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Original contribution

Preferential up-regulation of osteopontin in primary central nervous system lymphoma does not correlate with putative receptor CD44v6 or CD44H expression

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Summary Osteopontin (*SPP1*) is reportedly the most up-regulated gene in primary central nervous system lymphoma (PCNSL). Our objective was to confirm immunoexpression of osteopontin and determine if CD44v6 and CD44H played a significant role as receptors for osteopontin in PCNSL. Twenty PCNSL, 12 nodal diffuse large B-cell lymphoma (N-DLBCL), and 17 extra-nodal DLBCL (EN-DLBCL) archival pathology cases were examined. Osteopontin nuclear positivity was observed in 20 (100%) of 20 PCNSL cases, 16 (95 %) of 17 EN-DLBCL, and 3 of 12 (25%) N-DLBCL. The immunohistochemical score of osteopontin in PCNSL (7.0 ± 3.5) and EN-DLBCL (4.4 ± 4.1) was significantly higher than N-DLBCL (0.3 ± 0.6). Sixteen cases were positive for CD44v6 (33%), including 6 PCNSL, and 5 each EN-DLBCL and N-DLBCL; no statistical difference was observed. CD44H was positive in all cases except one PCNSL but without any significant differences across the 3 groups. CD44H expression was significantly higher in non–germinal center B-cell (GCB) (score 12 ± 1.5) as compared to the GCB group (9.5 ± 3.1), and in non-GCB PCNSL (7.9 ± 4.2) as compared to non-GCB non-CNS lymphoma (2.8 ± 4.0) ($P = .009$); the differences were insignificant for osteopontin and CD44v6. Neither CD44H nor CD44v6 scores correlated with the osteopontin expression score or Ki-67 index. Osteopontin immunoexpression was highest in PCNSL, suggesting its probable role in its pathogenesis. However, its lack of correlation with CD44v6 excludes the latter as the likely osteopontin receptor in PCNSL. The significantly higher CD44H expression in the non-GCB than GCB group may contribute to the aggressiveness of the non-GCB DLBCL. Further studies are needed to elucidate the pathway and the prognostic/predictive role of osteopontin in PCNSL.

1. Introduction

Diffuse large B-cell lymphoma (DLBCL), a biologically heterogeneous lymphoma, can be classified into 2 categories with probable prognostic relevance: germinal center B-cell (GCB)-like and non-GCB type according to the gene expression profiles [1]. Primary central nervous system lymphoma (PCNSL) is an aggressive DLBCL mostly of non-GCB type [2,3], with poor prognosis, yet confined to the central nervous system (CNS) microenvironment. It is unclear whether a malignant clone occurs de novo in the CNS or a clone of systemic lymphoma is recruited to it [4].

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Osteopontin, a cell-matrix glycoprotein, is involved in almost all steps of tumor progression, including invasion, metastasis, and angiogenesis through binding to multiple integrins and some forms of CD44, especially variant 6 [5,6]. CD44 exists in several isoforms with different extracellular regions. The standard form of CD44 (CD44H or CD44s) is a ubiquitously expressed isoform, whereas CD44 variants (CD44v) are only detected on activated leukocytes, leukemia, lymphoma, and carcinoma cells, where they regulate migration, proliferation, and apoptosis [7,8]. OPN binds to naturally expressed and stably transfected variant CD44 isoforms in a specific, dose-dependent, and anti-CD44 antibody inhibitable manner and, by such an interaction, can mediate chemotaxis in soluble form and attachment in immobilized form [6]. High expression levels of osteopontin and CD44v are associated with progression, metastatic spread and poor prognosis in a variety of malignancies, including non-Hodgkin lymphoma. [9-14].

In recent cDNA microarray studies, osteopontin was the most up-regulated gene in PCNSL compared to nodal and extra-nodal DLBCL (N-DLBCL, EN-DLBCL) [15,16]. CD44v expression seems to be associated with an unfavorable prognosis in DLBCL, but the reported data are not wholly consistent [11,17-23]. The mediator(s) of osteopontin up-regulation in PCNSL is unclear, and CD44v has not been studied in PCNSL as compared with N-DLBCL or EN-DLBCL. Given that CD44v6 is one of the major receptors for osteopontin and it is expressed predominantly in non-GCB type DLBCL [19], we studied the protein expression of osteopontin and CD44v6 and investigated their association in PCNSL. This is the first study to examine osteopontin, CD44H, and CD44v6 expression in PCNSL in comparison with EN-DLBCL and N-DLBCL.

2. Material and methods

2.1. Case selection

A retrospective search for patients with PCNSL, EN-DLBCL, and N-DLBCL was undertaken using the Cerner powerchart database at our institutions (Georgia Health Science University and University Hospital). A total of 20 cases of PCNSL, 12 cases of N-DLBCL, and 17 cases of EN-DLBCL were collected for this study. The EN-DLBCL cases were from sinonasal cavity (4), supraglottis (1), ear (1), orbit (1), temporal scalp (1), tongue (1), stomach (1), colon (2), thigh (1), testicle (1), iliacus muscle (2), and spine (1). All patients were immunocompetent and HIV negative. The hematoxylin and eosin slides were reviewed independently by 2 pathologists, and the diagnosis was confirmed according to the 2008 World Health Organization classification [24]. The study was approved by our institutional review boards.

2.2. Immunohistochemistry

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue using the avidin-biotin-peroxidase technique. Sections were deparaffinized and then underwent antigen retrieval by using Target Retrieval Solution, pH 6.0 (Dako, Carpinteria, CA) in a rice steamer (Black and Decker rice steamer). Endogenous peroxidase was quenched with 0.3% H_2O_2. Sections were then incubated with primary antibody for 30 minutes at room temperature followed by a labeled polymer conjugated to goat anti-mouse immunoglobulins (Envision$^+$ HRP kit, Dako) for 30 minutes. Bound antibody was detected with DAB$^+$ substrate kit (Dako). Slides were then counterstained with hematoxylin. The following primary mouse monoclonal antibodies were used: osteopontin, Ki-67, CD44H, and CD44v6 (all purchased from R&D Systems, Minneapolis, MN; Dilution: 1:100).

Expression of osteopontin, CD44H, and CD44v6 in malignant cells was evaluated independently by 2 pathologists. Staining for osteopontin was semi-quantitatively stratified and scored both by percentage positivity of tumor cells (0%, 1%-25% = score 1, 26%-50% = 2, 51%-75% = 3, and 76%-100% = 4) and staining intensity (none = 0, weak = 1; moderate = 2; intense = 3), and an overall score (0-12) calculated for each case by multiplying percentage score with intensity score. Ki-67 is reported by percent positivity of tumor cells. The cases with discrepancies were resolved by consensus. The immunoprofiles of CD10, BCl-6, and MUM-1 are available in archives for all N-DLBCL, EN-DLBCL, and 13 PCNSL cases.

2.3. Statistical analysis

Because the immunostaining scores of CD44v6, CD44H, osteopontin, and Ki-67 were not normally distributed in each group, the nonparametric Kruskal-Wallis test was employed in order to evaluate differences among extra-nodal, PCNSL, and nodal DLBCL groups and differences between GCB and non-GCB groups. The Spearman correlation was used to determine the magnitude and direction of the association between osteopontin with CD44v6, CD44H, and Ki-67, respectively. Results were considered statistically significant if the $P \leq .05$. All tests were performed using the JMP5.0 Statistics software package (SAS Institute Inc, Cary, NC).

3. Results

The average immunostaining scoring of osteopontin, CD44H, and CD44v6 expression of 3 groups is listed in Fig. 1A. Osteopontin nuclear immune-expression was observed in 20 of 20 (100%) PCNSL cases (Fig. 2A and B), 16 (95 %) of 17 EN-DLBCL (Fig. 2C), and 3 (25%) of 12 N-DLBCL cases (Fig. 2D). Staining intensity was moderate