Clinicopathologic analysis of gastric mucosa-associated lymphoid tissue lymphoma with or without c-Met expression

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Abstract

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) lymphoma of the stomach is mainly associated with *Helicobacter pylori* infection, and *H. pylori* eradication therapy is often effective. However, 20–30% of the cases of MALT lymphoma are resistant to the eradication therapy, and translocation of the *API2-MALT1* gene is often found in these cases. Most cases without translocation of *API2-MALT1* are localized to the stomach, whereas some cases with this translocation are a more advanced stage of MALT lymphoma that spreads to other organs. The c-Met receptor is a prognostic factor involved in infiltration and metastasis in many malignant tumors, including gastric, pancreatic, lung, and kidney cancer. In the present study, the expression of c-Met in 43 cases of gastric MALT lymphomas was immunohistochemically examined and compared with clinicopathological factors. To elucidate the significance of c-Met in MALT lymphoma, the expression intensity of c-Met in 22 *API2-MALT1* translocation-positive and 21 *API2-MALT1* translocation-negative cases was scored, compared, and examined. The immunohistochemistry analysis revealed strong staining for c-Met in 21 *API2-MALT1*

translocation-positive cases and in 1 translocation-negative case ($P = 0.00$). This result indicates the relationship between strong expression of c-Met and the progression of MALT lymphoma with *API2-MALT1* gene translocation.

Introduction

Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) lymphoma occurs in the extranodal organs such as the lungs, throat, thyroid gland, and gastrointestinal tract around the stomach [1]. Approximately 90–95% of the gastric MALT lymphomas are associated with *Helicobacter pylori* infection [2, 3]. Most patients with this infection can be cured with *H. pylori* eradication therapy, but some patients without the infection do not respond well to eradication therapy, and advanced stages of the lymphoma often occur [4]. Nevertheless, some patients with MALT lymphoma not associated with *H. pylori* infection have responded to eradication therapy; therefore, regardless of the presence of infection, *H. pylori* eradication therapy is often selected as a first treatment [5]. Of these cases, 20–30% are resistant to this eradication therapy. These cases are related to the t(11; 18)(q21; q21) translocation, which is a product of the amino terminus of the *API2* gene fused to the carboxyl terminus of the *MALT1* gene. This translocation is involved in tumor development by directly activating canonical and non-canonical NF-κB pathways [6], but the mechanism of oncogenesis is not completely clear yet. Radiotherapy or chemotherapy is

performed as a second line therapy for treatment of sterilization resistance [7]. However, there are cases in which remission is not achieved even with these treatments. Thus, the development of further treatment options is necessary.

The c-Met receptor specifically binds to hepatocyte growth factor (HGF), which increases cell proliferation, survival and mobility and is involved in tumorigenesis. HGF/c-Abnormal activation of the MET pathway has been confirmed in various tumors. c-Met is a cancer gene that encodes a receptor tyrosine kinase that uses hepatocyte growth factor (HGF) as a ligand; c-Met specifically binds to HGF, resulting in the synthesis of the HGF/c-Met signal. Activation of this signal promotes the activation of pathways involved in carcinogenesis, such as the RAS, PI3K, STAT3, and β -catenin pathways, and is involved in tumor cell invasion and metastasis via angiogenesis and metalloprotease production. c-Met is predominantly present in epithelial cells and expressed in the liver and kidney more frequently, but expression is also found in the gastrointestinal tract, prostate gland, seminal vesicle, breast cell lines, brain microglial cells, monocytes, and macrophages [8]. Moreover, it is involved in the development of B-cell lymphoma [9].

Studies on various carcinomas have suggested that the inhibition of the HGF/c-Met signaling system may be a promising target for anticancer agents. Its effect on the inhibition of activation and tumor pathways was also observed in an animal model of MALT lymphoma associated with *Helicobacter heilmannii* [10]. In order to clarify the association of the c-Met receptor and gastric MALT lymphoma, we investigated the expression of c-Met in patients with gastric MALT lymphoma using clinicopathological factors.

Methods

Case selection

A total of 43 patients with gastric MALT lymphoma, pathologically diagnosed at the Department of Pathology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan, from January 2000 to January 2014, were included in this study. Of these, 22 cases were of gastric MALT lymphoma with *API2-MALT1* gene translocation, and 21 cases were of gastric MALT lymphoma without the translocation. In addition, 10 patients with gastritis who underwent biopsy

and tested negative for MALT lymphoma were also included as control cases.

Immunostaining was performed on paraffin-embedded sections for 43 patients and control cases. Diagnosis and evaluation of immunohistochemistry (IHC) staining were conducted by hematopathologists (Y.S., R.O., and T.Y). *H. pylori* infection was identified using serum antibody titer and and pathological examination. Clinical, imaging, and laboratory information was extracted from the patients' medical charts. Clinical stages were determined following the Lugano classification [11]. This study was approved by the institutional ethical review board of Okayama University.

Summary of the patients

Patients were investigated in gender, age, *API2-MALT1* transformation, *H.pylori* infection, clinical stage, location in the stomach, endoscopic findings and response of eradication (Table 1).

Among the 43 cases of gastric MALT lymphoma, 39 cases were stage I, 3 cases were stage II, and 1 case was stage IV. Stage I cases were limited to the stomach. Stage II showed infiltration in local lymph nodes, and stage IV showed infiltration in the lung. *H. pylori* infection was identified by serum antibody titer and pathological examination. 40

cases were investigated in *H.pylori* infection by serum antibody titer. 17 cases were positive, and five of them were positive for *API2-MALT1* gene translocation and twelve cases were negative. On the other hand, 43 cases were investigated in *H.pylori* infection by pathological examination. 21 cases were positive, and five of them were positive for *API2-MALT1* gene translocation and sixteen cases were negative. Regardless of *H. pylori* infection, eradication therapy was initially conducted in 41 cases, and complete and partial remission were achieved in 18 cases (6 of which were *H. pylori* negative). The remaining two cases did not follow clinical courses. Twenty-one cases showed resistance to the eradication therapy, but all cases achieved remission after radiation therapy or chemoradiotherapy. Finally, two patients did not receive treatment under the watch-and-wait approach; the progression of the disease was not studied (Figure 4).

Immunohistochemistry

All samples used in this study were gastric biopsy specimens. These specimens were fixed in 10% formaldehyde and embedded in paraffin. Serial 4 μm-thick sections were cut from the paraffin blocks and stained with hematoxylin and eosin. The sections were

immunohistochemically stained using an automated Bond Max instrument (Leica Biosystems, Wetzlar, Germany), and mouse anti-human c-Met antibody (clone 4AT44; 1:100; LifeSpan BioSciences, Seattle, WA, USA) was used as the primary antibody. According to previous reports, immunoreactivity for c-Met was observed as cytoplasmic or membrane staining in lymphoid cells. Staining results were assessed semi quantitatively using a modified McCarty's H-scoring system. The intensity score (0, none; 1, light brown; and 2, brown) and proportion score (0, 0–5%; 1, 6–25%; 2, 26–50%; 3, 51–100%) were added to yield an overall c-Met score [12]. Then, the total score was calculated, in which 0, 1–3, and 4–5 points were negative, weak positive, and strongly positive results, respectively (Figure 1, Figure 2).

Fluorescence in situ hybridization (FISH)

FISH analysis was performed on fresh biopsy samples of all patients using dual-color, dual-fusion translocation probes for *API2-MALT1*, Vysis LSI *BIRC3/MALT1* dual color dual fusion probes(Abbott Molecular Inc, USA) .These probes hybridize to chromosome *11q22*(*BIRC3* SpectrumGreen) and chromosome *18q21*(*MALT1* SpectrumOrange).

In a normal cell that lacks the *t(11;18)(q21;q21)* translocation, a two orange, two green signal pattern will be observed reflecting the two intact copies of *MALT1* and *BIRC3*, respectively.

In an abnormal cell containing the *t(11;18)(q21;q21)* translocation, a one orange (*MALT1*), one green (*BIRC3*), and two fusion (*BIRC3/MALT1 and MALT1/ BIRC3*) signal pattern will be observed.

Statistical analysis

Statistical analysis was performed using the SPSS statistics 24 for Windows (SPSS Inc., Chicago, IL, USA) software. Qualitative variables were compared using chi-square or Fisher's tests. Quantitative variables were compared using Student's t-test or a nonparametric Wilcoxon test, whereas a Kruskal-Wallis rank test was used for comparisons among multiple groups. Logistic regression analysis was used as multivariate analysis. *P* values under 0.05 were considered statistically significant.

Results

c-Met expression in MALT lymphomas

The c-Met staining scores of cases of MALT lymphoma positive and negative for *API2-MALT1* translocation and the cases of gastritis were compared (Figure). The mean score for translocation-positive MALT lymphoma was 4.73, which was significantly higher than the score for the translocation-negative cases (mean score = 1.13; $P < 0.001$). There was no significant difference in translocation-negative MALT lymphoma compared to gastritis (mean score = 0.55; $P = 0.283$).

In addition, according to the c-Met score, 43 cases of MALT lymphoma were stratified into three groups: no expression group (score 0), low expression group (score 1–3), and high expression group. No expression, low expression, and high expression groups comprised 7, 16, and 22 cases, respectively. The relationship between c-Met expression and clinicopathological factors are shown in Table 2.

Relationship between clinical findings and pathological findings

There was no significant association between c-Met expression and gender, age, *H. pylori* infection by the serum antibody titer and the pathological examination, clinical

stage, and endoscopic findings. Contrastingly, *API2-MALT1* translocation was significantly associated with c-Met expression: the intensity scores for translocation-positive MALT lymphoma included 21 cases of brown, 1 light brown, and 0 none, whereas for translocation-negative cases, 1 was strongly positive, 13 weakly positive, and 7 negative ($P < 0.000$). The expression of c-Met was significantly high in cases where MALT lymphoma infiltrated the middle (M) region of the stomach or was widely expanded including the M region ($P = 0.045$). Furthermore, of the 41 studied cases, 25 patients were resistant to eradication therapy, out of which 22 were strongly positive for c-Met, which was significantly higher ($P = 0.046$).

Moreover, univariate and multivariate analyses were performed between two groups—no or low c-Met expression versus high c-Met expression—for the factors that were significant or tended to be significant in the analysis of the three groups of different c-Met expression. The presence/absence of a cobblestone-like appearance, observed in the endoscopy, was added to the analysis. The univariate analysis resulted in significant differences between all factors. However, only *API2-MALT1*

translocation-positive cases were found to be significantly associated with high c-Met expression in the multivariate analysis ($P < 0.000$).

Discussion

c-Met was originally identified as an oncogene in the 1980s, and it was first isolated from a human osteosarcoma cell line [13, 14]. Thereafter, many solid cancers were reported to be associated with the HGF/c-Met signaling pathway, but the reports on B-cell lymphoma, particularly MALT lymphoma, were limited [13]. In this study, which focused on the expression of c-Met to elucidate the progression of MALT lymphoma, we showed that c-Met expression is significantly associated with *API2-MALT1* translocation.

Regarding the development of gastric MALT lymphoma, the persistent activation of the NF-κB pathway is known to be caused by inflammation associated with *H. pylori* infection. In addition to generating immune responses, the direct activation of both canonical and non-canonical NF-κB pathways by genetic abnormalities such as *API2-MALT1* gene translocation induces the genesis of MALT

lymphoma [6]. In this study, c-Met expression was scarcely observed in the cases of gastritis associated with *H. pylori* infection or in *API2-MALT1* translocation-negative cases. Conversely, c-Met expression was strong in *API2-MALT1* translocation-positive cases (Figure 3). This result suggests that c-Met is directly associated with *API2-MALT1* gene translocation and the accelerated tumorigenesis pathway even without the inflammation caused by *H. pylori* infection.

The result of the univariate analysis between c-Met expression and various factors is shown in Table 3. In the univariate analysis, significant correlation with c-Met expression was found for cases with infiltration of the M gastric region, resistance to the eradication therapy, presence of cobblestone appearance, and absence of *H. pylori* infection, in addition to that for *API2-MALT1* translocation-positive cases. However, only *API2-MALT1* gene translocation was significantly correlated with c-Met expression in the multivariate analysis. The absence of *H. pylori* infection, cobblestone appearance in endoscopy, and resistance to eradication therapy are well-known characteristics of MALT lymphoma with *API2-MALT1* gene translocation [3, 15, 16]. The strong correlation between c-Met expression and *API2-MALT1* translocation may

be the reason why several factors were considered significantly correlated to c-Met expression in the univariate analysis. These results suggest that the only factor clearly correlated with c-Met expression is *API2-MALT1* translocation. However, the detailed mechanisms through which *API2-MALT1* gene translocation causes the expression of c-Met are unknown. Therefore, future research on this matter is expected.

MALT lymphoma is considered an indolent lymphoma [17]. In the present study, the use of radiation and/or chemotherapy following eradication therapy was effective and achieved remission even in cases involving other organs and lymph nodes. All patients that underwent these treatments survived without disease progression. Past studies have reported cases with progression to high grade lymphoma; the results were fatal, but these were a minority. Therefore, the use of aggressive therapy with strong influence on the whole body after failed eradication therapy is still supported [17, 18].

Molecular targeted therapy, which has limited influence on the whole body, has been recently developed. Rituximab (or anti-CD20 antibody) has been suggested as a single-agent treatment for MALT lymphoma [19]. However, the effects of this

treatment are still controversial [17, 18]. Therefore, other molecular targets for single-agent treatments or combined therapies should be identified.

Conclusion

Molecular therapies targeting c-Met have been used in the treatment of solid lung tumors, and the application of these therapies to lymphoma is expected. In this study, we investigated the c-Met expression status in gastric MALT lymphoma. This study showed that the higher c-Met expression might to be correlate with *API2-MALT1* translocation and therapeutic resistance in gastric MALT lymphoma. Molecular therapy targeting c-Met is expected to be established as a new treatment for MALT lymphoma in the future.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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