MM could provide the rationale for treatment with imatinib mesylate. However, no immunohistochemical analysis assessing the in situ expression of KIT in MM has been performed prior to the study reported herein.

The aim of our study was to investigate KIT expression by immunohistochemical analysis in a large cohort of bone-marrow biopsies of patients with MM, monoclonal gammopathy of undetermined difference (MGUS), lymphoplasmacytic lymphoma (LPL) and reactive plasmacytosis (RP) to provide the histopathological basis for a putative molecular-targeted therapy with imatinib mesylate.

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Materials and methods

Patients

We retrospectively reviewed bone marrow biopsies of 100 patients, 90 with monoclonal gammopathies (72 MM, 8 LPL and 10 MGUS) and 10 with RP, retrieved from the archives of the Institute of Pathology, University of Basel.

The diagnoses of MM, LPL, MGUS and RP were established by histomorphology and immunohistochemistry. All specimens were

reviewed and reclassified according to the World Health Organization classification criteria [6].

Immunohistochemistry

Serial sections 4-μm thick of 99 SUSA- and 1 formalin-fixed paraffin-embedded bone-marrow biopsies were used for immunohistochemical studies. The primary antibodies used in this study and types of antigen retrieval performed are summarized in Table 1.

Heat-induced antigen retrieval was performed in a microwave in ethylene diamine tetraacetic acid buffer (pH 8.0). An automated modified streptavidin-biotin system (Ventana Medical Systems, Tucson, AZ) was used for immunohistochemical staining. Mast cells served as internal positive control and a mutation-confirmed GIST as external positive control for CD117. Moreover, to obtain definite specificity control for CD117, the reaction was blocked by pre-incubation of the primary antibody with the specific antigen (10 nmol/ml diluted antibody, Novocastra). Positivity was defined as more than 10% KIT-positive plasma cells, considering only a membranous staining pattern.

Polymerase chain reaction sequence analysis

One MM with moderate CD117 staining was selected for KIT mutation analysis [the MM with weak CD117 staining could not be analyzed by polymerase chain reaction (PCR) due to SUSA fixation of the bone marrow biopsy]. Deparaffinizing the formalin-fixed tissues and DNA extraction was performed using a commercial DNA extraction kit (Qia Amp DNA mini-kit, Cat. #51304, Qiagen GmbH, Hilden, Germany). Exons 2, 8, 9, 11, 13 and 17 were amplified using a semi-nested PCR approach and were sequenced using the Big Dye Terminators Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). The primers were designed based on a previously published sequence of the human c-kit gene [2]. Sequence products were analyzed on an ABI Prism 310 Genetic Analyzer (Applied Biosystems; Foster City, CA, USA).

Results

Patients

The median age of the various patient populations was: MM (41 males and 31 females) 66 years (range: 31–89 years), LPL (8 males) 70 years (range: 52–81 years), MGUS (3 males and 7 females) 64 years (range: 36–80 years) and RP (5 males and 5 females) 73 years (range: 52–81 years) (Table 2).

Immunohistochemistry

The monotypic immunoglobulin detected in the 72 MM patients was IgG in 53% (21% kappa and 32% lambda) and IgA in 28% (15% kappa and 13% lambda). Monotypic light chains only were observed in 19% (equal distribution of kappa and lambda). In the eight LPL patients, IgM-kappa was found in 75% and IgM-lambda in 25%. In the ten MGUS patients, the M-component was IgG in 60% (kappa in 50% and lambda in 10%), IgA-lambda in 20%, IgM-kappa in 10% and only light chain (lambda) in 10%, respectively. Of the 72 MM cases, two (2.8%) were immunohistochemically KIT positive. One case (IgG-kappa) showed a moderate membranous expression in nearly 100% of the plasma cells (Fig. 1A, B, C), and the other case (IgG-lambda) showed a weak expression in 50% of the plasma cells (Fig. 2). Of MM, 97% (70/72) were KIT negative, and 100% of LPL, MGUS and RP were KIT negative.

Table 1 Primary antibodies and antigen retrieval techniques. *MW* microwave-based antigen retrieval with ethylene diamine tetraacetic acid buffer, pH 8.0

Antibody	Clonality	Antigen retrieval	Dilution	Source	Code number
CD117	Polyclonal	MW; 100°C, 15′	1:40	Dako	A4502
CD138	Monoclonal	MW; 100°C, 15′	1:15	Dako	M7228
IgG	Polyclonal	None	1:5000	Dako	A0423
IgA	Polyclonal	None	1:10,000	Dako	A0262
IgM	Polyclonal	None	1:5000	Dako	A0425
Light chain λ	Polyclonal	None	1:10,000	Dako	A0193
Light chain κ	Polyclonal	None	1:10,000	Dako	A0191

Table 2 Patient characteristics. *LPL* lymphoplasmacytic lymphoma, *MGUS* monoclonal gammopathy of undetermined significance

	Multiple myeloma	LPL	MGUS	Reactive plasmocytosis
General data				
Number of patients	72	8	10	10
Median age, years (range)	66 (31–89)	70 (52–81)	64 (36–80)	73 (52–81)
Gender male	41	8	3	5
M-component				
IgG, kappa	21%		50%	
IgG, lambda	32%		10%	
IgA, kappa	15%		0%	
IgA, lambda	13%		20%	
IgM, kappa	0%	75%	10%	
IgM, lambda	0%	25%	0%	
Lambda, only	9.5%		10%	
Kappa, only	9.5%		0%	

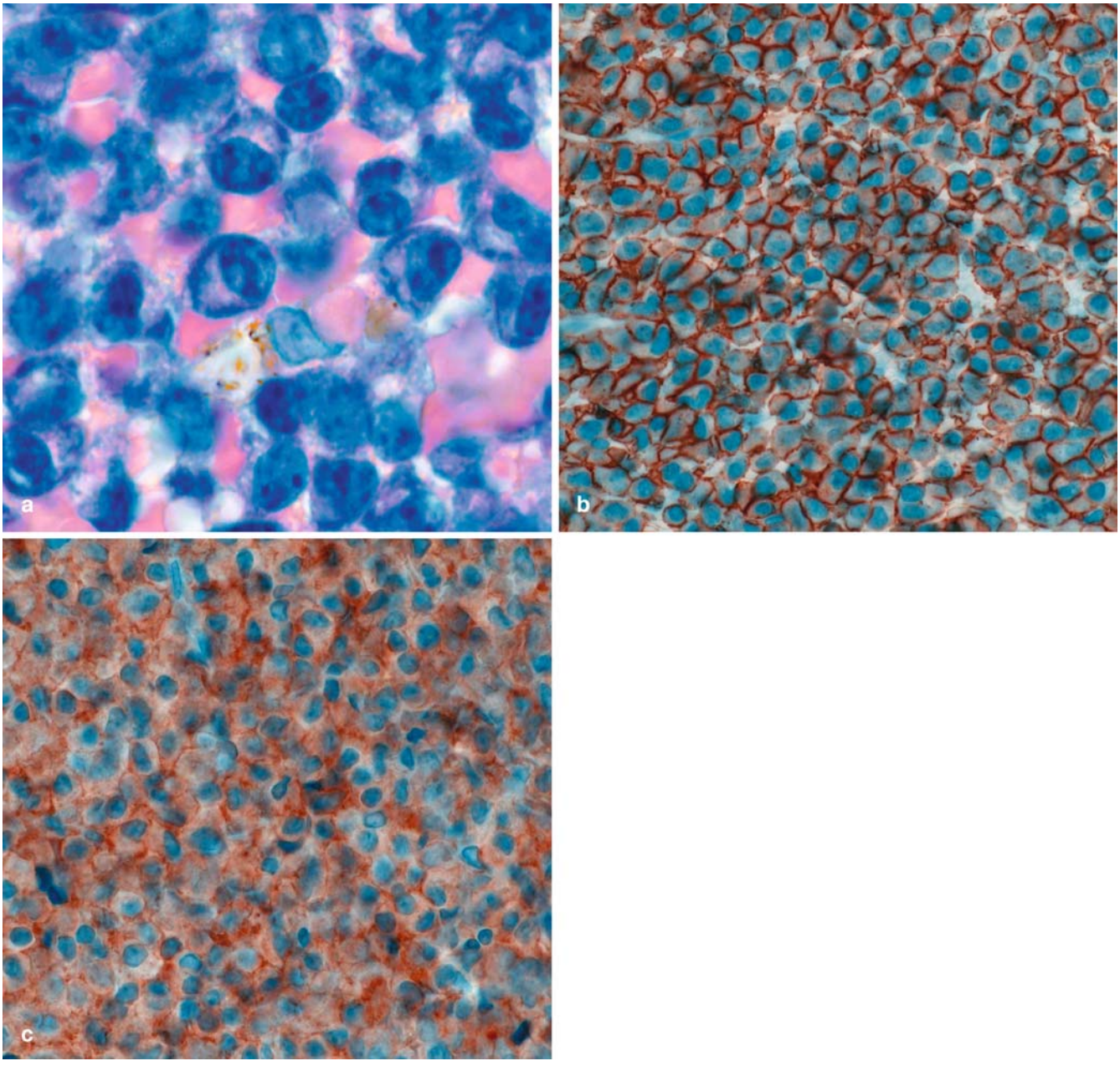

Fig. 1 Bone marrow with diffuse infiltration of atypical plasma cells in multiple myeloma (Giemsa, ×63) (**a**). Immunostaining for CD138 (×40), showing a strong positivity of malignant plasma cells (**b**). Immunostaining for CD117 (×40) with moderate positivity in malignant plasma cells (**c**)

KIT positivity in mast cells serving as internal control was detected in all cases. The definitive specificity control of the two positive MM cases was obtained by pre-incubation of the primary antibody.

PCR

DNA was extracted from the MM case with moderate KIT expression and amplified and sequenced as described. No mutation of the exon 2, 8, 9, 11, 13 and 17 of the c-kit gene was detected.

Discussion

In the present study, we have shown that KIT is rarely expressed in MM. Only 2 of 72 cases (2.8%) demonstrated immunohistochemically detectable expression, and all cases of MGUS, RP and LPL were KIT negative.

To our knowledge, this is the first study to systematically analyze KIT expression in plasma cells on paraffin sections. Yang et al. assessed the immunophenotype of cutaneous and extracutaneous mast cell disease on paraffin sections and included in their study, four cases of MM without finding KIT expression [17]. Natkunam et

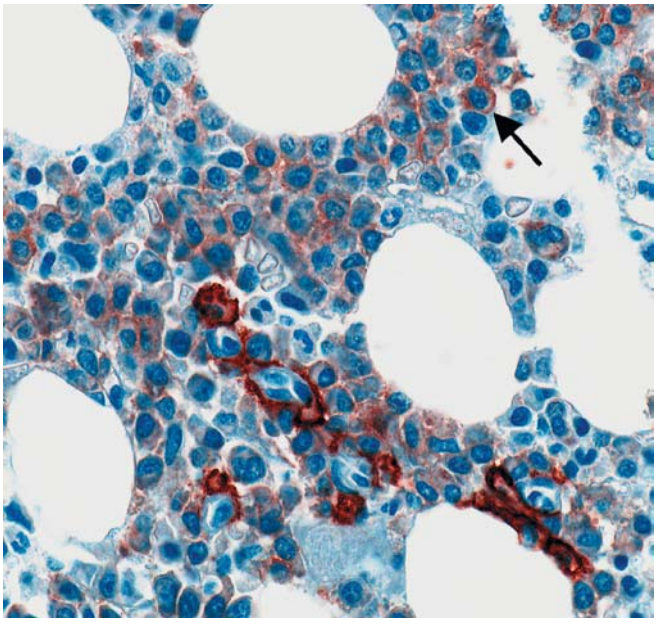

Fig. 2 Immunostaining for CD117 (×40) with weak positive malignant plasma cells (*arrow*) and adjacent strong positive mast cells as internal control

al. analyzed the utility of paraffin section immunohistochemistry for KIT in the differential diagnosis of systemic mast cell disease involving the bone marrow and found no expression of KIT in various B-cell lymphomas or plasma cell tumors [8]. The slight discrepancy in KIT detection between immunohistochemistry and flow cytometry is not unusual and may be related to fixation and processing or to the specificity of the anti-CD117 antibodies employed.

Based on our large series of 72 cases and these few reports from the literature, we conclude that MM is not a candidate disease for treatment with imatinib mesylate, as the majority of cases do not express the KIT target. This conclusion is clearly corroborated by the fact that in the B2225 study, an international multi-center trial assessing the efficacy of imatinib mesylate in advanced/metastatic malignancies, all six patients with MM (some expressing KIT in >50% of plasma cells) showed disease progression while under treatment with imatinib (personal communication with Novartis).

Thus, for the few cases of MM that show KIT expression, demonstration of the imatinib target is not, in itself, sufficient to achieve the expected clinical effects. Obviously, the tyrosine kinase of the involved receptor should be functionally active, such as in most cases of GIST [13]. In general, activating oncogenic mutations of target genes is a prerequisite for a clinical response to imatinib therapy [3, 4, 9, 14, 15].

In agreement with the clinical data from the B2225 study (myeloma patients with disease progression while under treatment), PCR analysis of our MM case with moderate KIT expression revealed a wild-type gene configuration, i.e., no mutations in exons 2, 8, 9, 11, 13 and 17. Another possible pathway of successful treatment of MM patients with imatinib could be targeting PDGFR signaling. We, therefore, tested several anti-PDGFR antibodies, but did not obtain reliable and reproducible results. Continuative mutational analysis was not possible, due to RNA/DNA degradation in SUSA-fixed tissue. However, based on the clinical findings from the B2225 study mentioned above, a pathogenetic and therapeutic role of PDGFR in MM is unlikely.

In summary, most cases of MM are immunohistochemically KIT negative. The very few positive cases probably do not show activating KIT mutations and, therefore, will not respond to imatinib treatment.

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