Relationship Between O-GlcNAcase Expression and Prognosis of Patients With Osteosarcoma

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Abstract: Several studies have demonstrated a role of O-GlcNacyla tion (O-GlcNAc) in tumorigenesis of various carcinomas by modification of tumor-associated proteins. However, its implication in the pathogenesis of osteosarcoma remains unclear. This study aimed to investigate the levels of O-GlcNAc and the expressions of O-linked N-acetylglucosamine transferase (OGT) and O-GlcNAcase (OGA) in human osteosarcoma tissues, by using immunohistochemistry; and to find correlations between the levels of expressions and several clinicopathologic parameters. There were 109 first diagnosed osteosarcoma patients, including Enneking stage IIB (n = 70) and III (n = 39). Correlations between the immunoreactive score (IRS) and clinicopathologic parameters, overall survival, and metastasis-free survival were evaluated. A positive correlation was found between the IRS of OGA and the percentage of postchemotherapeutic tumor necrosis (r = 0.308; P = 0.017). Univariate analysis revealed significantly lower OGA IRS in metastatic patients (P = 0.020) and poor chemotherapeutic-responder patients (P = 0.001). By multivariate analysis, presence of tumor metastasis (P = 0.002) and lower OGA IRS (P = 0.004) was significantly associated with shorter overall survival. Subgroup analysis in stage IIB osteosarcoma (n = 70) demonstrated that male sex (P = 0.019), presence of tumor recurrence (P = 0.026), poor chemotherapeutic responder (P = 0.022), and lower OGA IRS (P = 0.019) were significantly correlated with short metastasis-free survival. But, lower OGA IRS was the only independent predictor for short metastasis-free survival (P = 0.006). Our findings suggested that O-GlcNAc pathway, especially OGA, may involve in pathogenesis and aggressiveness of osteosarcoma. Low level of OGA expression may be used as a poor prognostic indicator.  

Key Words: immunohistochemistry, O-GlcNAcase, O-GlcNac ylation, O-linked N-acetylglucosamine transferase, osteosarcoma  

O-GlcNAcyla tion (O-GlcNAc) is a posttranslational modification of proteins associated with regulation of various protein functions by an addition of N-acetylglucosamine (GlcNAc) to the hydroxyl group of serine or threonine residues of the target proteins. This modification is a dynamic and reversible process, strictly regulated by 2 important enzymes, O-linked N-acetylglucosamine transferase (OGT) and O-GlcNAcase (OGA), which add and remove the GlcNAc moieties, respectively.1 O-GlcNAc is involved in many cellular processes, such as signal transduction, transcription, cell cycle control, and epigenetic control of gene expressions in response to stress and nutrient imbalance.2,3 In this regard, O-GlcNAc homeostasis needs to be maintained to achieve proper cellular functions.  

Aberrations of O-GlcNAc are associated with the pathogenesis of many human diseases, including diabetes, neurodegenerative diseases and cancers.4,5 With respect to cancer pathogenesis and progression, several key players, such as p53, c-Myc, Forkhead box M1 etc., have been shown to be modified via the O-GlcNAc pathway.5–8 Recently, several cancer studies have reported that elevated O-GlcNAc is associated with tumorigenesis and cancer cell metastasis.8–16 Moreover, several previous studies have shown that hyper-O-GlcNAc and overexpression of OGT were associated with worse clinicopathologic outcomes in various cancers.12,16–18
Osteosarcoma is most commonly found in children, adolescents and young adults, accounting for ~20% of all primary bone tumors.19 At present, the treatment strategy for osteosarcoma includes surgical resection of primary bone tumor combined with polychemotherapy.19–21 In patients with nonmetastatic osteosarcoma, the 5-year survival rate has reported as 71%.22 However, the 5-year survival rate is as low as ~30% in metastatic cases.23 The clinical prognostic indicators for osteosarcoma include extent of disease (tumor staging) at the time of diagnosis, size and location of the tumor, surgical remission and/or responses to chemotherapy, and the presence of metastasis.20,24

Although there are many studies that have investigated the relationship between O-GlcNAc and various cancers, the role of O-GlcNAc in the pathogenesis of osteosarcoma remains largely unknown. Based on our previous study,25 it was found that O-GlcNAc levels and OGT expression were increased in primary cancer cells isolated from patients with osteosarcoma in association with metastasis and poor response to chemotherapy. Furthermore, the degrees of OGT expression were

### Table 1. Clinicopathological Characteristics of 109 Osteosarcoma Cases

| Characteristics                  | Enneking Stage |  |  |  |
|----------------------------------|----------------|---|---|---|
|                                  | IIB (n = 70)*  | III (n = 39)* | P  |  |
| Age (Mean ± SD (y))              | 22.21 ± 15.91  | 19.30 ± 16.25  | 0.360† |  |
| Sex                              |                |                | 0.171‡ |  |
| Male                             | 32 (45.7%)     | 23 (58.9%)     |  |  |
| Female                           | 38 (54.3%)     | 16 (41.1%)     |  |  |
| Tumor location                   |                |                | 0.419‡ |  |
| Extremities                      | 63 (90%)       | 34 (87.2%)     |  |  |
| Axial                            | 7 (10%)        | 5 (12.8%)      |  |  |
| Neoadjuvant chemotherapy         |                |                | 0.997‡ |  |
| Cis/Doxo based§                  | 51 (72.9%)     | 29 (74.4%)     |  |  |
| Others||                      | 9 (12.9%)      | 5 (12.8%)      |  |  |
| None                             | 10 (14.2%)     | 5 (12.8%)      |  |  |
| Surgical resection               |                |                | 0.075‡ |  |
| Yes                              | 56 (80%)       | 26 (66.7%)     |  |  |
| No                               | 14 (20%)       | 13 (33.3%)     |  |  |
| Lung metastasis (later detected during a follow-up) |                |                |  |  |
| Yes                              | 34 (48.6%)     | NA             | NA |  |
| No                               | 36 (51.4%)     | NA             | NA |  |
| Bone metastasis (later detected during a follow-up) |                |                |  |  |
| Yes                              | 3 (4.3%)       | NA             | NA |  |
| No                               | 67 (95.7%)     | NA             | NA |  |
| Recurrence                       |                |                | 0.836‡ |  |
| Yes                              | 8 (11.4%)      | 4 (10.3%)      |  |  |
| No                               | 62 (88.6%)     | 35 (89.7%)     |  |  |
| Tumor necrosis after chemotherapy (n = 60) |                |                | 0.678‡ |  |
| ≥90%                             | 9 (20.9%)      | 3 (17.6%)      |  |  |
| <90%                             | 34 (79.1%)     | 14 (82.4%)     |  |  |

*Stage IIB is a high-grade and extracompartamental osteosarcoma lesion without distant metastases at the diagnostic period, while stage III is an osteosarcoma lesion diagnosed as any grade with distant metastases.

†Independent samples t test.

‡Two-tailed Pearson χ² test.

§Common drugs used in chemotherapy for 94 cases, including carbo = carboplatin; cis = cisplatin; doxo = doxorubicin.

||Others = metrotrexate, ifosfamide, etoposide, docetaxel and gemcitabine.

NA indicates not applicable.
significantly higher in these cancer cells derived from poor responders than in those from good responders. However, due to a small sample size in that study as well as very limited knowledge regarding the role of O-GlcNAc together with that of the 2 relevant enzymes in the pathogenesis and prognosis of osteosarcoma, a study with a

**FIGURE 1.** Representative images of O-GlcNAc (O-GlcNAcylation) levels, OGT (O-linked N-acetylglucosamine transferase) and OGA (O-GlcNAcase) expressions from different osteosarcoma specimens, categorized as stage IIIB or III according to the Enneking classification, showing distinct immunostaining intensities, scored as strong, moderate or weak intensity. Negative = specimens reacted without the primary antibodies; scale bar = 50 µm.
larger sample size is required to gain further insights. In this study, the expressions of OGT and OGA, and the levels of O-GlcNAc were, therefore, examined in 109 bone specimens, histopathologically diagnosed as osteosarcoma using immunohistochemistry, and the correlations were determined between the O-GlcNAc profiles and several clinicopathologic characteristics at the time of diagnosis as well as patients’ treatment outcomes.

**MATERIALS AND METHODS**

**Tissue Samples**

The study cohort included bone biopsies taken from 109 patients with osteosarcoma, who were diagnosed and treated at the Maharaj Nakorn Chiang Mai Hospital, Thailand, between 1999 and 2020. All patients were followed-up for survival analysis until January 11, 2020. According to the Enneking tumor staging,26 all of these cases were classified as either stage IIB or III (Table 1). Of 109 patients, 73 cases (66.9%) had received standard treatment, comprising surgical tumor resection in combination with neoadjuvant chemotherapy. The remaining 36 patients (33.1%) refused either chemotherapy (n = 9), surgical therapy (n = 21) or both (n = 6). All specimens were fixed in formalin and embedded in paraffin before being obtained from tissue archive of the Department of Pathology, Faculty of Medicine, Chiang Mai University. The study protocol was approved by The Research Ethics Committee of the Faculty of Medicine, Chiang Mai University (no. 062/2015). Demographic and clinicopathologic features were retrieved from hospital records and are summarized in Table 1. Using the previously published protocol,27 the percentage of postchemotherapeutic tumor necrosis was calculated by certified pathologists. Of 73 cases who received both chemical and surgical therapies, tumor necrosis data of 60 cases were only available for determination of their correlation with the levels of the 3 molecules.

**Immunohistochemistry**

Immunohistochemistry protocol was described as in previous study.28 It was performed on the 3-μm tissue sections using the Ventana automated stainer (Ventana Medical Systems, Tucson, AZ). Optimized conditions, including dilution, incubation time, and positive control tissues for each antibody used in the study are summarized in Table 2.12,14,16,29-35 Tissue sections were first deparaffinized using EZ Prep (Ventana Medical Systems) at 75°C for 8 minutes. Heat-induced antigen unmasking was performed using Cell Conditioning Solution CCI (Ventana Medical Systems) at 95°C for 30 minutes, followed by incubation with UV inhibitor (Ventana Medical Systems) for 4 minutes at 37°C to block endogenous peroxidase activity. Each section was incubated at 37°C for 32 minutes with the prediluted primary antibody. After washing, the section was incubated with either HRP-labeled anti-rabbit or HRP-labeled anti-mouse IgG (UV HRP Multimer, Ventana Medical Systems) at 37°C for 8 minutes. Immunoreactivity was detected using the Ultraview Universal DAB Detection Kit (Ventana Medical Systems). The section was then counterstained and postcounterstained with hematoxylin and bluing reagent,
respectively, and mounted with a cover slip. The section was first oriented using a bright field microscope (Carl Zeiss, Oberkochen, Germany) at ×10 magnification, and 3 images that represented the immunostaining of each section were taken using an attached digital camera (Axiocam Icc5, Carl Zeiss) at ×40 magnification. Tissue sections from breast (3 cases), lung (2 cases) and colon (1 case) cancers known to express O-GlcNAc, OGT, and OGA \(^{8,9,11,12,14-16}\) were used as a positive control (Supplemental Fig. 1, Supplemental Digital Content 1, http://links.lww.com/AIMM/A314), whereas omission of these antibodies during the immunoreaction resulted in no staining (data not shown).

**Determination of Immunoreactive Score (IRS)**

The IRS was based on the 3 representative images as aforementioned, and the average IRS from these images was used to semiquantitatively assess immunohistochemical staining. The IRS (0 to 12) in each digitized image was determined by multiplication of the percentage score of positive cells as follows, 0 = 0%, 1 = < 10%, 2 = 10% to 50%, 3 = 51% to 80%, 4 = > 80%, with the intensity score as follows, 0 = no staining, 1 = weak (light yellow), 2 = moderate (brown), 3 = strong (dark brown). The immunostaining in the nucleus and/or the cytoplasm in a cancer cell was counted as a positive cell, regardless of its intensity. All immunostained specimens were examined by 2 independent observers (T.S. and N.C.), who were trained and calibrated before IRS analysis period with J.S., a Thai Board-certified pathologist, without prior knowledge of clinical data. The intraclass correlation coefficients for intraobserver and interobserver calibration were equal to 0.93 and 0.81, respectively.

**FIGURE 2.** A moderately positive correlation between the immunoreactive scores (IRS) of OGA (O-GlcNAcase) and the percentage of postchemotherapeutic tumor necrosis.

**FIGURE 3.** The immunoreactive scores (IRS) of O-GlcNAc (O-GlcNAcylation) or those of OGT (O-linked N-acetylglucosamine transferase) were not significantly associated with increased overall survival (A, B, respectively), whereas the higher OGA (O-GlcNAcase) IRS than its cutoff value was significantly associated with increased overall survival (C). The cutoff value of each biomolecule was estimated from the time-dependent receiver operating characteristic curve and its P-value was derived from the log-rank test. *P < 0.05.
The statistical analysis was performed using IBM SPSS Statistics version 23 (Chicago, IL). The demographic and clinicopathologic features were compared between the 2 tumor stages (IIB vs. III) using Independent samples t test or $\chi^2$ analysis. The mean IRSs were compared between the 2 tumor stages using independent samples t test. Correlations between the IRS and the clinicopathologic characteristics of patients were analyzed using Pearson correlation or Spearman Rank correlation analysis. Univariate analyses of overall and metastasis-free survival in association with the IRS were performed using the Kaplan-Meier method together with the log-rank test. Multivariate survival analysis was carried out by Cox regression of proportional hazards to indicate significance at the 95% confidence interval (CI) using Stata version 16 (StataCorp, College Station, TX). The results with $P$ values < 0.05 were considered statistically significant.

### RESULTS

#### Positive Correlation Between the IRSs of OGA Expression and the Percentages of Tumor Necrosis

The clinicopathologic data of this cohort are summarized in Table 1. The follow-up period of 109 patients with osteosarcoma after initial diagnosis ranged from 1 to 199 months with an average of 39.5 months. Varying intensities from weak to strong immunostaining of O-GlcNAc levels, OGT and OGA expressions were observed in 109 osteosarcoma specimens as shown by their representative images in Figure 1. The same immunoreactive patterns, found both in the nucleus and in the cytoplasm, are also reported in other cancers using 3 primary antibodies mentioned in Table 2. Between the 2 stages, no significant differences were found in any of the clinicopathologic parameters (Table 1) and in the mean IRS of the 3 biomolecules (Supplemental Fig. 2, Supplemental Digital Content 1, http://links.lww.com/AIMM/A314). By univariate analysis (Table 3), 4 significant associations between the IRS of O-GlcNAc levels, that of OGT expression, or that of OGA expression performed using the Kaplan-Meier method with the log-rank test. Multivariate survival analysis was carried out by Cox regression of proportional hazards to indicate significance at the 95% confidence interval (CI) using Stata version 16 (StataCorp, College Station, TX). The results with $P$ values < 0.05 were considered statistically significant.

### Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics version 23 (Chicago, IL). The demographic and clinicopathologic features were compared between the 2 tumor stages (IIB vs. III) using Independent samples $t$ test or $\chi^2$ analysis. The mean IRSs were compared between the 2 tumor stages using independent samples $t$ test. Correlations between the IRS and the clinicopathologic characteristics of patients were analyzed using Pearson correlation or Spearman Rank correlation analysis. Univariate analyses of overall and metastasis-free survival in association with the IRS were performed using the Kaplan-Meier method together with the log-rank test. Multivariate survival analysis was carried out by Cox regression of proportional hazards to indicate significance at the 95% confidence interval (CI) using Stata version 16 (StataCorp, College Station, TX). The results with $P$ values < 0.05 were considered statistically significant.
and the clinicopathologic parameters were noted, including the \( \text{O-GlcNAc} \) IRS and tumor locations \((P = 0.029)\), the OGT IRS and the presence of recurrence during the follow-up period \((P = 0.001)\), the OGA IRS and the presence of metastasis during the follow-up period \((P = 0.020)\), and the OGA IRS and the percentage of postchemotherapeutic tumor necrosis \((P = 0.001)\). In particular, a moderate and positive correlation was found between the IRS of OGA expression and the percentage of postchemotherapeutic tumor necrosis in 60 cases \((r = 0.308, P = 0.017; \text{Fig. 2})\), whereas the \( \text{O-GlcNAc} \) IRS or the OGT IRS was not found to be correlated with the percentage of postchemotherapeutic tumor necrosis (data not shown).

**Survival Analysis Reveals Significant Association Between the IRSs of OGA Expression and Patients’ Overall Survival**

Using the Kaplan-Meier method and the log-rank test, the IRS cutoff points for \( \text{O-GlcNAc} \), OGT, and OGA, derived from receiver operating characteristic curves, were 6, 2.5, and 5, respectively. By univariate analysis of patients’ overall survival, high degrees of OGA expression \((\text{IRS} > 5; \text{Fig. 3C})\), but not high \( \text{O-GlcNAc} \) levels \((\text{Fig. 3A})\) or OGT expressions \((\text{Fig. 3B})\), were found to be significantly associated with long overall survival in patients with osteosarcoma \((P = 0.002, \text{Table 4})\). In addition to the IRS of OGA, good chemotherapeutic response with the percentages of tumor necrosis \( \geq 90 \), the absence of metastasis, and the low Enneking stage were found to be significantly associated with long overall survival in patients with osteosarcoma \((P = 0.008, < 0.001, \text{and} < 0.001, \text{respectively}; \text{Table 4})\). By multivariate analysis, the low IRS of OGA and the presence of metastases were found to be an independent prognostic marker for short overall survival in patients with a hazard ratio of 2.07 \((95\% \text{ CI: } 1.26-3.42, P = 0.004)\) and 3.33 \((95\% \text{ CI: } 1.56-7.10, P = 0.002)\), respectively \((\text{Table 5})\).

**Survival Analysis Reveals Significant Association Between the IRSs of OGA Expression and Metastasis-free Survival in Stage IIB Osteosarcoma**

Analysis of metastasis-free survival was only performed in stage IIB cases \((n = 70)\). By univariate analysis, high degrees of OGA expression \((\text{IRS} > 5)\) were found to be significantly associated with long metastasis-free survival \((P = 0.019; \text{Fig. 4C, Table 6})\), whereas neither high \( \text{O-GlcNAc} \) levels \((\text{Fig. 4A})\) nor OGT expressions \((\text{Fig. 4B})\) were found to be associated with metastasis-free survival. Moreover, good chemotherapeutic response with
the percentages of tumor necrosis ≥ 90 was found to be a significantly associated with long metastasis-free survival ($P = 0.022$; Table 6), whereas male patients and the presence of recurrence during the follow-up period were found to be significantly associated with short metastasis-free survival ($P = 0.019**$ and $0.026$, respectively; Table 6). By multivariate analysis, low degrees of OGA expression (IRS $\leq 5$) were the only independent prognostic factor for short metastasis-free survival in the stage IIB patients with a hazard ratio of 2.85 (95% CI: 1.36-6.00, $P = 0.006$; Table 7).

**DISCUSSION**

In this study, the mean IRS of these 3 biomolecules were not found to be different between the 2 stages. However, 4 significant associations between the IRS of the 3 biomolecules and some clinicopathologic parameters were found by univariate analysis. Particularly, high expressions of OGA, but not those of O-GlcNAc or OGT, were found to be associated with the high percentage of postchemotherapeutic tumor necrosis. Consistently, the high OGA expressions were only found to be significantly associated with a longer overall survival and metastasis-free period in stage IIB. In addition, by multivariate analysis, the low OGA expression was found to be an independent prognostic factor for a shorter in both overall and metastasis-free survivals of patients with osteosarcoma.

In carcinoma, elevated levels of O-GlcNAc and high degrees of OGT expression, whilst decreased expression of OGA, are demonstrated in cancerous tissues of pancreas, whereas only the O-GlcNAc levels and the OGT expressions are reported to be increased in lung, prostate, breast, colon and gall bladder cancers. However, from our recent study of O-GlcNAc in oral cancer, the O-GlcNAc levels have not been found to be significantly enhanced upon increased histopathologic severities of oral

**TABLE 6. Metastasis-free Survival Analyses of Patients With Stage IIB Osteosarcoma (n = 70)**

| Factor                           | Patients | Events of Metastasis (n) | Median of Metastasis-free Period (mo)* | $P$  |
|----------------------------------|----------|-------------------------|----------------------------------------|------|
| Age at diagnosis (y)             |          |                         |                                         |      |
| $\leq 15$                        | 33       | 21                      | 101.8                                   | 0.058|
| $> 15$                          | 37       | 13                      | 24.8                                    |      |
| Sex                              |          |                         |                                         |      |
| Male                             | 32       | 13                      | 24.8                                    | 0.019**|
| Female                           | 38       | 21                      | 157.4                                   |      |
| Site                             |          |                         |                                         |      |
| Extremities                      | 63       | 32                      | 38.3                                    | 0.933|
| Axial                            | 7        | 2                       | Undefined†                               |      |
| Recurrence                       |          |                         |                                         |      |
| No                               | 62       | 26                      | 62.3                                    | 0.026**|
| Yes                              | 8        | 8                       | 20.9                                    |      |
| Cheomoresistance (n = 43)‡       |          |                         |                                         |      |
| Good responders                  | 9        | 2                       | 101.8                                   | 0.022**|
| Poor responders                  | 34       | 24                      | 27.1                                    |      |
| O-GlcNAc                         |          |                         |                                         |      |
| High (immunoreactive score $> 6$)| 32       | 12                      | 157.4                                   | 0.091|
| Low (immunoreactive score $\leq 6$)| 38     | 22                      | 27.8                                    |      |
| OGT                              |          |                         |                                         |      |
| High (immunoreactive score $> 2.5$)| 34    | 16                      | 56.2                                    | 0.806|
| Low (immunoreactive score $\leq 2.5$)| 36  | 18                      | 27.8                                    |      |
| OGA                              |          |                         |                                         |      |
| High (immunoreactive score $> 5$)| 37       | 13                      | 157.4                                   | 0.019**|
| Low (immunoreactive score $\leq 5$)| 33      | 21                      | 20.9                                    |      |

*Median of metastasis-free period (months) from available data of either 70 or 43 patients.
†Median survival cannot be determined if survival of patients is > 50% at the longest time point.
‡Available data of the percentage of postchemotherapeutic tumor necrosis in patients with stage IIB.
$P$ values were calculated with the log-rank test.
**$P < 0.05$.
OGA indicates O-GlcNAcase; O-GlcNAc, O-GlcNAcylation; OGT, O-linked N-acetylglucosamine transferase.

**TABLE 7. Multivariate Analysis of Metastasis-free Survival in Patients With Stage IIB Osteosarcoma (n = 70)**

| Factor                           | Metastasis-free Survival |
|----------------------------------|--------------------------|
| Age at diagnosis (y)             | 1.00                     |
| ≤ 15                             | 1.00                      |
| > 15                             | 1.60 (0.75-3.38)          |
| Sex                              | 1.00                     |
| Male                             | 0.50 (0.24-1.05)          |
| Female                           | 1.00                     |
| Site                             | 1.00                     |
| Extremities                      | 1.11 (0.25-4.89)          |
| Axial                            | 0.893                    |
| Recurrence                       | 1.00                     |
| No                               | 1.00                     |
| Yes                              | 1.85 (0.82-4.17)          |
| OGA                              | Low (immunoreactive score $\leq 5$) | 2.85 (1.36-6.00) |
| High (immunoreactive score $> 5$)| 1.00                     |

**$P < 0.01$.
CI indicates confidence interval; HR, hazard ratio; OGA, O-GlcNAcase.
squamous cell carcinoma, nor have the OGT or the OGA expressions. Consistent with these findings, the O-GlcNAc levels, the OGT or the OGA expressions were not found to be different between the 2 distinct clinical severities of osteosarcoma. Therefore, involvement of O-GlcNAc along with OGT and OGA expressions in the pathogenesis of osteosarcoma still requires further investigations.

By univariate analysis, the present study showed that high degrees of OGA expression, but not those of O-GlcNAc or OGT, were found to be correlated with increased overall survival with osteosarcoma. Moreover, high OGA expressions were found to be associated with increased metastasis-free survival in patients with stage IIB. These findings are consistent with the results of a previous retrospective study in liver cancer that showed high OGA expressions in patients with hepatocellular carcinoma, who exhibited 3-year cumulative recurrence-free survival longer than those with low OGA expressions, implying that OGA expression in the specimens might be useful as a prognostic indicator of tumor-free survival in both hepatocellular carcinoma and osteosarcoma.

By multivariate analysis, OGT overexpression was found to be associated with prostate cancer progression and recurrence, and high O-GlcNAc levels were found to be an independent prognostic factor for poor survival. Increased O-GlcNAc levels were also associated with poor survival in patients with cholangiocarcinoma. The O-GlcNAc levels and the OGT expressions were up-regulated in breast cancer cells, in which these 2 biomolecules appeared to contribute to tumor cell growth and invasion. In lung cancer, high OGT expressions were associated with poor survival of patients and with short recurrence-free survival. In contrast to these findings from various types of carcinoma, our study using multivariate analysis instead demonstrated that OGA expression in osteosarcoma specimens is an independent prognostic indicator. This discrepancy may be due to different origins of tumor cells.

Several studies have shown that the histologic response to presurgical chemotherapy, as a standard intervention for patients with osteosarcoma, is one of the most important predictors for clinical outcomes and prognosis of osteosarcoma. By comparison with poor responders, good responders have significantly higher overall and disease-free survival rates. In agreement with those studies, our data showed that a good chemotherapeutic response with the percentages of tumor necrosis ≥ 90 was found to be a significant positive predictor in both overall survival of all patients with osteosarcoma and metastasis-free survival in patients with stage IIB. In addition, high OGA expressions in osteosarcoma specimens were positively correlated with the good chemotherapeutic response with the percentages of tumor necrosis ≥ 90. Consequently, it is suggested by this study that both OGA expression, as a molecular marker, and the good chemotherapeutic response, as a clinical indicator, are useful for prognosis of patients with osteosarcoma. Consistently, inhibition of OGA expressions by treatment with Thiamet-G remarkably enhanced cancer cell migration and invasion in lung, colon, and ovarian cancers, which may likely decrease a patients’ survival rate.

In conclusion, our findings demonstrate that the high degrees of OGA expression are associated with good clinical outcomes and prognosis of patients with osteosarcoma. Low level of OGA expression may be useful as a poor prognostic marker for overall and metastasis-free survival in patients with osteosarcoma. Note that comparisons of O-GlcNAc, OGT, and OGA immunostaining in osteosarcoma specimens between preoperative and postoperative chemotherapies were not analyzed because of the heterogeneities and frequent alterations in the postoperative chemotherapeutic regimens for patients with osteosarcoma depending on clinicians’ judgement during a long follow-up period from 1999 to 2020. To better understand the molecular mechanism(s) of OGA in association with specific tumor-associated protein(s) in osteosarcoma for patients’ survival enhancement, additional in vitro investigations are required to address a possibility for OGA, functioning to reduce growth, migration and invasion of tumor cells, isolated from osteosarcoma lesions.

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