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The Global Histone Modification Patterns of Osteosarcoma

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Background: Epigenetic alteration may affect a patient's prognosis by altering the development and progression of the tumor. Some recent reports have identified a correlation between histone modification and patient outcome. However, no studies have been conducted on global histone modification in osteosarcomas. **Methods:** We investigated histone modification in 54 cases of osteosarcoma by performing immunohistochemical staining. The immunohistochemical expression of four histone modification markers, acetylated H4 lysine 12 (H4K12Ac), acetylated H3 lysine 18, trimethylated H3 lysine 27, and dimethylated H3 lysine 4 were evaluated. **Results:** High H4K12Ac expression was correlated with patient age ($p = 0.011$). However, the other histone modification markers showed no correlation with any of the clinicopathological data such as survival, tumor grade, tumor site, metastasis, age, or gender. **Conclusions:** Our study showed that all four histone modification markers are expressed in osteosarcoma (median expression rate, 40 to 60%). However, we did not find a correlation with the clinicopathological factors except for age. Further study to evaluate the reason for the association between H4K12Ac and patient age is needed.

Key Words: Histones; Osteosarcoma; Histone H3; Histone H4

Osteosarcomas are primary high-grade malignant tumors in which the neoplastic cells produce osteoid, and they are the most common non-hematopoietic primary malignant tumor of bone. Osteosarcoma can occur at any age, but 60% of patients are under the age of 25-years. Treatment for osteosarcoma requires a combined approach including surgery for the primary tumor and systemic chemotherapy.[1] The response to preoperative chemotherapy is one of the most important prognostic factors. The survival rate is less than 20% when treatment for osteosarcoma consists of only surgery.[2,3] Osteosarcoma molecular markers have recently been a focus of research, including the traditional prognostic factors such as tumor grade, metastasis and chemotherapy response. For example, matrix metalloproteinase (MMP)-2, MMP-9, urokinase plasminogen activator, chemokine receptor 4, and ezrin are molecular prognostic factors for osteosarcoma and potential therapy targets.[4-7]

A recent study suggested that epigenetic changes may contribute to the development and progression of cancers including leukemia,[8] indicating that molecular mechanisms are present that regulate gene expression without changing the DNA sequence. Alterations in DNA methylation, modification of histone tails, chromatin remodeling, and micro RNAs have been investigated as mechanisms of epigenetic change.[9-11] Epigenetic alterations may affect a patient's prognosis by involving cell growth, differentiation, and cell death of the tumor.[12] Histone modifications seem to have a role regulating transcription and other nuclear processes.[13] Some recent reports have identified a correlation between histone modification and outcomes from prostate cancer, non-small cell lung cancer, and esophageal cancer.[14-16] However, no study has been conducted on global histone modification in osteosarcoma. We performed immunohistochemistry, analyzed the expression pattern of modified histones, and investigated whether a correlation exists between modified histone expression and clinicopathological parameters.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board of Kyung Hee University. Fifty-four formalin-fixed, paraffin-embedded osteosarcoma specimens from 52 patients were used for immunohistochemical staining, and the specimens were obtained prior to chemotherapy. Samples were collected from 1983 to 2005 in the Department of Pathology at Kyung Hee University Hospital and the University of Buenos Aires Hospital. All cases were evaluated by two investigators (DSI and PYK), using hematoxylin and eosin-stained sections. The hospital records were also reviewed for each case. Immunohistochemical stains were performed on unstained slides. Briefly, 4-μm tissue sections were cut from the microarray paraffin blocks, dried, deparaffinized and washed, and then endogenous peroxidase activity was blocked with 3% hydrogen peroxidase. After being washed with Tris-buffer, the slides were blocked with protein serum (Dako, Glostrup, Denmark). The sections were then incubated with a primary antibody for acetylated (Ac) H4 lysine (Lys) 12 (H4K12Ac; 1:50, Cell Signaling Technology Inc., Danvers, MA, USA), Ac H3 Lys 18 (H3K18Ac; 1:500, Cell Signaling Technology Inc.), trimethylated (triMe) H3 Lys 27 (H3K27-triMe; 1:200, Cell Signaling Technology Inc.) and dimethylated (diMe) H3 Lys 4 (H3K4diMe; 1:500, Abcam Inc., Cambridge, UK). The primary binding antibody was visualized using the LSAB kit detection system (Dako). Nuclear staining utilized the normal histone staining pattern for each antibody. The staining results were reviewed by two investigators (DSI and PYK), and the percentage of tumor cells showing positive immunoreactivity was determined at ×200 magnification using a microscope. We calculated the percentage of nuclear-stained tumor cells and all cases were dichotomized into high expression (higher than the median) and low expression (lower than or equal to the median) for the correlation with the clinicopathological parameters.

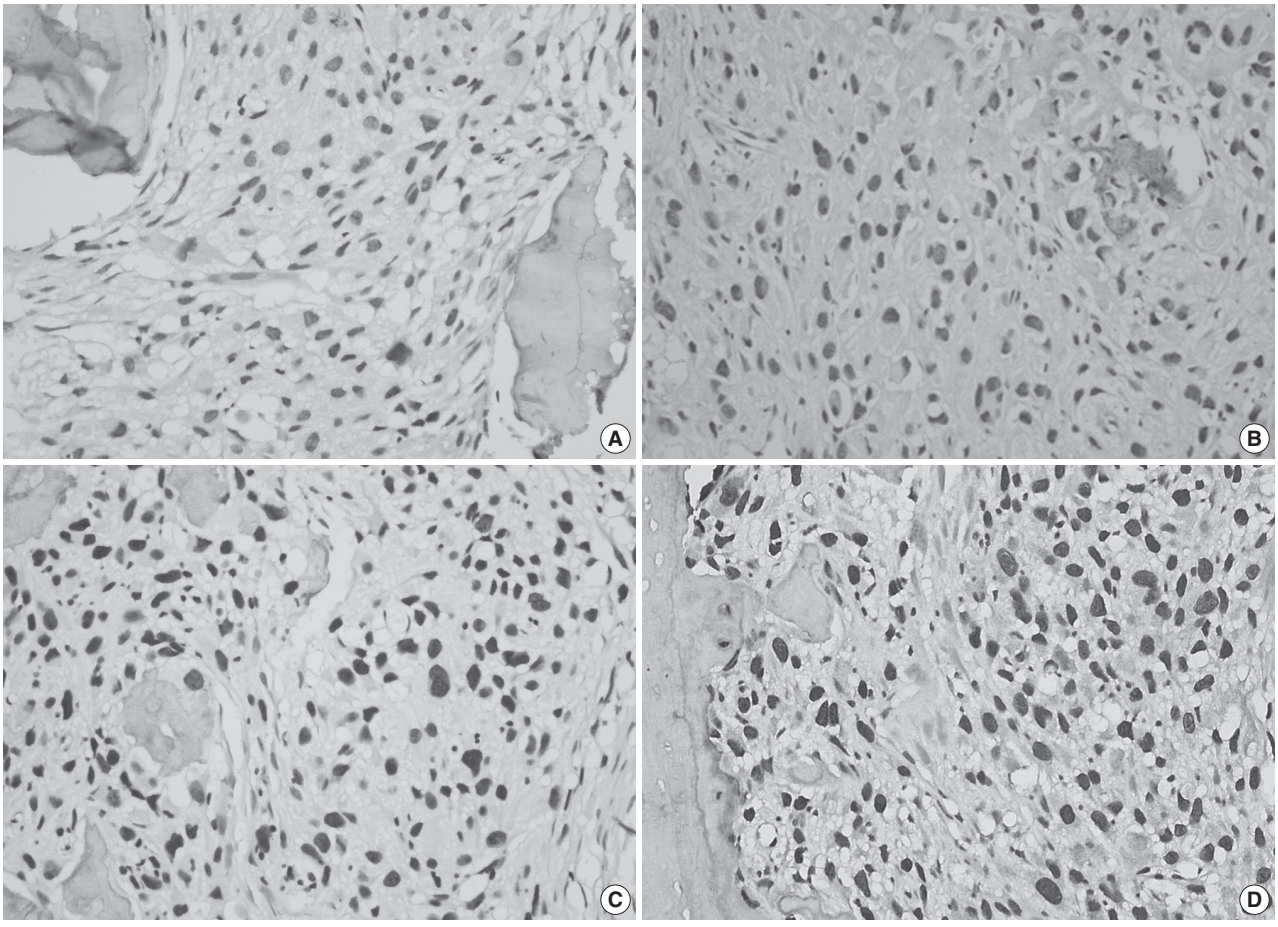

Fig. 1. Immunohistochemical staining for (A) acetylated H4 lysine 12, (B) acetylated H3 lysine 18, (C) trimethylated H3 lysine 27, and (D) dimethylated H3 lysine 4 antibodies in osteosarcoma shows strong nuclear expressions.

Statistics

Statistical analyses were conducted using the SPSS ver. 17 (SPSS Inc., Chicago, IL, USA). A $p < 0.05$ was regarded as significant.

RESULTS

We analyzed 54 cases of osteosarcoma from 52 patients that ranged in age from 6 to 66 years, with a mean age of 22 years. Of these cases, 29 were male, and 23 were female. The follow-up period ranged from 1 to 205 months, and the mean follow-up time was 64 months. Of the tumors, 52 cases were conventional osteosarcomas (46 osteoblastic type, one fibroblastic type, four chondroblastic type, and one giant-cell rich osteosarcoma), and two were the telangiectatic type of osteosarcoma. The femur was the most common site for a tumor, followed by the pelvic bone and humerus. Forty-nine of the 54 cases had high grade tumors at the time of diagnosis, and five cases were low grade.

The immunohistochemistry staining pattern showed nuclear expression and similar intensity for all four makers. The percentage of positive nuclear-stained cells for the anti-H4K12Ac, H3K18Ac, H3K27triMe, and H3K4diMe antibodies ranged from 0 to 100%. The median positive percentage for each antibody was 60%, 40%, 60%, and 60%, respectively. High expression of each antibody was seen in 25, 26, 25, and 22 of the 54 cases, respectively (Fig. 1). H4K12Ac was correlated with age ($p = 0.011$), whereas H3K27triMe and H3K4diMe showed low p-values with age ($p = 0.061$ and $p = 0.052$, respectively) (Table 1). The other clinicopathological variables, including survival, showed no correlation with expression of the H4K12Ac, H3K18Ac, H3K27triMe and H3K4diMe antibodies. A Ka-

Table 1. Correlation between histone expression and the clinicopathological parameters

Characteristics	n	%	Histone modification patterns											
			H4K12Ac			H3K18Ac			H3K27triMe			H3K4diMe		
			Low	High	p-value	Low	High	p-value	Low	High	p-value	Low	High	p-value
Age[a] (yr)														
< 40	45	83.3	21	24	0.011	24	21	0.698	23	22	0.117	26	19	0.228
≥ 40	7	16.7	7	0		3	4		6	1		6	1	
Sex														
Male	29	55.8	17	12	0.438	17	12	0.278	19	10	0.112	19	10	0.58
Female	23	44.2	11	12		10	13		10	13		13	10	
Metastasis														
Yes	14	32.6	8	6	0.927	7	7	0.916	8	6	0.903	8	6	0.594
No	29	67.4	17	12		15	14		16	13		19	10	
Stage[b]														
I	16	35.6	10	6		9	7		8	8		12	4	
II	18	40	6	12	0.406	8	10	0.789	9	9	0.406	9	9	0.508
III	4	8.9	4	0		3	1		4	0		3	1	
IV	7	15.5	5	2		4	3		4	3		4	3	
Grade[a]														
High	49	90.7	26	23	1.000	24	25	0.353	26	23	1.000	29	20	1.000
Low	5	9.3	3	2		4	1		3	2		3	2	
Type[a]														
Osteoblastic	46	85.2	23	23		22	24		24	22		26	20	
Chondroblastic	4	7.3	2	2		2	2		1	3		2	2	
Telangiectatic	2	3.7	2	0	0.445	2	0	0.404	2	0	0.307	2	0	0.552
Fibroblastic	1	1.9	1	0		1	0		1	0		1	0	
Giant cell rich	1	1.9	1	0		1	0		1	0		1	0	
Site[a]														
Femur	29	58	15	14		14	15		17	12		17	12	
Humerus	4	8	2	2		2	2		2	2		3	1	
Tibia	6	12	3	3	0.263	3	3	0.882	3	3	0.584	3	3	0.383
Pelvis	7	14	2	5		3	4		2	5		3	4	
Face	4	8	4	0		3	1		3	1		4	0	

[a]Fisher's exact test; [b]Linear by linear test.
Low, lower than and equal to; High, higher than median percentage of tumor cells showing positive for histone markers (chi-square test).

plan-Meier analysis also showed no correlation between survival and H4K12Ac, H3K18Ac, H3K27triMe, and H3K4diMe ($p = 0.70$, $p = 0.08$, $p = 0.09$, and $p = 0.40$, respectively).

DISCUSSION

Osteosarcomas are the most common primary malignant tumors of bone. Preoperative chemotherapy has improved the overall survival of patients with osteosarcoma. Yet because this type of tumor shows highly aggressive behavior, such clinicopathological factors as tumor site, stage, metastasis, recurrence, and the response to preoperative chemotherapy have been suggested as prognostic factors.[17] Furthermore, molecular factors such as vascular endothelial growth factor, pigment epithelium derived factor, MMPs, urokinase plasminogen activator, and ezrin have also been evaluated for their therapeutic potential in osteosarcoma.[17] Histone modification has recently been suggested as a new prognostic marker. Seligson *et al.*[14] reported that histone modification is correlated with prostate cancer recurrence, while Barlési *et al.*[15] reported on the correlation between histone modification and the prognosis of non-small cell lung cancer. Tzao *et al.*[16] revealed a correlation between histone modification and an esophageal cancer prognosis. In our study, histone modification markers were selected based on previous studies, and our results showed that all four markers were expressed at a median from 40-60%. However, our study only showed a correlation between H4K12Ac modification and age ($p = 0.011$). The group under age 40-years showed higher H4K12Ac expression than those over 40-years. Additionally, none of patients over age 40 expressed H4K12Ac. However, H3K27triMe and H3K4diMe were correlated with patient age. The former studies showed no correlation between histone modification and patient age in several human carcinomas.[14-16] In our study, other clinicopathological factors such as gender, metastasis, tumor stage, histological type, and site were not correlated with histone modification. We performed a Kaplan-Meier survival analysis to determine whether histone modification predicted patient outcome, but it also did not reveal a correlation. We had thought that these results were evidences that histone modification affects the development of osteosarcoma in young-age patients. However, patient age is still debatable as a prognostic factor for osteosarcoma. Bacci *et al.*[18] reported a worse prognosis for patients aged 14-years and younger, but Carsi and Rock[19] reported a poor 5-years survival for patients older than 40-years. Although previous studies did not reveal a correlation between histone modification and age in human cancer, several studies have revealed decreased histone modification in the elderly of an animal model.[20,21] They suggested that decreased histone modification is an aging process and that decreased chromatin function with age is due to such epigenetic changes. Therefore, further studies are needed to determine why histone modification is correlated in patients with an osteosarcoma. In conclusion, we demonstrated that all four histone modification markers (H4K12Ac, H3K18Ac, H3K27triMe, and H3K4diMe) were expressed in osteosarcomas (median expression rate, 40 to 60%). However, the expression was not associated with any clinicopathological factor, except age.

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