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Global Histone Modification Profiles are Well Conserved Between Normal B Lymphocytes and Neoplastic Counterparts

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Abstract The identification of cell type is essential in diagnostic tumor histopathology. We hypothesized that some patterns of global histone modification are specific to both particular cell types of non-neoplastic tissues and their neoplastic counterparts. To examine the hypothesis in lymphoid cells, global histone modification patterns of germinal center and mantle zone B cells in reactive lymphoid hyperplasia (RLH) were compared with those of follicular lymphoma (FL) and mantle cell lymphoma (MTL) cells by immunohistochemistry. We revealed that FL cells and MTL cells exhibited the similar histone modification pattern to that of germinal center B lymphocytes and mantle zone B lymphocytes in RLH, respectively. These results indicate that global histone modification profiles specific to non-neoplastic germinal center B lymphocytes and mantle zone B lymphocytes are well conserved in corresponding neoplastic lymphoma cells, and suggest that they will be indicative of tumor cell type at least in B cell lymphoma.

Keywords Diagnostic histopathology · Tumor · Cell type · Global histone modification · Immunohistochemistry · Reactive lymphoid hyperplasia · Germinal center B cell · Mantle zone B cells · Follicular lymphoma · Mantle cell lymphoma

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Abbreviations

Ab	Antibody
CK	Cytokeratin
FL	Follicular lymphoma
HE	Hematoxylin and eosin
H3	Histone 3
H4	Histone 4
H3K18Ac	Acetylated histone 3 lysine 18
H3R17diMe	Asymmetric dimethylated histone 3 arginine 17
H4K20triMe	Trimethylated histone 4 lysine 20
MTL	Mantle cell lymphoma
RLH	Reactive lymphoid hyperplasia

Introduction

The identification of tumor cell type is essential for diagnostic histopathology. It could lead us to appropriate treatments of the tumor including molecular target therapy and allow us to predict its prognosis. In the case of metastatic tumors, it may provide useful information on their primary sites. Currently, the tumor cell type identification is largely based on analysis of gene expression profile of the tumor by immunohistochemistry, cDNA microarray, and so on. For example, expression pattern of cytokeratin (CK) 7 and CK20 by immunohistochemistry in metastatic tumors might suggest candidates of the possible primary organ. Also, immunohistochemistry of tissue-specific proteins, such as prostate-specific antigen (PSA) and thyroid transcription factor (TTF)-1 could identify primary sites of tumors of unknown origin. However, combination of CK7 and CK20 immunohistochemistry unfortunately gives only four expression patterns, and is

of limited use to determine the definite primary organ. Although immunohistochemistry of tissue-specific proteins is in fact very useful for identification of tumor origin, the diagnostic applications have been so far limited to several cell types including the prostate, thyroid, and lung. Gene expression profiling using cDNA microarray has provided means for tumor subtyping, but necessity of handling RNA, challenging data analysis, and high cost hamper its routine clinical use. Thus, tumor cell subtyping based on gene expression profiling needs to be compensated by alternative and more versatile methods.

The posttranslational modifications of histones, such as phosphorylation, acetylation, and methylation, are epigenetic mechanisms regulating chromatin structure and gene expression. Global histone modifications are defined as modifications of histones that occur globally over large regions of chromatin including coding and non-coding sequences [1]. Presumably, different patterns of global histone modification would result in different distribution of eu- and hetero-chromatin in the nucleus, or chromatin pattern, which is routinely observed in tissue samples stained with hematoxylin and eosin (HE).

To clarify the relationship between cell type and global histone modification pattern, we compared global histone modification patterns of reactive lymphoid hyperplasia (RLH), follicular lymphoma (FL), and mantle cell lymphoma (MTL) through immunohistochemistry. Among various posttranslational modifications of histones, we focused on acetylation and methylation of lysine and arginine residues of histones H3 and H4. Here we showed that particular profiles of global histone modifications are associated with particular cell types of non-neoplastic B cells and the neoplastic counterparts, by immunohistochemistry with specific antibodies (Abs) against individual histone modifications. The results suggest that global histone modification pattern is indicative of subtypes of malignant lymphoma.

Materials and Methods

Case Selection

Seven cases of RLH (chronic tonsillitis), 8 cases of FL, and 7 cases of MTL were retrieved from the files of the Department of Surgical Pathology, Hyogo College of Medicine Hospital, except 4 cases of MTL retrieved from the files of Kobe City Medical Center General Hospital. Lymphomas were classified according to the current WHO classification criteria [2] with the aid of immunohistochemistry. Briefly, RLH consisted of multiple hyperplastic lymphoid follicles with germinal centers where many tingible body macrophages are present. FL had follicle-like structures composed of centrocyte-like tumor cells expressing Bcl-2 protein. MTL consisted of diffuse neoplastic expansion of B lymphoid cells that express CD5 and Cyclin D1 proteins, which resulted in obscurity of normal follicular structures. Seven RLH cases included 3 males and 4 females with age from 41 to 56, the median of which was 43. Eight FL cases included 4 males and 4 females with age from 58 to 74, the median of which was 64. All of seven MTL cases were males with age from 45 to 74, the median of which was 66. Among 8 FL cases, 3 cases were histologically classified as Grade 1, while the remaining 5 cases as Grade 2.

Immunohistochemical Analyses and Counting of the Positive Cells

Immunohistochemistry was performed on sections (3 micrometer thick) of formalin-fixed and paraffin-embedded tissues. Standard two-step indirect method using Envision System (DAKO) was employed as described [3]. For antigen retrieval, the sections were placed in 0.01 M sodium citrate buffer pH 6.0 at 95°C for 40 min. Primary rabbit polyclonal Abs specific for the following particular posttranslational modifications of histones H3 or H4 were applied for 30 min at room temperature at the following dilutions: Ab for acetylated (Ac) H3 lysine 18 (K18) (H3K18Ac; Abcam Inc., Cambridge, MA, USA) at 1:200, Ab for asymmetric dimethylated (diMe) H3 arginine 17 (R17) (H3R17diMe; Abcam Inc.) at 1:400, and Ab for trimethylated (triMe) H4 lysine 20 (K20) (H4K20triMe; Abcam Inc.) at 1:200. Immunoreactive cells for each antibody were quantified using the image analysis software, WinROOF (MITANI CORPORATION, Fukui, Japan). Values of mean percentage of positive cells ± s.e.m. were calculated for each antibody for each cell type.

Results

In order to examine the relationships between non-neoplastic lymphoid cell type and global histone modification pattern, immunohistochemistry was performed using RLH cases with a variety of specific Abs against acetylation or methylation of histones H3 and H4 such as H3K18Ac, H3R17diMe and H4K20triMe. We identified three representative patterns of global histone modification in histological structure of RLH cases (Fig. 1a). In the first pattern (Fig. 1b), both germinal center B cells and lymphocytes outside the germinal center, including mantle zone B cells, were evenly positive. This pattern was observed in H3K18Ac immunohistochemistry. In the second pattern (Fig. 1c), germinal center B cells were stained stronger than lymphocytes outside the germinal center including mantle zone B cells. This pattern was obtained by H3R17diMe. Finally in the third pattern

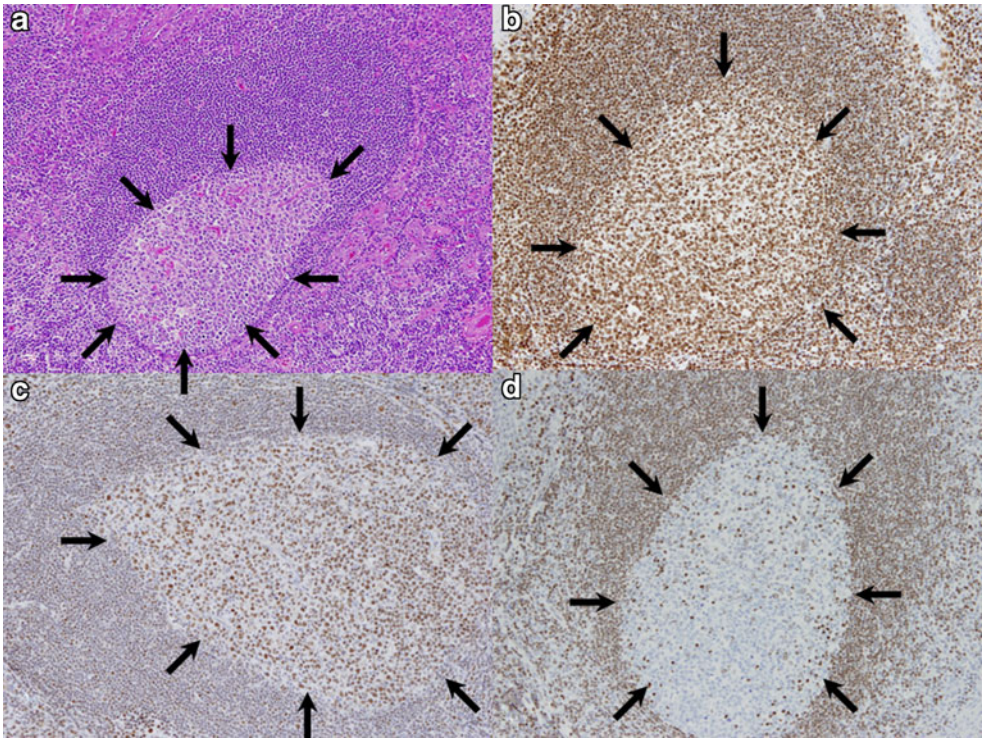

Fig. 1 The representative histone modification profile of reactive lymphoid hyperplasia (RLH). Germinal center is indicated by arrows. An area outside the germinal center is mantle zone. **a** HE image (original magnification: ×100). **b** Immunostaining for H3K18Ac (original magnification: ×100). **c** Immunostaining for H3R17diMe (original magnification: ×100). **d** Immunostaining for H4K20triMe (original magnification: ×100). Brown nuclei are shown to be positive in immunostaining. Both germinal center B cells and mantle zone B cells were evenly positive for H3K18Ac (**b**). Germinal center B cells were stained stronger than mantle zone B cells for H3R17diMe (**c**). On the other hand, germinal center B cells were not stained but mantle zone B cells were stained in H4K20triMe (**d**)

(Fig. 1d), germinal center B cells were not stained but lymphocytes outside the germinal center including mantle zone B cells were stained. This was a pattern by H4K20triMe immunohistochemistry. These results indicated that global histone modification patterns of germinal center B cells are different from those of mantle zone B cells in the non-neoplastic lymphoid tissue.

Next, we examined global histone modification patterns in malignant lymphoma cases of FL (Fig. 2a–d) and MTL (Fig. 3a–d) by immunohistochemistry, and compared the

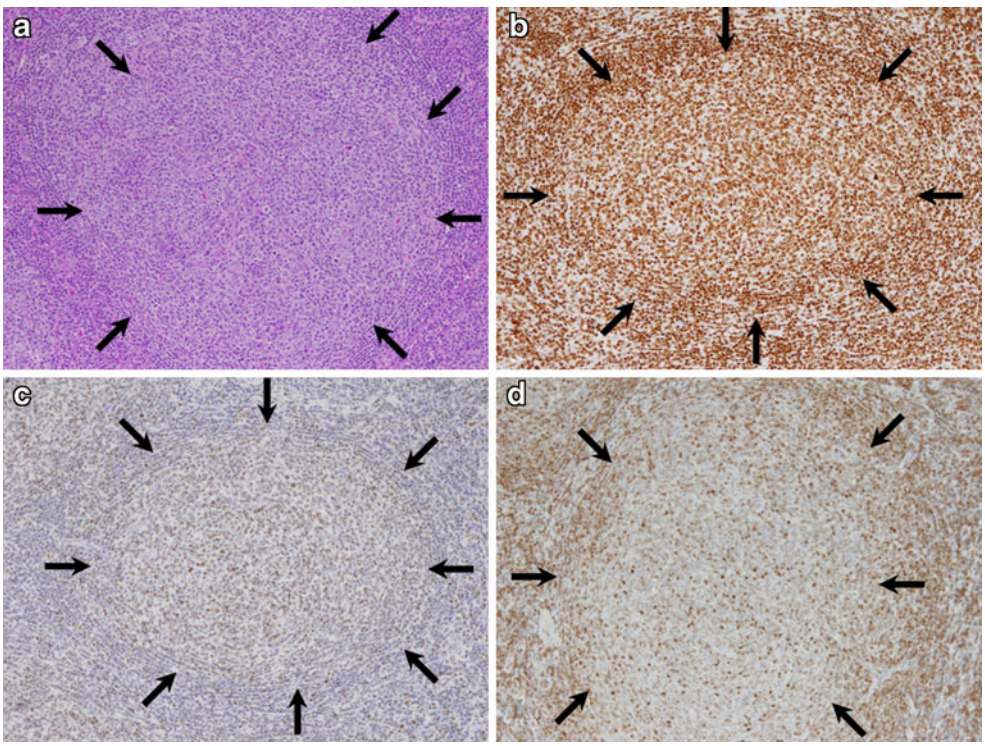

Fig. 2 The representative histone modification profile of follicular lymphoma (FL). Germinal center-like tumor nodule is indicated by arrows. **a** HE image (original magnification: ×100). **b** Immunostaining for H3K18Ac (original magnification: ×100). **c** Immunostaining for H3R17diMe (original magnification: ×100). **d** Immunostaining for H4K20triMe (original magnification: ×100). Brown nuclei are shown to be positive in immunostaining. FL cells were immunohistochemically positive for H3K18Ac (**b**) and H3R17diMe (**c**), but negatively for H4K20triMe (**d**). FL cells were stained stronger than non-neoplastic lymphocytes surrounding the nodules by H3R17diMe (**c**)

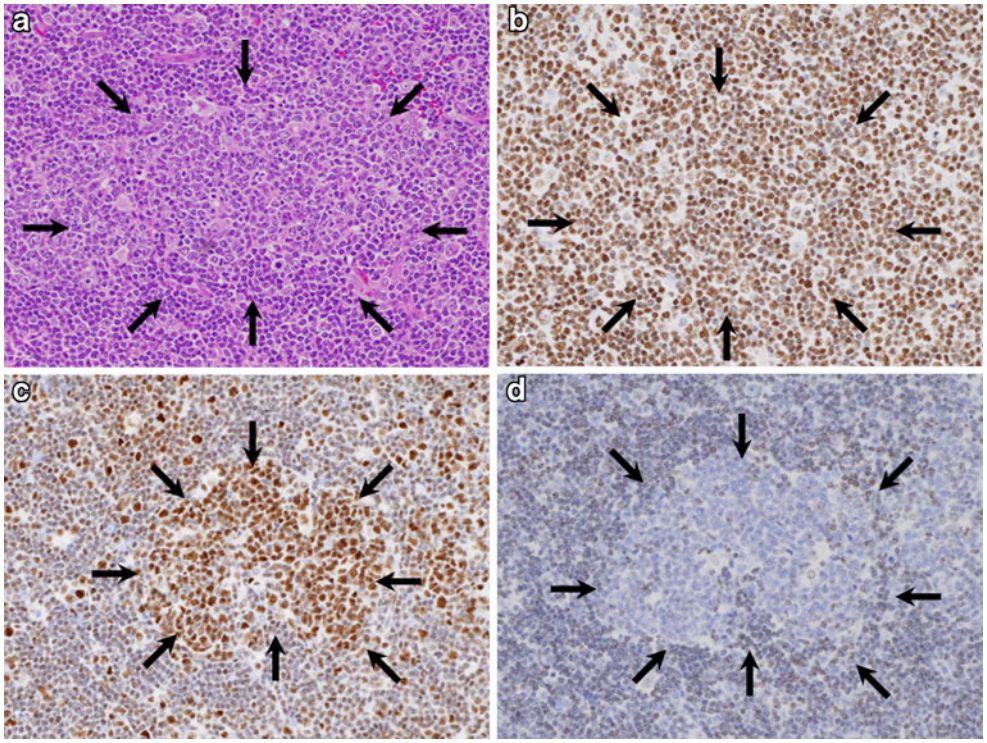

Fig. 3 The representative histone modification profile of mantle cell lymphoma (MTL). Residual germinal centers are indicated by arrows. **a** HE image (original magnification: ×100). **b** Immunostaining for H3K18Ac (original magnification: ×100). **c** Immunostaining for H3R17diMe (original magnification: ×100). **d** Immunostaining for H4K20triMe (original magnification: ×100). Brown nuclei are shown to be positive. MTL cells surrounded a residual germinal center, and were immunohistochemically stained by H3K18Ac (**b**), H3R17diMe (**c**) and H4K20triMe Abs (**d**). They were positive for H3K18Ac in similar magnitude as the germinal center B cells (**b**). Two subpopulations of MTL cells were observed (**c**). MTL cells with weak H3R17diMe signals directly surrounded germinal centers. Outside of these were MTL cells with equivocal H3R17diMe signals to germinal center B cells (**c**). MTL cells were stained weakly but apparently positive for H4K20triMe, while the residual germinal center B cells were not (**d**)

patterns with those observed in RLH (Fig. 1a–d). FL cells were immunohistochemically positive for H3K18Ac (Fig. 2b) and H3R17diMe (Fig. 2c), but negative for H4K20triMe (Fig. 2d). FL cells were stained stronger than non-neoplastic lymphocytes surrounding the tumor nodules by H3R17diMe immunohistochemictry (Fig. 2c). These staining patterns of FL cells were quite similar to those of germinal center B cells in RLH (Fig. 1a–d).

On the other hand, MTL cells (Fig. 3a) were immunohistochemically stained by H3K18Ac (Fig. 3b), H3R17diMe (Fig. 3c), and H4K20triMe Abs (Fig. 3d). Proliferating MTL cells surrounded a residual germinal center, and were stained with H3K18Ac in similar magnitude as the germinal center B cells (Fig. 3b). In Fig. 3c, two subpopulations of MTL cells were observed, namely, those with weaker or equivocal H3R17diMe staining, when compared with germinal center B cells. MTL cells with weak H3R17diMe staining directly surrounded germinal centers (Fig. 3c). Outside of these were MTL cells with equivocal H3R17diMe staining to germinal center B cells (Fig. 3c). The two subpopulations of MTL cells were observed in 3 out of 7 MTL cases. MTL cells were weakly but apparently positive for H4K20triMe, while the residual germinal center B cells were not (Fig. 3d). These staining patterns of MTL cells were reminiscent of mantle zone B lymphocytes in RLH (Fig. 1a–d).

Quantitative data of the positive cells for each antibody for each cell type were summarized in Table 1.

Table 1 Percentage of positive cells of immunohistochemistry for H3K18Ac, H3R17diMe, and H4K20triMe in the cases of reactive lymphoid hyperplasia, follicular lymphoma, and mantle cell lymphoma

	H3K18Ac	H3R17diMe	H4K20triMe
GC of reactive lymphoid hyperplasia	87.8±2.7	71.1±6.2	14.0±3.1
Mantle zone of reactive lymphoid hyperplasia	83.1±3.6	69.4±6.1	69.8±7.0
Follicular lymphoma cells	81.5±4.2	73.6±5.8	23.5±4.7
Mantle cell lymphoma cells	91.3±2.4	75.0±6.1	66.9±6.3

The values show mean percentage ± s.e.m.

GC germinal centers

Discussion

In this paper, we demonstrated global histone modification profiles specific either to germinal center B lymphocytes or to mantle zone B lymphocytes using immunohistochemical methods. FL cells and MTL cells exhibited specific patterns of global histone modification corresponding to their normal counterparts, namely, germinal center B cells and mantle zone B cells, respectively. These results support our hypothesis that some patterns of global histone modifications are specific to particular cell types of non-neoplastic tissues and their neoplastic counterparts. The results also imply that immunohistochemical profiling of global histone modification is indicative of tumor cell types in diagnostic histopathology. It was reported that global histone modification patterns are predictive of clinical outcome and prognosis in cancers of prostate, lung, and esophagus [3–5]. In contrast, to our knowledge, this is the first report on possible application of global histone modification patterns for cell type identification.

It is well known that tumorigenesis is attributed to various genetic aberrations including mutations, change of gene copy number and chromosomal derangements. In addition to these genetic differences between neoplastic and non-neoplastic cells, contributions of epigenetic differences between them have been recently recognized [1]. The epigenetic differences between neoplastic and non-neoplastic cells include differences in histone modification patterns. For example, an aberrant pattern of histone methylation in some types of acute myelogenous leukemia is conferred by chromosomal translocation involving MLL (mixed lineage leukemia) histone methyltransferase gene and various fusion partner genes [6]. In the case of lymphoma, Pal et al. reported that expression of protein arginine methyltransferase (PRMT) 5, which methylates the arginine 8 of histone H3 and the arginine 3 of histone H4, is increased in MTL [7]. In contrast to differences in global histone modification between neoplastic and non-neoplastic counterparts, we explored conserved patterns of global histone modification between neoplastic and non-neoplastic cells in the present study.

As described above, it was reported that global histone modification patterns are predictive of clinical outcome and prognosis in solid cancers of prostate, lung, and esophagus [3–5, 8]. In the case of prostate cancer, global histone modification patterns are predictive of clinical outcome in the low grade cancer, but not in the high grade cancer [8]. In the cases of lung and esophagus cancers, global histone modification patterns are predictive of clinical outcome especially in the early stage cancer [4, 5]. These results are consistent with the hypothesis that epigenetic mechanisms including global histone modification play important roles in the initiation, not in the promotion, stage of tumorigenesis, before genomic changes occur [1]. Presumably, when tumorigenesis proceeds and cancer phenotypes are stabilized, global histone modification patterns of neoplastic cells may be similar to, and not significantly different from, those of non-neoplastic counterparts. The conserved pattern of global histone modification between neoplastic cells and non-neoplastic counterparts can be therefore observed when the neoplasms are in the promotion stage, as in the cases of lymphoma in this paper.

In conclusion, we propose that analysis of global histone modification patterns could become a compensatory diagnostic tool for identifying tumor cell types in tumor histopathology. There are various Abs available that are specific to individual histone modifications. Combinatorial diversity of global histone modifications will compensate the limited availability of the current methods of cell type identification. The number of cases examined in this study is so limited that we should confirm the results in larger number of cases. Moreover, we have to examine whether global histone modification patterns could generally provide cell type identification of tumors by examining many types of tumors other than lymphoma. Comprehensive catalogue of global histone modification patterns of various cell types might provide an excellent database not only for the diagnostic identification of primary sites of metastatic tumors, but also for the contribution to cell-type specific therapy.

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References

1. Jones PA, Baylin SB (2007) The epigenetics of cancer. Cell 128:683–692
2. Swerdlow SH, Campo E, Harris NL et al (eds) (2008) WHO classification of tumours of haematopoietic and lymphoid tissues, 4th edn. WHO, Geneva
3. Seligson DB, Horvath S, Shi T et al (2005) Global histone modification patterns predict risk of prostate cancer recurrence. Nature 435:1262–1266
4. Barlesi F, Giaccone G, Gallegos-Ruiz MI et al (2007) Global histone modifications predict prognosis of resected non-small-cell lung cancer. J Clin Oncol 25:4358–4364
5. Tzao C, Tung HJ, Jin JS et al (2009) Prognostic significance of global histone modifications in resected squamous cell carcinoma of the esophagus. Mod Pathol 22:252–260
6. Krivtsov AV, Armstrong SA (2007) MLL translocations, histone modifications and leukemia stem-cell development. Nat Rev Cancer 7:823–833
7. Pal S, Balocchi RA, Byrd JC et al (2007) Low levels of miR-92b/96 induce PRMT5 translation and H3R8/H4R3 methylation in mantle cell lymphoma. EMBO J 26:3558–3569
8. Seligson DB, Horvath S, McBrian MA et al (2009) Global levels of histone modifications predict prognosis in different cancers. Am J Pathol 174:1619–1628