MET and MST1R as prognostic factors for classical Hodgkin's lymphoma

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MST1R (RON) and MET are receptor tyrosine kinase gene family members that form a noncovalent complex on the cell surface, a critical step in tumor progression. A recent study suggested a prognostic role of MET expression in Hodgkin/Reed–Sternberg (HRS) cells in classical Hodgkin's lymphoma (cHL). The purpose of this study was to examine the prognostic significance of MET and MST1R expression in cHL. The prognostic impact of MET and MST1R was examined in 100 patients with cHL (median age: 32 years) by immunohistochemistry and mRNA *in situ* hybridization. The median follow-up time was 95 months (interquartile range: 42–126 months). MET or MST1R protein expression was associated with high MET or MST1R mRNA expression, respectively. Thirty-eight patients (38%) expressed MET protein in HRS cell, which was associated with better overall survival ($P=0.004$). Twenty-six patients (26%) expressed MST1R protein, which was associated with better overall survival ($P=0.022$) and event-free survival ($P=0.021$). Multivariate analysis identified MET protein as an independent prognostic factor for overall survival and MST1R protein as an independent prognostic factor for event-free survival. Subgroup analysis according to Ann Arbor stage showed that expressions of MET and MST1R protein have prognostic impact in the advanced stage only. In particular, coexpression of MST1R and MET protein was associated with a better survival outcome than MET or MST1R expression alone or no expression. This study suggests that MET and MST1R are independent prognostic factors in classical cHL, and may allow the identification of a subgroup of cHL patients who require more intensive therapy.

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Classical Hodgkin lymphoma (cHL) is characterized by disruption of the normal lymph node architecture by the minority of malignant Hodgkin/Reed–Sternberg (HRS) cells. HRS cells are found in a background of bystander reactive cells composed of T and B lymphocytes and other cell types, including macrophages, eosinophils, basophils, and plasma cells. Deregulated signaling pathways,^{1,2} Epstein–Barr virus (EBV) infection,^{3–5} and an inflammatory microenvironment^{6–8} contribute to the survival and proliferation of HRS cells.

Despite important advances in the treatment of cHL, 5 to 10% of patients are resistant to initial therapy and 10 to 30% will relapse after initial remission.⁹ The standard stratification system for survival in patients with cHL is the International

Prognostic Score (IPS), which is limited to patients with advanced-stage disease.¹⁰ Increased hematologic toxicity and secondary malignancies are major problems in cHL. Several clinical, biological, and laboratory prognostic factors have been examined for their prognostic utility. Although some prognostic markers have been reported, including early interim positron emission tomography,¹¹ tumor-associated macrophages,^{6,8} and EBV status,^{5,12} their predictive clinical use in patients with cHL is still unclear.

The HGF/MET signaling pathway, which is a subfamily of receptor tyrosine kinases (RTKs), has drawn special attention to the association among tumor cell proliferation, survival, invasion, and metastasis.^{13,14} Deregulation and the consequent aberrant signaling of MET are caused by different mechanisms, including gene amplification, overexpression of activating mutations, and increased autocrine or paracrine ligand-mediated stimulation. These events can play important roles in the pathogenesis of cancer.¹⁵ Recent studies reveal that MET is overexpressed in gastric,¹⁶ colon,¹⁷ breast,¹⁸

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ovary,¹⁹ kidney,²⁰ lung,²¹ and thyroid carcinomas.²² Downregulation of MET expression by an antagonistic anti-MET antibody or small interfering RNA inhibits cellular proliferation and motility in lung cancer²³ and medullary thyroid cancer.²⁴ Previous studies also report that MET/HGF has prognostic significance in many malignant tumors.^{19,20,25}

MST1R (RON), an RTK with homology to MET, is involved in tumor progression and metastasis.^{26,27} MST1R is overexpressed in human epithelial malignancies.^{28,29} The expressions of MET and MST1R have been detected in HRS cells in patients with cHL.^{30,31} Recently, MET expression was reported to be a prognostic factor for clinical outcomes in cHL.³² Although a relationship between MST1R and MET is observed in several malignancies,^{33,34} no study has examined the prognostic significance of MET and MST1R in cHL patients. Thus, the aim of the present study was to determine the clinical significance of MET/MST1R protein and mRNA expression in patients with cHL.

Materials and methods

Patients

The present report involved a retrospective study of 100 consecutive patients with cHL diagnosed at Asan Medical Center between 1990 and 2009. All of the patients met the following criteria: pathologically confirmed cHL; no previous treatment; no previous history of malignancy; and treatment with combination chemotherapy, with or without radiation treatment. Paraffin-embedded tumor tissues and follow-up data were available for all included patients.

Clinical information, including age, gender, Ann Arbor stage, presence or absence of B symptoms, IPS, serum lactate dehydrogenase (LDH) level, treatment regimen, and survival data, were obtained from the medical records. The median follow-up time for surviving patients was 95 months (interquartile range: 42–126 months). Response criteria were based on standard guidelines. Routine follow-up imaging analyses were performed every 3 months for the first 2 years, every 6 months for the next 3 years, and then annually (or whenever clinically indicated) thereafter.

Histopathological Analysis and Immunohistochemistry

The histological data of all patients were reviewed by three pathologists (JH and YWK). Histological subtype was classified according to the World Health Organization (WHO) criteria as follows: nodular sclerosis (NS), lymphocyte-rich (LR), mixed cellularity (MC), lymphocyte-depleted (LD), or not otherwise specified cHL.

A representative tumor section paraffin block (donor block) was collected from each case, and three 1-mm-diameter tumor cores were obtained with a trephine apparatus (Seoungkohn, Seoul, Korea). Trephinated paraffin tissue cores were consecutively placed in recipient plastic molds (tissue array mold; Seoungkohn), which were filled with liquid paraffin and cooled.

For the automatic immunohistochemistry staining device (Benchmark XT, Ventana Medical System, Tucson, AZ, USA), a protocol using formalin-fixed, paraffin-embedded tissue sections was used. Briefly, 5- μ m-thick sections were transferred onto poly-L-lysine-coated adhesive slides and dried at 62 °C for 30 min. After standard heat epitope retrieval for 30 min in ethylene diamine tetra-acetic acid (pH 8.0) in the autostainer, the samples were incubated with antibodies against cleaved MET (dilution 1:50) and MST1R (dilution 1:25; both from Santa Cruz Biotechnology, Santa Cruz, CA, USA). The sections were subsequently incubated with biotinylated anti-rabbit immunoglobulins, peroxidase-labeled streptavidin (LSAB kit, DAKO, Glostrup, Denmark), and 3,3'-diaminobenzidine. Slides were counterstained with Harris hematoxylin.

The expression of MET or MST1R was evaluated using the criteria suggested by Xu *et al*.³² A sample was considered MET or MST1R positive if >30% of definitive HRS cells showed immunohistochemical reactivity with MET and MST1R antibodies (Figures 1a and b, respectively). A sample was considered MET or MST1R negative if MET or MST1R expression was negative or <30% positive for HRS cells, or positive for bystander cells only. The median number of tumor cells for each case was 24. All interpretations of MET and MST1R staining patterns were performed by a colleague blinded to knowledge of the clinical outcomes.

The *in situ* hybridization (ISH) analysis for EBV-encoded RNA-1 and RNA-2 (EBER) was performed and scored as described elsewhere.³⁵

mRNA ISH

RNA ISH was conducted using a commercially available mRNA ISH kit (QuantiGeneViewRNA, Paranomix, Fremont, CA, USA) according to the manufacturer's instructions. To remove the paraffin from the samples, the slides were deparaffinized in xylene. The samples were incubated with a pre-treatment solution followed by protease digestion. MET or MST1R gene-specific probe was designed. A probe set was hybridized and amplifier molecules were hybridized to each pair of oligonucleotides. The fast red substrate (MET prove) and the fast blue substrate (MST1R prove), alkaline phosphatase breaks down the substrate to form a precipitate. The slides were counterstained with hematoxylin.

The expression level was semiquantitatively evaluated based on the number of blue or red dots in the

Figure 1 MET and MST1R expression in cHL tissue. (a) HRS cells showing strong MET immunostaining on the cell membranes (original magnification $\times 400$). (b) MST1R expression on the cell membranes (original magnification $\times 400$). (c) High expression of MET mRNA (red dot) and MST1R mRNA (blue dot) on tumor cells (original magnification $\times 1,000$). (d) No MET/MST1R mRNA expression on tumor cells (original magnification $\times 1,000$).

tumor cells (0, 1+, 2+, and 3+). In normal lymphoid tissue, the expression of MET/MST1R mRNA was 1+. For the purpose of further analysis, the data were grouped into two categories using a cutoff value equal to the MET/MST1R mRNA level in normal lymphoid tissue: negativity (0 and 1+) and positivity (2+ and 3+) (Figures 1c and d).

Statistical Analysis

Overall survival (OS) was defined as the time between the first day of diagnosis and the date of death from any cause. The follow-up of patients still alive was censored at their last follow-up date. Event-free survival (EFS) was defined as the interval between the first day of diagnosis and the date of disease progression, relapse, or death from any cause. The follow-up of patients still alive without event was censored at their last follow-up date. The OS and EFS were analyzed with Kaplan–Meier curves, which were compared by log-rank testing.

Multivariate prognostic analyses were performed on OS and EFS with the Cox proportional hazards regression model. Categorical variables were compared using the χ^2 test. All statistical analyses were performed by the SPSS statistical software program (version 18.0; SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Patient Characteristics

The clinical characteristics of the 100 patients included in the present study are summarized in Table 1. Patient age ranged from 4 to 77 years (median, 32 years). We recorded 36 patients experiencing relapse, disease progression, or death. Median OS and EFS were not reached. The estimated 5-year OS and EFS were 81.1 and 61.9%, respectively.

Table 1 Demographic and clinical characteristics of patients

Characteristic at diagnosis	No. of patients (%)
Age, median (range, years)	33 (4–77)
Male gender	57 (57%)
<i>Histologic subtype</i>	
Nodular sclerosis	61 (61%)
Mixed cellularity	24 (24%)
Lymphocyte rich	7 (7%)
Lymphocyte depleted	2 (2%)
Not classifiable	6 (6%)
<i>Ann Arbor stage</i>	
I	15 (15%)
II	30 (30%)
III	30 (30%)
IV	25 (25%)
<i>Stage (limited vs advanced)</i>	
Limited	41 (41%)
Advanced	59 (59%)
B symptoms present	38 (38%)
International Prognostic Score ≥ 4 (high risk)	16 (16%)
EBER positivity	48 (48%)
<i>Primary treatment</i>	
Chemotherapy	64 (63.4%)
Chemoradiotherapy	34 (33.7%)
Radiotherapy only	3 (3%)
<i>Chemotherapeutic regimen</i>	
ABVD	68 (68%)
ABVD/C-MOPP hybrid	10 (10%)
BEACOPP	7 (7%)
C-MOPP	6 (6%)
Other regimen	9 (9%)

EBER, Epstein–Barr virus-encoded RNA-1 and RNA-2 assessed by *in situ* hybridization; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone; C-MOPP, cyclophosphamide, vincristine, prednisone, and procarbazine.

MET Protein Expression in cHL Tissue

Thirty-eight patients (38%) showed cytoplasmic and membranous positivity for MET. The clinicopathological factors of patients according to MET expression are summarized in Table 2. The MET-negative patients were more likely to have the NS subtype (67.7 vs 50%) and less likely to have the LR subtype (3.2 vs 13.2%; $P = 0.026$) than MET-positive patients. No distinction between the groups was observed in terms of age ($P = 0.211$), sex ($P = 0.303$), B symptoms ($P = 0.396$), Ann Arbor stage ($P > 0.999$), IPS ($P = 0.09$), LDH ($P = 0.666$), EBER ($P = 0.412$), bulky disease ($P = 0.498$), treatment method ($P = 0.631$), or primary chemotherapeutic regimen ($P = 0.354$).

MST1R Protein Expression in cHL Tissue

Twenty-six patients (26%) showed cytoplasmic and membranous positivity for MST1R. The clinicopathological factors of patients according to MST1R expression are summarized in Table 2. MST1R-positive patients were more likely to have the NS subtype (80.8 vs 54.1%), and were less likely to have

the LR subtype (0 vs 9.5%; $P = 0.128$) than MST1R-negative patients. No distinction between the groups was observed in terms of age ($P = 0.353$), sex ($P = 0.819$), B symptoms ($P = 0.483$), Ann Arbor stage ($P = 0.644$), IPS ($P = 0.227$), LDH ($P = 0.625$), EBER ($P = 0.504$), bulky disease ($P = 0.121$), treatment method ($P = 0.401$), or primary chemotherapeutic regimen ($P = 0.582$). Coexpression of MET and MST1R in tumor cells was observed in 13 cases (13%). In all, 25 cases (25%) showed MET expression only, 13 cases (13%) showed MST1R expression only, and 49 cases were negative for both MET and MST1R.

Correlation Between MET or MST1R Protein Expression and MET or MST1R mRNA Expression

MET or MST1R protein expression was associated with high MET or MST1R mRNA expression, respectively (Table 3, $P < 0.001$ and $P = 0.029$, respectively).

Prognostic Significance of MET and MST1R Protein Expression

MET-negative patients had lower 5-year OS rates than MET-positive patients (73.8 vs 93.7%, $P = 0.004$; Figure 2a), although the 5-year EFS rates were comparable (59.4 vs 65.4%, $P = 0.131$; Figure 2b). MST1R-negative patients had lower 5-year OS rates (76.1 vs 96.2%, $P = 0.022$; Figure 3a) and 5-year EFS rates (55.6 vs 81.4%, $P = 0.021$; Figure 3b) than MST1R-positive patients. To evaluate the relative importance of MET and MST1R, we combined the dichotomized MET and MST1R, and stratified patients into four groups (MET + /MST1R +, MET + /MST1R –, MET – /MST1R +, and MET – /MST1R –). Patients with MET + /MST1R + had better OS ($P = 0.004$; Figure 4a) and EFS rates ($P = 0.085$; Figure 4b) compared with the other expression patterns, although the statistical significance of the EFS was marginal.

To further assess the additional prognostic information regarding MET and MST1R, we performed subgroup analyses according to the Ann Arbor stage. In advanced-stage disease, MET-negative cases showed worse OS rates than MET-positive cases ($P = 0.002$; Supplementary Figure 1b). However, in limited-stage disease, MET expression was not significantly associated with OS ($P = 0.619$; Supplementary Figure 1a). In both stages, MET expression was not significantly associated with EFS ($P = 0.452$ for limited stage; Supplementary Figure 1c and $P = 0.212$ for advanced stage; Supplementary Figure 1d).

In patients with advanced-stage disease, MST1R-negative cases showed a worse trend for OS rate compared with MST1R-positive cases ($P = 0.071$; Supplementary Figure 2b), but MST1R expression was not significantly associated with OS rate in patients with limited-stage disease ($P = 0.178$; Supplementary Figure 2a). For advanced-stage

Table 2 Correlation of MET and MST1R protein expression with clinicopathologic variables

Characteristic	MET expression		P-value	MST1R expression		P-value
	Negative (n = 62)	Positive (n = 38)		Negative (n = 74)	Positive (n = 26)	
Age			0.211 ^a			0.353 ^a
Age < 40	34 (54.8%)	26 (68.4%)		42 (56.8%)	18 (69.2%)	
Age ≥ 40	28 (45.2%)	12 (31.6%)		32 (43.2%)	8 (20.8%)	
Gender			0.303 ^a			0.819 ^a
Male	38 (61.3%)	19 (50%)		43 (58.1%)	14 (53.8%)	
Female	24 (38.7%)	19 (50%)		31 (41.9%)	12 (46.2%)	
Disease subtype			0.026 ^b			0.128 ^b
Nodular sclerosis	42 (67.7%)	19 (50%)		40 (54.1%)	21 (80.8%)	
Mixed cellularity	16 (25.8%)	8 (21.1%)		19 (25.7%)	5 (19.2%)	
Lymphocyte rich	2 (3.2%)	5 (13.2%)		7 (9.5%)	0 (0%)	
Lymphocyte depleted	1 (1.6%)	1 (2.6%)		2 (2.7%)	0 (0%)	
Not classifiable	1 (1.6%)	5 (13.2%)		6 (8.1%)	0 (0%)	
B symptom			0.396 ^a			0.483 ^a
Absent	36 (58.1%)	26 (68.4%)		44 (59.5%)	18 (69.2%)	
Present	26 (41.9%)	12 (31.6%)		30 (40.5%)	8 (30.8%)	
Ann Arbor stage			> 0.999 ^a			0.644 ^a
Limited	25 (40.3%)	16 (42.1%)		29 (39.2%)	12 (46.2%)	
Advanced	37 (59.7%)	22 (57.9%)		45 (60.8%)	14 (53.8%)	
IPS			0.09 ^a			0.227 ^b
< 4	49 (79%)	35 (92.1%)		60 (81.1%)	24 (92.3%)	
≥ 4	13 (21%)	3 (7.9%)		14 (18.9%)	2 (7.7%)	
LDH (U/l)			0.666 ^a			0.625 ^a
< 250	19 (32.2%)	14 (36.8%)		23 (31.9%)	10 (40%)	
≥ 250	40 (67.8%)	24 (63.2%)		49 (68.1%)	15 (60%)	
EBER			0.412 ^a			0.504 ^a
Negative	30 (48.4%)	22 (57.9%)		40 (54.1%)	12 (46.2%)	
Positive	32 (51.6%)	16 (42.1%)		34 (45.9%)	14 (53.8%)	
Bulky disease			0.498 ^b			0.121 ^b
Absent	57 (91.9%)	33 (86.8%)		69 (93.2%)	21 (80.8%)	
Present	5 (8.1%)	5 (13.2%)		5 (6.8%)	5 (19.2%)	
Primary treatment			0.631 ^b			0.401 ^b
Chemotherapy	39 (62.9%)	24 (63.2%)		49 (66.2%)	14 (53.8%)	
Chemoradiotherapy	22 (35.5%)	12 (31.6%)		23 (31.1%)	11 (42.3%)	
Radiotherapy	1 (1.6%)	2 (5.3%)		2 (2.7%)	1 (3.8%)	
Primary chemotherapeutic regimen			0.354 ^b			0.582 ^b
ABVD	38 (62.3%)	30 (83.3%)		48 (66.7%)	20 (80%)	
ABVD/C-MOPP hybrid	8 (13.1%)	2 (5.6%)		8 (11.1%)	2 (8%)	
BEACOPP	5 (8.2%)	2 (5.6%)		6 (8.3%)	1 (4%)	
C-MOPP	5 (8.2%)	1 (2.8%)		6 (8.3%)	0 (0%)	
Other regimen	5 (8.2%)	1 (2.8%)		4 (5.6%)	2 (8%)	

IPS, International Prognostic Score; LDH, lactate dehydrogenase; EBER, Epstein-Barr virus-encoded RNA-1 and RNA-2 assessed by *in situ* hybridization; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; BEACOPP, bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, and prednisone; C-MOPP, cyclophosphamide, vincristine, prednisone, and procarbazine.

^aThe χ^2 test by two-sided Pearson's test.

^bThe χ^2 test by two-sided Fisher's test.

Table 3 Correlation of MET and MST1R protein expression with mRNA expression

Characteristic	MET expression		P-value		MST1R expression		P-value
	Negative (n = 62)	Positive (n = 38)			Negative (n = 74)	Positive (n = 26)	
MET mRNA			< 0.001 ^a	MST1R mRNA			0.029 ^a
Negative	56 (90.3%)	23 (60.5%)		Negative	55 (74.3%)	13 (53.6%)	
Positive	6 (9.7%)	15 (39.5%)		Positive	19 (25.7%)	13 (46.4%)	

^aThe χ^2 test by two-sided Pearson's test.

Figure 2 Comparison of survival rates according to MET and MST1R protein expression. (a) Overall survival according to MET. (b) Event-free survival according to MET.

disease, MST1R-negative cases showed worse EFS rates than MST1R-positive cases ($P=0.041$; Supplementary Figure 2d). For limited-stage disease, MST1R expression was not significantly associated with EFS rate ($P=0.336$; Supplementary Figure 2c).

In univariate analysis, both OS and EFS were associated with IPS (≥ 4). Age (>40 years), B symptoms, advanced stage, abnormal LDH, and ABVD-based chemotherapeutic regimen (doxorubicin, bleomycin, vinblastine, and dacarbazine) were associated with OS but not with EFS (Table 4). In multivariate analysis, MET expression was an independent prognostic marker for OS, along with high-risk IPS (≥ 4), B symptoms, age (>40 years),

Figure 3 Comparison of survival rates according to MST1R protein expression. (a) Overall survival. (b) Event-free survival.

abnormal LDH, and ABVD-based chemotherapeutic regimen (Table 5). In multivariate analysis, MST1R expression was an independent prognostic marker for EFS, along with high-risk IPS (≥ 4), B symptoms, age (>40 years), abnormal LDH, and ABVD-based chemotherapeutic regimen (Table 6).

Prognostic Significance of MET and MST1R mRNA Expression

Although there was no statistically significant difference, patients with MET positivity had a trend toward better OS than the MET-negative group (5-year OS rate: 89.9 vs 78.6%, $P=0.361$; Supplementary

Figure 4 Comparison of survival rates according to MET/MST1R protein expression pattern. (a) Overall survival. (b) Event-free survival.

Figure 3a). Patients with MST1R positivity also had a trend toward better OS than the MST1R-negative group (5-year OS rate: 86.9 vs 78.3%, $P=0.367$; Supplementary Figure 3c), although it was statistically nonsignificant. The MET or MST1R mRNA expression was not significantly associated with EFS ($P=0.772$; Supplementary Figure 3b and $P=0.897$; Supplementary Figure 3d, respectively).

Discussion

We found that the expression of MET, MST1R, or both was significantly associated with favorable clinical outcomes, which supports the hypothesis

that a MET/MST1R-related signaling pathway plays an important role in the progression of cHL. Evaluation of the MST1R and MET expression status may aid in selecting patients who could benefit from intensive therapy. Limitations of this study include the retrospective nature of the study design, the short follow-up period, and the relatively small sample size.

In striking contrast to our results, expression of MET/HGF in solid malignancies has been associated with unfavorable clinical outcomes,^{16,17,19,20,22,25} although a few studies identified a favorable prognostic impact of MET.^{36,37} Expression of MST1R is also associated with unfavorable clinical outcomes^{38–40} or not associated with prognosis.⁴¹ However, a recent study found that MET expression is associated with a favorable prognosis in cHL.³² These findings in cHL suggest that the prognostic impact of MET and MST1R in cHL may be significantly different from those in other solid malignancies.

There are several possible explanations for the favorable outcome associated with MET expression in patients with cHL. The unique histological features of cHL could play an important role. Because cHL is composed, on average, of <1% malignant HRS cells and a majority of reactive cells, including T and B lymphocytes and other cell types, the reactive cells could play an important role in disease progression. Xu *et al*³² suggested that the activation of MET in HRS cells might change the immunosuppressive function of the reactive cells by causing the secretion of factors that alter the microenvironment. This process could result in a decreased risk of cHL progression. In a mouse model, HGF gene transfer attenuates acute liver injury and hepatic fibrosis^{42,43} and suppresses TGF- β levels.⁴⁴ HRS cells may produce TGF- β to downmodulate the immune response.⁴⁵ Therefore, MET expression in HRS cells could reduce TGF- β production and help recover immune function. A previous study has revealed that MET inhibitor SU11274 suppressed cell growth by inducing G2/M cell cycle arrest in cHL cell line.³² Other studies have also found that MET inhibitor PHA665752 causes cell death and induces apoptosis in diffuse large B-cell lymphoma cell line.³⁷ MST1R expression is also required for the growth of lymphoblastoid cell line and posttransplantation lymphoproliferative disorder.⁴⁶

In a study of diffuse large B-cell lymphoma by Uddin *et al*,³⁷ the oncogenic roles of the MET and/or MST1R in promoting tumor cell growth and proliferation were reflected in the higher proliferation rates of the tumors that expressed MET protein. The authors ascribed the superior survival of diffuse large B-cell lymphoma cases with MET expression to the increased sensitivity to chemotherapy associated with higher proliferation rates (Uddin *et al*³⁷). Although we did not examine the proliferation rates of HRS cells in this series,

Table 4 Univariate analyses with MET or MST1R protein expression for overall survival (OS) and event-free survival (EFS)

Covariate		Univariate analysis					
		OS			EFS		
		HR	95% CI	P-value ^a	HR	95% CI	P-value ^a
Age (years)	<40 vs ≥40	3.823	1.48–9.85	0.006	1.549	0.80–2.97	0.19
Sex	Female vs male	2.717	0.99–7.43	0.052	1.172	0.60–2.27	0.639
B symptoms	(–) vs (+)	2.86	1.18–6.9	0.02	1.429	0.74–2.75	0.288
IPS	<4 vs ≥4	4.719	1.98–11.2	<0.001	2.137	1.00–4.55	0.049
Ann Arbor stage	Limited vs advanced	3.165	1.06–9.4	0.038	1.801	0.88–3.66	0.104
Bulky disease	<10 vs ≥10	2.151	0.28–16.0	0.455	1.152	0.35–3.75	0.815
LDH (U/l)	Normal vs abnormal	5.168	1.19–22.2	0.028	1.616	0.75–3.45	0.216
EBER	(–) vs (+)	1.277	0.63–2.56	0.491	0.948	0.58–1.53	0.827
MET expression	(–) vs (+)	0.156	0.03–0.67	0.012	0.576	0.27–1.19	0.138
MST1R expression	(–) vs (+)	0.136	0.01–1.01	0.052	0.316	0.11–0.08	0.03
Chemo. regimen	Non-ABVD vs ABVD	0.415	0.17–0.97	0.044	0.895	0.44–1.81	0.758

HR, hazard ratio; CI, confidence interval; IPS, International Prognostic Score; LDH, lactate dehydrogenase; EBER, Epstein–Barr virus-encoded RNA-1 and RNA-2 assessed by *in situ* hybridization; Chemo, chemotherapeutic; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine.

^aCox univariate analysis.

Table 5 Multivariate analysis with MET protein expression for overall survival (OS) and event-free survival (EFS)

Prognostic factor	OS			EFS		
	P-value	HR	95% CI	P-value	HR	95% CI
MET positive	0.025	0.187	0.043–0.811	0.203	0.62	0.297–1.295
IPS ≥4	0.003	3.815	1.597–9.113	0.087	1.949	0.908–4.185
MET positive	0.015	0.163	0.038–0.702	0.151	0.585	0.282–1.215
Presence of B symptoms	0.03	2.654	1.098–6.419	0.356	1.364	0.706–2.637
MET positive	0.02	0.176	0.041–0.76	0.171	0.599	0.288–1.247
Age ≥40	0.01	3.484	1.348–9.002	0.201	1.534	0.796–2.957
MET positive	0.015	0.163	0.038–0.701	0.127	0.564	0.271–1.176
LDH abnormal	0.032	4.947	1.146–21.34	0.214	1.619	0.757–3.462
MET positive	0.022	0.179	0.041–0.78	0.152	0.579	0.274–1.223
ABVD regimen	0.166	0.542	0.228–1.289	0.95	1.023	0.494–2.119

IPS, International Prognostic Score; LDH, lactate dehydrogenase; EBV, Epstein–Barr virus; HR, hazard ratio; CI, confidence interval; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine.

Table 6 Multivariate analysis with MST1R protein expression for overall survival (OS) and event-free survival (EFS)

Prognostic factor	OS			EFS		
	P-value	HR	95% CI	P-value	HR	95% CI
MST1R positive	0.074	0.159	0.021–1.193	0.04	0.335	0.118–0.951
IPS ≥4	0.001	4.149	1.743–9.876	0.095	1.908	0.893–4.078
MST1R positive	0.058	0.144	0.019–1.07	0.032	0.321	0.113–0.909
Presence of B symptoms	0.028	2.687	1.113–6.492	0.379	1.344	0.696–2.597
MST1R positive	0.07	0.156	0.021–1.163	0.035	0.326	0.115–0.923
Age ≥40	0.008	3.593	1.392–9.272	0.209	1.522	0.791–2.93
MST1R positive	0.073	0.158	0.021–1.185	0.047	0.347	0.122–0.986
LDH abnormal	0.034	4.852	1.125–20.92	0.307	1.487	0.694–3.186
MST1R positive	0.071	0.157	0.021–1.174	0.035	0.323	0.113–0.922
ABVD regimen	0.088	0.473	0.200–1.117	0.933	1.031	0.505–2.108

IPS, International Prognostic Score; LDH, lactate dehydrogenase; EBV, Epstein–Barr virus; HR, hazard ratio; CI, confidence interval; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine.

previous studies have reported prognostic relevance of proliferation rates of HRS cells in cHL.^{47–49} It is conceivable that the superior survival of cHL with

MET and/or MST1R expression in the present study may also involve higher proliferation rate of HRS cells.

In our study, MET or MST1R protein correlated with each level of mRNA. However, MET or MST1R mRNA expression was not associated with the survival outcome of patients. Alternative splicing was reported in MET and MST1R, and this process could result in the different expression of protein.⁵⁰ MET protein levels are affected by various factors including the levels of MET mRNA expression and stability; the translational activity, which may be regulated by exogenous and endogenous microRNAs; and proteasomal degradation.^{51–53} Therefore, several protein regulating factors may be responsible for the differences between protein and mRNA levels.

Amplification, mutation, and overexpression of MET were described in various malignancies and such dysregulation was also associated with tumor progression.^{54,55} Amplification, mutation, and overexpression of the MST1R have also been reported in renal and lung cancer and implicated in transformation, tumor formation, and metastasis.^{39,56} Furthermore, amplification of 3p, which is a the chromosome region containing MST1R, is a common event in lung, renal, and breast cancer, occurring in 15–42.5% of the samples examined.⁵⁷ MET signaling is mainly mediated by the RAS–MAPK and PI3K–AKT pathways and affects gene expression and cell cycle progression.^{58,59} Xu *et al*³² also reported that HGF stimulation induced p-AKT upregulation in cHL cell line. MST1R is also a strong inducer of both MAPK and the PI3K signaling pathways.⁶⁰ Therefore, coexpression of MET and MST1R finally would result in tumor progression.

Intriguingly, patients with coexpression of MST1R and MET showed better survival outcomes than those with MET or MST1R expression alone, or no expression. MST1R and MET are expressed on the cell membrane surface as preformed dimers before ligand stimulation. A bidirectional transphosphorylation occurs between MET and MST1R after exposure of cells to either HGF or macrophage-stimulating protein.⁶¹ Although MST1R is less efficient than MET as a kinase, the formation of MET/MST1R complexes leads to a more efficient MST1R transphosphorylation by MET, resulting in a more sustained signal than that induced by the MST1R/MST1R homodimer. Activation of both MET and MST1R may induce a cooperative or synergistic response to their ligands. Patients with tumors coexpressing MET and MST1R reportedly have a worse prognosis than those with single receptor-positive tumors.^{34,62,63} The synergistic effect of MET and MST1R might induce a favorable outcome, as MET expression is associated with a better prognosis in cHL.

A previous study demonstrated a correlation of MET expression with EBV positivity in cHL patients,³⁰ whereas a larger cohort showed no association.⁶⁴ In the present study, we observed no correlation between MET expression and EBV status, in agreement with the data of previous studies.^{32,64} No studies have examined the correlation between

MST1R expression and EBV status in cHL patients. Our result found no correlation between MST1R expression and EBV status. These results suggest that MET and MST1R expression was associated with survival outcome independent of EBV status.

In the present study, MET or MST1R expression was significantly associated with better prognosis, especially for patients with advanced-stage disease, which is consistent with results from another study in patients with cHL.³² Although IPS is the standard stratification system for survival in patients with advanced-stage disease, it is limited to selecting distinct subgroups of patients at very high risk of recurrence.⁶⁵ This result suggests that MET or MST1R positivity provides additional prognostic information, independent of IPS.

In summary, our results suggest the prognostic significance of the coexpression of MST1R and MET in cHL patients with advanced-stage disease. Coexpression of MST1R and MET can be used to identify a subgroup of cHL patients at high risk for recurrence or progression who may benefit from aggressive chemotherapy. Further studies, including prospective clinical trials, are needed to investigate the effects of MET or MST1R expression on clinical outcomes and to confirm the present findings.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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