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Original

Mast Cell Infiltration is Associated with Myelofibrosis and Angiogenesis in Myelodysplastic Syndromes

Mayumi HOMMA[1,2], Masafumi TAKIMOTO[1], Hirotsugu ARIIZUMI[1,2], Eisuke SHIOZAWA[1], Toshiko YAMOCHI-ONIZUKA[1], Miki KUSHIMA[3], Norimichi HATTORI[2], Takashi MAEDA[2], Hidetoshi NAKASHIMA[2], Bungo SAITO[2], Kouji YANAGISAWA[2], Isao MATSUDA[2], Tsuyoshi NAKAMAKI[2], Shigeru TOMOYASU[2] and Hidekazu OTA[1]

Abstract : Myelodysplastic syndromes are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by persistent peripheral cytopenia with morphological and functional abnormalities of hematopoietic cells. Mast cells infiltrate into or around tumor tissues and play a role in remodeling of the stromal microenvironment, contributing to tumor progression. Increased mast cell numbers are associated with fibrosis, angiogenesis and a poor prognosis in human carcinomas. The aim of this study was to determine whether mast cell infiltration contributes to myelofibrosis or angiogenesis in myelodysplastic syndromes. We evaluated the correlation between mast cell density and the extent of myelofibrosis and angiogenesis in myelodysplastic syndromes. Fifty bone marrow biopsies taken from patients with a diagnosis of myelodysplastic syndromes were examined. Grading of myelofibrosis was evaluated by silver impregnation staining. Mast cell density and microvessel density were evaluated by immunohistochemistry. Human mast cells have been divided into two phenotypes. We designated a tryptase-positive mast cell as MC_T and a chymase-positive mast cell as MC_{TC}. Microvessels were identified by CD34-positive endothelial cells. Microvessel density and the extent of myelofibrosis were significantly greater in patients with high MC_T and MC_{TC} density compared to those with low MC density. Based on this, we suggest that the presence of high mast cell numbers is associated with myelofibrosis and angiogenesis in myelodysplastic syndromes.

Key words : angiogenesis, bone marrow, mast cell, myelodysplastic syndromes, myelofibrosis

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders characterized by persistent peripheral cytopenia with morphological and

[1] *Second Department of Pathology, Showa University School of Medicine,* 1-5-8 Hatanodai, Shinagawa-ku Tokyo 142-8555, Japan.
[2] *Division of Hematology, Department of Medicine, Showa University School of Medicine.*
[3] *Division of Diagnostic Pathology, Showa University Hospital.*

functional abnormalities of hematopoietic cells. Patients with MDS often have bone marrow (BM) hypercellularity with an increased risk of transformation into acute myeloid leukemia (AML)[1]. The pathogenesis of MDS is a multi-step process, including a series of genetic mutations within hematopoietic stem cells and abnormalities of BM microenvironment. Components of the BM microenvironment include macrophages, fibroblasts, vascular endothelial cells and mast cells (MCs).

MCs are derived from precursors of the hematopoietic lineage and complete their differentiation in peripheral tissues[2]. These cells have been implicated in the pathogenesis of a variety of chronic inflammatory diseases. MCs contain various biochemical mediators in cytoplasmic granules, including histamine, heparin, tryptase, chymase, and cytokines such as interleukin (IL)-4, IL-5, IL-6 and IL-8. The release of these substances from MCs in inflammatory lesions is thought to play an important role in acceleration of the inflammatory process, angiogenesis and fibrosis. Human MCs have been divided into two phenotypes based on protease content : a tryptase-positive, chymase-positive phenotype, termed MC_{TC}, and a tryptase-positive, chymase-negative phenotype, termed MC_T[3]. Both the MC_{TC} and MC_T phenotypes are present in almost all human tissues.

Elevated MC numbers in human carcinomas, including hepatocellular carcinoma, cholangiocarcinoma[4], oral squamous cell carcinoma[5], and pulmonary adenocarcinoma[6], are associated with fibrosis, angiogenesis and a poor prognosis. MCs may also be associated with fibrosis and angiogenesis in lymphomas. Molin and colleagues found a higher number of MCs in nodular sclerosis classical Hodgkin lymphoma than in other types of Hodgkin lymphoma, and patients with higher MC infiltration have reduced relapse-free survival[7]. In B-cell non-Hodgkin's lymphoma, the MC density is correlated with microvessel density (MVD), and both densities increase in parallel with increasing grade of malignancy[8]. Fukushima and colleagues reported that MCs are associated with fibrosis in diffuse large B-cell lymphoma[9]. An increase in the MC count is also seen in MDS[10, 11]. There are some reports that MC numbers often increase in MDS with myelofibrosis[12] and the extent of angiogenesis correlates with the number of MCs in MDS[13]. In this study, we evaluated the correlation of MC_T and MC_{TC} density with the extent of myelofibrosis and angiogenesis in MDS, to investigate whether MC infiltration contributes to myelofibrosis and angiogenesis in MDS and to clarify the alteration of BM microenvironment in MDS.

Methods

Patients

We examined paraffin-embedded trephine BM biopsies from 72 patients with a diagnosis of MDS at Showa University Hospital (Tokyo, Japan) from 1997 to 2007. Patients provided informed consent at the time the BM examination was performed. BM trephine biopsies were obtained from the posterior iliac crest, and aspiration samples were obtained simultaneously for cytology. BM smears, prepared without anticoagulant, were fixed and

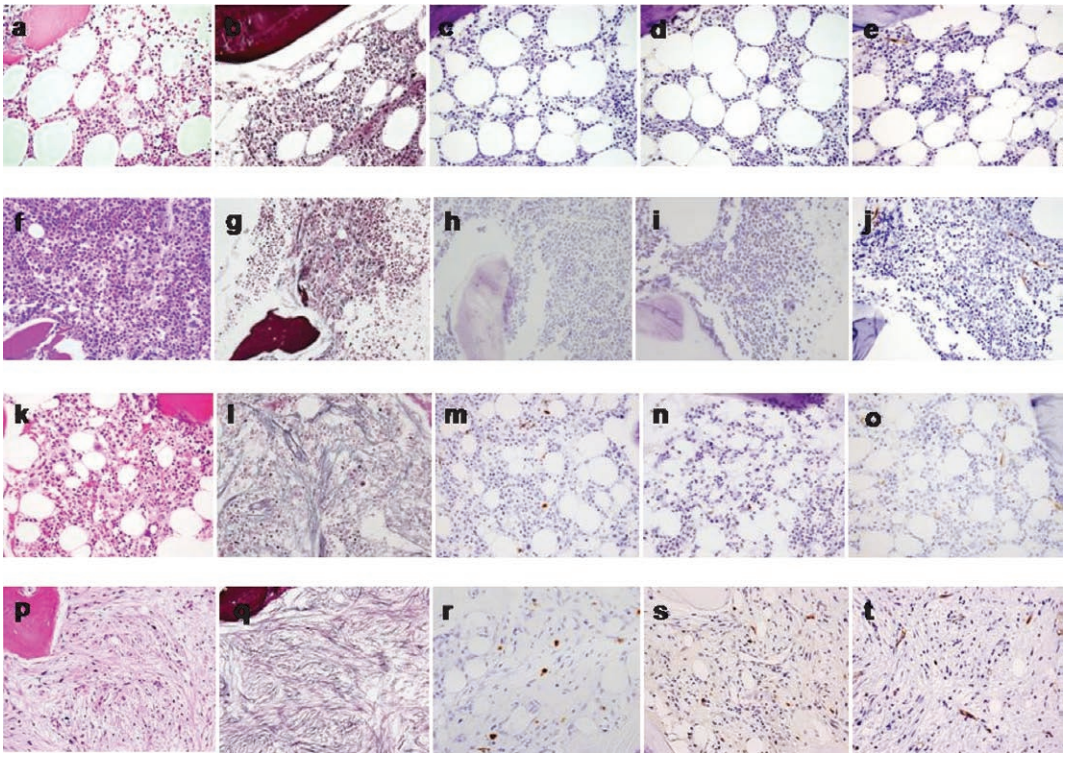

Fig. 1. Myelofibrosis and immunohistochemical staining of mast cells (MCs) and microvessels in bone marrow (BM). MC_T was detected as a tryptase-positive MC and MC_{TC} was detected as a chymase-positive MC. BM microvessels were identified as CD34-positive endothelial cells. Both MC_T and MC_{TC} increased with extent of myelofibrosis and microvessel density (MVD). MF-0; (a) HE, (b) silver impregnation, (c) tryptase, (d) chymase, (e) CD34: MF-1; (f) HE, (g) silver impregnation, (h) tryptase, (i) chymase, (j) CD34: MF-2; (k) HE, (l) silver impregnation, (m) tryptase, (n) chymase, (o) CD34: MF-3; (p) HE, (q) silver impregnation, (r) tryptase, (s) chymase, (t) CD34 (×400).

stained by the May-Giemsa method. Twenty-two patients were excluded from the study due to insufficient materials or inadequate follow-up, leaving 50 patients for analysis. The patients included 33 men and 17 women, ranging in age from 40 to 90 years (mean 72.2 years). Their survival period ranged from 2 to 49 months (mean 12 months). Peripheral blood (PB) cell counts and BM smear counts were also obtained, including the percentage of myeloblasts in the PB and BM, the type and degree of dysplasia, the presence of ring sideroblasts, and karyotype. Based on the BM myeloblast count, number of PB cytopenias and karyotype, the patients were stratified using the International Prognostic Scoring System (IPSS)[14] into four risk groups: low risk (score 0; n=7), intermediate-1 (score 0.5-1.0; n=17), intermediate-2 (score 1.5-2.0; n=19), or high risk (score ≥2.5; n=7). Patients were classified according to the French-American-British (FAB)[15] and World Health Organization (WHO)[1] criteria. They were divided into FAB subgroups as follows: refractory anemia (RA), n=11; RA with ring sideroblasts (RARS), n=6; RA with excess blasts (RAEB),

n＝19; RAEB in transformation (RAEB-t), n＝8; AML arising from MDS, referred to as MDS overt leukemia (MDS-OL), n＝3. Patients were further classified according to WHO criteria as follows: refractory cytopenia with unilineage dysplasia (RCUD), actually RA, n＝1; RARS, n＝1; refractory cytopenia with multilineage dysplasia (RCMD), n＝14; RAEB-1, n＝14; RAEB-2, n＝6; MDS associated with isolated deletion of 5q, n＝1; and AML with myelodysplasia-related changes, n＝10. Although chronic myelomonocytic leukemia has recently been removed from the definition of MDS, 3 cases were included in the study to examine possible differences between chronic myelomonocytic leukemia and MDS subtypes. As controls, BM biopsies from 5 patients with no evidence of lymphoma cell infiltration, performed for diagnostic purposes as a part of staging procedures for classical Hodgkin lymphoma (n＝1) and non-Hodgkin lymphoma (n＝4), were also evaluated.

Bone marrow analysis

The specimens were fixed in 10% buffered formalin, decalcified in 10% buffered EDTA (pH 7.2), and embedded in paraffin. The paraffin-embedded tissue sections were stained using hematoxylin and eosin (HE), Giemsa stain, naphthol-ASD-chloroacetate, and silver impregnation for histological investigation. Semiquantitative evaluation of BM cellularity was performed with a scoring system based on cell numbers in normal BM: 0, no increase in comparison with the normal state; ＋1/－1, mild to moderate increase/decrease; ＋2/－2, marked increase/decrease[16]. Immunohistochemical staining was performed on the same tissue sections. Examination of the biopsies was carried out independently by two investigators unaware of the patients' diagnosis and clinical data. Patient samples were taken at the time of initial diagnosis. In the cases that progressed to AML (n＝14), we examined the nine BM biopsies at the time of overt leukemia development if they were available.

Myelofibrosis grading

Grading of myelofibrosis (accumulation of reticulin and collagen fibers) followed the semiquantitative scoring system[17]: MF-0, scattered linear reticulin with no intersections (cross-overs) corresponding to normal BM; MF-1, loose network of reticulin with many intersections, especially in perivascular areas; MF-2, diffuse and dense increase in reticulin with extensive intersections, occasionally with only focal bundles of collagen and/or focal osteosclerosis; and MF-3, diffuse and dense increase in reticulin with extensive intersections with coarse bundles of collagen, often associated with significant osteosclerosis.

Immunohistochemistry

Formalin-fixed, paraffin-embedded specimens of BM biopsies were examined by immunohistochemical staining procedures. We used monoclonal antibodies against tryptase (10D11, Novocastra, Newcastle-upon-Tyne, UK; diluted 1: 150) to detect MC_T, chymase (CC1, MBL, Nagoya, Japan; diluted 1: 100) to detect MC_{TC}[18], and CD34 (NU-4A1, Nichirei, Tokyo,

Japan ; diluted 1 : 50) to detect blood vessels identified by CD34-positive endothelial cells. Tissue sections were deparaffinized with xylene, rehydrated in a series of graded alcohols, and then immersed in 3% H_2O_2 to quench the endogenous peroxidase activity. For CD34 staining, sections were then subjected to microwave antigen retrieval in citric acid buffer at pH 7.0 for 30 min at 98℃. After incubation with primary monoclonal antibodies for 90 min at room temperature, immunohistochemical staining was performed using an Envision Kit (DakoCytomation, Glostrup, Denmark). Diaminobenzidine was used for color development, and hematoxylin was used for counterstaining.

Mast cell counts

The number of tryptase-positive and chymase-positive MCs in the specimens were quantified using a computerized morphometry system (WinROOF ver. 5.01, Mitani Corp., Tokyo, Japan). MC_T and MC_{TC} densities were expressed as the absolute number of tryptase-positive or chymase-positive MCs per 1 mm^2 of hematopoietic area in BM biopsies.

Quantification of microvessel density

CD34 is a useful antigen for assessing intratumor angiogenesis in solid tumors and meets the high quality requirements for BM angiogenesis research. Blood vessels were defined by CD34-positive endothelial cells forming a structure with a clearly discernible lumen. This definition is important to discriminate blood vessels from CD34-positive myeloid progenitors. Myeloid stem cells are also CD34-positive, but these cells can be excluded by their morphology, even if highly abundant[19]. Slides were first scanned at 100× magnification and 3 areas with abundant microvessels were chosen and defined as "hot spots". The number of microvessels in each of these hot spots was then determined at 400× magnification. The final MVD (microvessels per field at 400× magnification) was determined by taking the average of 3 separate visual counts. Large vessels and vessels in the periosteum or bone were not counted.

Statistical analysis

All statistical analyses were performed using StatView software (ver. 5.0, SAS Institute Inc. Raleigh, NC, USA). Patient characteristics and MC density were compared using the Chi-squared test with Fisher's exact test. Comparisons between two groups were performed using the Mann-Whitney U-test and comparisons among more than three groups were made with the Kruskal-Wallis test. P values < 0.05 were considered to be statistically significant.

Results

Clinical and pathological parameters of the fifty MDS patients investigated in this study are shown in Table 1. More tryptase-positive MCs than chymase-positive MCs were detected in the paraffin-embedded MDS specimens (MC_T : 2.27 ± 3.50 / mm^2 versus MC_{TC} : $1.19 \pm$

Table 1. Clinical and histological findings of patients with myelodysplastic syndromes at the initial diagnosis and the time of overt leukemia

no.	Age (years)	Sex	IPSS	WHO	FAB	cellularity	MC_T (/ mm^2)	MC_{TC} (/ mm^2)	myelofibrosis	MVD (/ × 400 field)
Initial diagnosis										
1	74	F	Low	RCUD(RA)	RA	−1	13.46	13.69	MF-2	17
2	62	M	Low	RCMD	RA	0	4.92	1.47	MF-2	6
3	74	F	Int-1	RCMD	RA	−2	0.13	0.13	MF-1	9.33
4	85	M	Int-1	RCMD	RA	−1	1.04	0.96	MF-0	8.33
5	58	M	Int-1	RCMD	RA	0	0	0	MF-1	12.33
6	60	M	Int-2	RCMD	RA	+1	0.29	0.22	MF-2	10.33
7	68	F	Int-1	RARS	RARS	0	4.98	3.23	MF-1	8
8	69	M	Low	RCMD	RARS	−2	6.32	4.07	MF-0	12
9	74	M	Int-1	RAEB-1	RAEB	+1	2.21	0.80	MF-2	20
10	80	M	Int-1	RAEB-1	RAEB	−1	1.92	2.40	MF-1	20.33
11	80	F	Int-1	RAEB-1	RAEB	−2	0	0	MF-0	15
12	80	M	Int-1	RAEB-1	RAEB	+1	0.21	0.07	MF-1	11
13	83	M	Int-1	RAEB-1	RAEB	−1	1.14	0.25	MF-0	8
14	73	M	Int-1	RAEB-1	RAEB	+2	0	0	MF-1	8.67
15	86	F	Int-2	RAEB-1	RAEB	−2	3.06	0.36	MF-1	10
16	86	F	Int-2	RAEB-1	RAEB	−1	0	0	MF-0	11.67
17	53	M	Int-2	RAEB-1	RAEB	−1	0.93	1.03	MF-2	17.33
18	69	M	Int-2	RAEB-1	RAEB	+1	0.20	0.07	MF-1	5.33
19	79	M	Int-2	RAEB-1	RAEB	0	7.13	0.41	MF-2	10
20	76	F	Int-1	RAEB-2	RAEB	+2	7.58	3.83	MF-3	29.67
21	66	F	Int-2	RAEB-2	RAEB	+1	0.63	0	MF-1	5.67
22	74	M	Int-2	RAEB-2	RAEB	−1	0.98	0.22	MF-1	10.33
23	69	M	Int-2	RAEB-2	RAEB	+2	2.62	0.19	MF-1	11.33
24	50	M	Int-2	RAEB-2	RAEB-t	−1	1.20	0	MF-1	11.67
25	81	M	High	RAEB-2	RAEB	+1	2.31	0.45	MF-2	3.67
26	73	F	High	AML-MDS	RAEB-t	−1	0	0	MF-1	11
27	62	M	Int-2	AML-MDS	RAEB-t	−2	0.20	0.14	MF-0	10.67
28	90	M	Int-2	AML-MDS	RAEB-t	−1	0.37	0.28	MF-0	7.67
29	40	M	Int-2	AML-MDS	RAEB-t	−1	2.64	1.48	MF-1	21.67
30	77	M	Int-2	AML-MDS	MDS-OL	+1	2.08	2.91	MF-2	11.67
31	62	M	High	AML-MDS	RAEB-t	−2	0.91	0	MF-2	13.67
32	69	F	High	AML-MDS	MDS-OL	+1	0.94	0.22	MF-1	4
33	87	F	High	AML-MDS	RAEB-t	0	1.62	1.00	MF-1	0.67
34	71	M	High	AML-MDS	RAEB-t	−1	1.06	0.79	MF-1	13
35	77	M	High	AML-MDS	MDS-OL	+1	0.24	0.08	MF-1	6.33
36	64	M	Low	CMML	CMML	0	0.75	0.52	MF-1	13
37	81	F	Int-1	RCMD	RA	+2	1.16	0.88	MF-1	17.33
38	81	M	Low	RCMD	RA	+1	1.87	1.19	MF-1	11.67
39	62	F	Int-1	RCMD	RA	+1	0.89	0.36	MF-1	3
40	63	M	Int-2	RCMD	RA	0	2.99	0.34	MF-1	11.67
41	80	F	Int-1	RCMD	RARS	+2	17.87	14.17	MF-1	6.67
42	66	M	Int-1	RCMD	RARS	0	0.54	0	MF-0	12.33
43	65	M	Int-2	CMML	CMML	0	0.49	0.49	MF-1	4.33
44	84	M	Low	CMML	CMML	+1	0	0	MF-0	6.67
45	78	M	Int-2	RAEB-1	RAEB	0	1.49	0.37	MF-2	1.67
46	73	F	Int-2	RAEB-1	RAEB	−1	2.78	0.29	MF-2	6.67
47	70	F	Int-2	RCMD	RARS	−1	0	0	MF-0	6.33
48	84	M	Int-1	RAEB-1	RAEB	+2	8.94	0.17	MF-2	16.33
49	63	M	Low	5q-	RARS	−2	0.45	0	MF-0	14.33
50	77	F	Int-1	RCMD	RA	−2	0	0	MF-1	10.67
at the time of overt leukemia										
37'	NA									
38'						0	0	0	MF-2	8
39'						+2	0.27	0.03	MF-0	3.67
40'						0	9.22	0.24	MF-1	2.67
41'						0	3.65	3.16	MF-2	5.33
42'						0	0.12	0.12	MF-1	18.33
43'	NA									
44'						−1	0.15	0.08	MF-0	14
45'						+2	0.87	0	MF-2	24
46'						−1	0.35	0.07	MF-0	6.33
47'						+2	0.31	0.54	MF-1	7.67
48'	NA									
49'	NA									
50'	NA									

IPSS : International Progonostic Scoring System, Int : intermediate, WHO : World Health Organization, FAB : French-American-British, MC_T : tryptase-positive, chymase-negative mast cell, MC_{TC} : tryptase-and chymase-positive mast cell, MVD : microvessel density, RCUD : refractory cytopenia with unilineage, RA : refractory anemia, RCMD : refractory cytopenia with multilineage dysplasia, RARS : RA with ring sideroblasts, RAEB : RA with excess blasts, RAEB-t : RAEB in transformation, AML-MDS : acute myeloid leukemia with myelodysplasia-related changes, MDS-OL : myelodysplastic syndromes overt leukemia, CMML : chronic myelomonocytic leukemia, 5q- : MDS associated with isolated del (5q), NA : not available

Table 2. Density of mast cells in myelodysplastic syndromes

myelofibrosis	n	MC_T	P-value	MC_{TC}	P-value
MF-0,1	37	1.58±3.09	*0.002	0.92±2.42	*0.033
MF-2,3	13	4.23±3.95		1.97±3.70	

Number of MCs (/mm^2) (mean±SD)

MVD(/×400 field)	n	MC_T	P-value	MC_{TC}	P-value
<16	42	1.78±3.08	*0.03	0.84±2.29	*0.014
≧16	8	4.86±4.59		3.04±4.45	

Number of MCs (/mm^2) (mean±SD)

MC_T: tryptase-positive, chymase-negative mast cell,
MC_{TC}: tryptase- and chymase-positive mast cell,
MCs: mast cells, MVD: microvessel density

2.81/mm^2, $P=0.004$). Morphologically, both round to oval MCs and spindle MCs were detected. Qualitatively, we detected more round to oval MCs than spindle MCs. The extent of myelofibrosis and MVD were significantly higher in patients with high MC_T and MC_{TC} densities (>1.5 MC_T/mm^2 or >0.37 MC_{TC}/mm^2) than in those with low MC_T density (myelofibrosis $P=0.018$; MVD $P=0.041$) or MC_{TC} density (myelofibrosis $P=0.027$; MVD $P=0.007$). The MC_T density was higher in the high-fibrosis group (MF-2, MF-3) than in the low-fibrosis group (MF-0, MF-1). The MC_{TC} density was also higher in the high-fibrosis group (MF-2, MF-3) than in the low-fibrosis group (MF-0, MF-1). The MC_T and MC_{TC} densities were higher in patients with high MVD (MVD ≥16 per 400× field) than in patients with low MVD (MVD <16 per 400× field) (Table 2). The MC_T or MC_{TC} density were not correlated with age, sex, IPSS classification, BM cellularity, survival period, presence of MDS-OL transformation, or time to transformation to MDS-OL. There was no significant correlation between the grade of myelofibrosis and MVD. Neither FAB nor WHO classifications of MDS were significantly correlated with the MC_T or MC_{TC} density, the grade of myelofibrosis or MVD. Among the patients who progressed to AML, there was no significant difference in the MC_T or MC_{TC} density, the grade of myelofibrosis, or MVD before and after transformation to MDS-OL.

Discussion

The BM microenvironment is regulated by stromal cells, including endothelial cells and macrophage-lineage cells. The stromal microenvironment is composed of vessels, fibroblasts and several kinds of inflammatory cells, and it influences tumor growth and progression. MCs infiltrate into or around tumor tissues and play a role in remodeling of the stromal microenvironment, which leads to tumor progression. Human MCs are conventionally divided into two types depending on the expression of different proteases in their gran-

ules : the MC_T type contains tryptase and is predominantly located in the lungs and small intestinal mucosa, whereas the MC_{TC} type contains both tryptase and chymase and is predominantly found in connective tissue areas such as skin, heart, synovia and small intestinal submucosa[2,3]. Tryptase is a serine protease and a mitogen and comitogen for fibroblasts and tracheal smooth muscle cells, while chymase is a chymotrypsin-like serine protease that converts angiotensin I to angiotensin II. Angiotensin II stimulates fibroblast proliferation through activation of transforming growth factor-β1[9]. These findings suggest that both MC tryptase and MC chymase play important roles in the development of fibrosis in human disease.

Horny and colleagues reported that the predominant BM MC type is MC_{TC} in normal or reactive states, and MC_T in neoplastic states such as MDS[20]. In our study, more tryptase-positive MCs than chymase-positive MCs were detected in MDS cases. A few quantitative immunohistochemical analyses of the tissue distribution of MC_T and MC_{TC} have been published. Terada and Matsunaga[4] showed that the percentages of MC_T and MC_{TC}, approximately 20% and 80% respectively, were almost the same in normal liver, hepatocellular carcinoma and intrahepatic cholangiocarcinoma. Fukushima and colleagues[9] reported a preponderance of MC_T over MC_{TC}, both in diffuse large B-cell lymphoma lymph nodes and in reactive lymph nodes. However, the pathophysiological basis of the lack of chymase expression in MCs involved in MDS remains unknown.

Myelofibrosis is present in approximately 10% of MDS cases[1], and histopathological and clinical differences between fibrotic and non-fibrotic MDS have been reported. BM cellularity, white blood cell counts, and the percentage of myeloblasts in PB and BM are significantly higher in MDS with myelofibrosis compared to MDS without myelofibrosis[12]. In our study, however, the only significant difference between MDS cases with and without myelofibrosis was that the BM smear nucleated cell count was lower in high-fibrosis cases than in low-fibrosis cases. The extent of the myelofibrosis can be such that BM aspiration results in either "dry taps" or extremely scanty and non-representative aspirates, which may produce lower BM smear nucleated cell counts in MDS with myelofibrosis. A previous study linked myelofibrosis to a poor prognosis in terms of life expectancy and time to leukemic transformation[21], however Pagliuca and colleagues found relatively long survival in a series of 10 MDS patients with myelofibrosis[22], and Rios and colleagues did not find a significant correlation between myelofibrosis and reduced survival[23]. Therefore, the relationship between the occurrence of myelofibrosis in MDS and survival is still controversial.

MC density is also correlated with the extent of pathological angiogenesis, such as that in chronic inflammatory diseases and tumors. In our study, both the MC_T and MC_{TC} densities were higher in the high MVD group than in the low MVD group. MCs contain many angiogenic factors, such as heparin and histamine, and a variety of cytokines and chemokines, such as transforming growth factor-β, tumor necrosis factor-α, IL-8, basic fibroblast growth factor (also known as fibroblast growth factor-2) and vascular endothelial growth

factor[13]. Tryptase acts as a mitogen for fibroblasts, smooth muscle cells and epithelial cells, so tryptase released from MCs also plays an important role in neovascularization.

Abnormal angiogenesis has been implicated in the pathogenesis of MDS, and increased MVD in the BM of patients with MDS has also been described[24]. We found no significant correlation between MVD and clinico-histological parameters. Some studies have reported increased MVD in hypercellular MDS than normocellular or hypocellular cases[25, 26], however other studies found no significant correlation between MVD and BM cellularity, PB counts, or percentage of BM myeloblasts[19, 24]. Similarly, most studies have failed to show a significant correlation between MVD and prognostic factors, including overall survival, progression-free survival and IPSS score. Ribatti and colleagues, however, showed that angiogenesis in MDS was correlated with total metachromatic and tryptase-positive MC counts and that microvessels and MC counts increased together with MDS progression[13]. In our study, there was no significant correlation between MVD and MDS progression, however MC_{TC} densities were higher in patients with high MVD, suggesting that MC chymase may play an important role in angiogenesis in MDS.

The accumulation of MCs in different tumors has been documented. Under normal conditions MCs are absent from the PB and they are rarely seen in BM[27], and the number of MCs is lower than that of any other hematopoietic cells in BM, even in cases in which the BM MC count increases. Increased numbers of BM MCs were found in 45 (2.2%) of 2000 BM specimens obtained from patients with hematologic disorders[10]. In our study, there was no significant difference in MC density between MDS cases and controls, however our control samples were BM biopsies from patients with malignant lymphoma, which may be somewhat biased, even without infiltration of lymphoma cells.

Stem cell factor, which is produced mainly by stromal cells, is the principal growth factor for human MCs[28], while MCs also induce stem cell factor. In MDS, levels of stem cell factor may increase as the period of ineffective hematopoiesis and cytopenia becomes longer. Therefore, MCs may also increase with the period of cytopenia. MCs induce various biochemical mediators to promote fibrosis, while stem cell factor is produced by mainly fibroblasts. Consequently, whether the increase of MCs contributes to the development of fibrosis or results from fibrosis is still a matter of discussion.

Recently, Chiu and colleagues suggested that the fibrogenetic mechanism in systemic mastocytosis is most likely different from that of other BM neoplasms which are also associated with myelofibrosis[29]. Systemic mastocytosis is a stem cell disorder characterized by a pathological accumulation of clonal MCs in one or more organ system. The MC aggregates are accompanied by fibrosis. In their study, there was no significant expression of type IV collagen or laminin in BM of systemic mastocytosis, compared with primary myelofibrosis or metastatic malignancy. Normal BM MCs are reported to be round to oval in shape[30]. Horny and colleagues reported round to oval shape MCs in many cases of MDS, while in the cases of mastocytosis, the MC shape showed variability ; spindle shaped cells were the

predominant cell type in most cases[20]. These differences in MC morphology may affect the different mechanisms of myelofibrosis in MDS and systemic mastocytosis. In our study, both round to oval MCs and spindle MCs were detected, but there were more round to oval MCs than spindle MCs. From this, we infer that the increase in MC numbers in MDS is reactive, and not due to clonal proliferation.

Based on the results of this study, we suggest that MC_T and MC_{TC} densities are correlated with the extent of myelofibrosis and angiogenesis in MDS. Evaluation of possible correlations between the outcome of patients with MDS and MC density and the extent of myelofibrosis or angiogenesis will require a study with a larger series of cases.

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