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Short communication

Expression pattern of adaptor protein PAG: Correlation between secondary lymphatic follicle and histogenetically related malignant lymphomas

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Abstract

Transmembrane adaptor protein PAG, also known as Csk-binding protein (Cbp), which binds and activates the cytoplasmic tyrosine kinase Csk, the major negative regulator of Src-family kinases, was found to be expressed in germinal centers of lymphoid follicles as well as in follicular, but not mantle cell lymphomas. Expression of PAG may reflect its role in regulation of proliferation and differentiation of germinal center B-cells. From the routine histopathology point of view, PAG might be a new positive marker of follicular lymphoma and a negative marker of mantle cell lymphoma.

Keywords: Germinal center; Lymphoma; PAG; Histopathology

1. Introduction

Phosphoprotein associated with glycosphingolipid enriched microdomains (PAG), also known as Csk-binding protein (Cbp), is a broadly expressed transmembrane adaptor protein present in membrane microdomains called membrane rafts or glycosphingolipid enriched microdomains [1,2]. Tyrosine phosphorylated PAG binds a tyrosine kinase Csk, the major negative regulator of Src-family kinases [1–7]. This brings the cytoplasmic Csk to the proximity of its membrane-localized substrates; furthermore, complexing with PAG increases the intrinsic activity of Csk [6]. In resting peripheral blood T lymphocytes, relatively high level of the phospho-PAG/Csk complex appears to suppress activity of Src-kinases Lck and Fyn, and thereby helps to set an activation threshold in these cells. Following ligation of T-cell receptor (TCR), PAG becomes dephosphorylated which leads to release of Csk and activation of the Src-kinases; this may play a role in the onset of T-cell-based immune responses [1,3,7,8]. Suppression of PAG expression may participate in pathologic B-cell lymphoproliferation, as recently demonstrated for theileriosis [9]. Therefore, it may be expected that PAG expression (and phosphorylation) may participate in regulation of lymphoid cell proliferation and differentiation, and that is why we wished to determine the PAG expression in various compartments of secondary lymphatic follicles and in lymphomas derived from them. Our present data demonstrate that the protein is strongly expressed in germinal centers of lymphoid follicles and also in follicular, but not mantle cell lymphomas.

2. Materials and methods

Thirty malignant lymphomas diagnosed in the Department of Pathology, Faculty Hospital Královské Vinohrady, Prague, since May 2001 to March 2004 were tested for the

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PAG expression. The diagnoses were established according to the WHO classification [10] after consensus of at least two pathologists on the basis of histological features and immunohistochemical profiles. Diagnoses of five mantle cell lymphomas were further confirmed by PCR and/or FISH detection of *t*(11; 14) translocation and by flow-cytometric immunophenotyping in two other cases. In addition, one superficial and four deep lymph nodes displaying follicular pattern of hyperplasia and an appendix with hyperplastic lymphoid tissue were included in the study.

Tissue samples were formalin fixed and routinely processed. The immunohistochemical reaction included an antigen retrieval step heating in citrate buffer pH 6.0 for 20 min. Mouse IgG2a monoclonal antibody MEM-255 (Exbio Praha, Czech Republic) was prepared from a mouse immunized with recombinant human PAG as described elsewhere [1]; it reacts optimally with denatured PAG (under the conditions of Western blotting) whether tyrosine phosphorylated or not. MEM-255 was used here for the tissue section immunostaining as a primary antibody. The reaction was visualized by the Universal LSAB+ kit (DAKO, Carpinteria, CA) according to the manufacturer's recommendation.

The samples were considered positive if the brown reaction product clearly highlighted cytoplasmic membranes in a distinct linear pattern in more than 10% of the neoplastic cells. Each reaction was compared to the negative control treated in the same procedure, but the primary antibody was omitted.

3. Results

In hyperplastic lymphatic tissue, the germinal centres of secondary lymphatic follicles were strongly positive with the PAG-specific mAb MEM-255, clearly contrasting with essentially negative small lymphocytes in the follicle mantle that contained only scattered clearly positive cells whose distribution correlated with $CD3^+$ T-cells (Fig. 1A and B and data not shown). The paracortical T-cell area displayed a weak positive reaction. Malignant lymphomas followed the same pattern of expression: follicular lymphomas (four cases grade 1, four cases grade 2, one case grade 3) were strongly positive (9/9) highlighting neoplastic "germinal centers" (Fig. 1D), whereas mantle cell lymphomas (7/7) (Fig. 1C), small lymphocytic lymphomas (3/3), and extranodal marginal zone lymphomas (3/3), plasmocellular neoplasms—plasma cell myeloma and plasmocytoma (2/2) were completely negative (not shown). Diffuse large B-cell lymphomas (DLBCL) were heterogenous—two cases were positive and three negative. Classic Hodgkin lymphomas (7/7) were PAG-negative (not shown). The results are summarized in the Table 1.

4. Discussion

The strong expression of PAG in proliferating cells of germinal centers and in follicular lymphomas derived from these cells is in striking contrast to the expectation based on the assumption that PAG participates at negative regulation of Src-kinases (thus, proliferating cells should express little PAG in order to keep the Src-kinases active). Similarly, surprising is the observed lack of PAG expression (or at least substantially lower expression not detectable under the conditions used) by mantle cells that in fact represent a virgin cell population being in a resting not activated state. It should be noted that PAG is relatively strongly expressed and constitutively phosphorylated in resting peripheral blood T-cells [1,3,5,7]. The present observation that PAG is much more strongly expressed in proliferating centrocytes and centroblasts than in resting B-cells could be explained by the hypothesis that Src-family kinases may play somewhat different roles in B-cells than in T-cells. Supposedly, following activation of resting peripheral T-cells, Src-kinases Lck/Fyn phosphorylate immunoreceptor tyrosin-based activation motifs (ITAMs) of signalling subunits of the T-cell receptor, and thus propagate the signal leading, finally to activation of nuclear transcription factors AP1, NFAT and NF κB. In B-cells, however, the phosphorylated Src-kinases may preferentially phosphorylate immunoreceptor tyrosin-based inhibitory motifs (ITIMs) of negative signalling regulators such as CD22 or PIR-B [11]. The latter possibility concurs well with the observation that cross-linking of the B-cell receptor results in phosphorylation of PAG, followed by recruitment of Csk to the membrane rafts [12] resulting in suppression of the Src-kinases that in its effect may temporarily block the negative regulatory signals based on ITIM phosphorylation. Moreover, molecular profiling of a various DLBCL revealed three subgroups of these lymphomas—germinal center B-cell-like, activated B-cell-like, and type 3 DLBCL; four groups of genes predictive of

Table 1
Expression of PAG in various types of lymphoma as determined in the present study

Diagnosis (WHO classification)	Location	Number of cases	PAG
Small lymphocytic lymphoma/chronic lymphocytic leukemia	Lymph node	3	Negative
Mantle cell lymphoma	Lymph node	7	Negative
Extranodal marginal zone lymphoma	Stomach	1	Negative
	Nasopharynx	1	Negative
Splenic marginal zone lymphoma	Spleen	1	Negative
Follicular lymphoma (grade 1–3)	Lymph node	9	Positive
Diffuse large B-cell lymphoma	Lymph node	5	Two positive, three negative

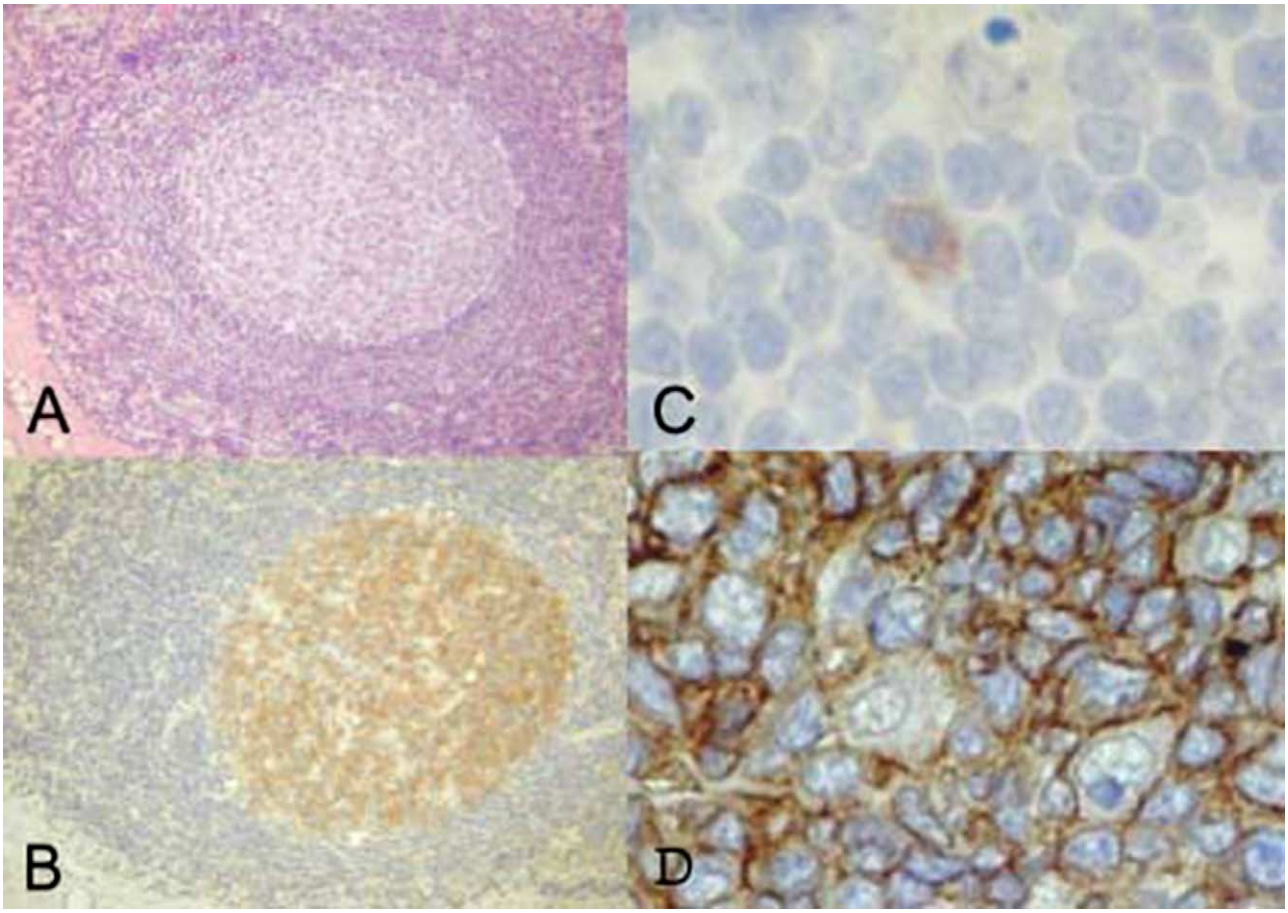

Fig. 1. PAG is relatively specifically expressed in germinal centers of lymph node lymphoid follicle and in follicular lymphoma. Quandrants (A and B): a secondary lymphatic follicle containing a germinal center surrounded by a mantle zone, note strong positivity in the germinal center and negativity in the mantle zone. Quadrant (C): mantle cell lymphoma, note negativity of the lymphoma cells, the single positive cell in the centre is probably a T lymphocyte. Quadrant (D): follicular lymphoma grade 2, note strong positivity of the lymphoma cells. (B–D) Immunostaining with MEM-255 and (A) hematoxylin-eosin staining. Magnification: (A and B) 200× and (C and D) 600×.

chemotherapy were identified [13]. We suspect that the heterogeneous expression of PAG among DLBCL cases might reflect different oncogenic pathways. Further analysis is needed to determine possible correlation of PAG expression with these subtypes and/or chemotherapy response. Recent results on signaling perturbations in lipid rafts of anaplastic large cell lymphoma and Hodgkin lymphoma cell lines involving also PAG [14] lend further support to the idea that PAG may be involved in neoplastic lymphoproliferation. Mechanisms participating in regulation of PAG expression in various subsets and differentiation stages of B lymphocytes remain to be elucidated. Finally, it should be acknowledged that the reactivity of the mAb under the particular conditions used in this study may not perfectly reflect the expression of the PAG molecule. It is imaginable that a critical post-translational modification of a subset of PAG molecules (perhaps differentially occurring in different cell types) may affect the recognition by MEM-255. Nevertheless, based on Western blotting data, MEM-255 reactivity is apparently not compromised by phosphorylation, the major physiological modification of PAG.

From the routine histopathology point of view, PAG (or at least the MEM-255 epitope of PAG) might be a new positive marker of follicular lymphoma and a negative marker of some “small cell lymphomas”.

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References

[1] Brdicka T, Pavlistova D, Albrecht L, Bruyns E, Korinek V, Angelisova P, Scherer J, Shevchenko A, Hilgert I, Cerny J, Drbal K, Kuramitsu Y, Kornacker B, Horejsi V, Schraven B. Phosphoprotein

associated with glycosphingolipid - enriched microdomains (PAG), a novel ubiquitously expressed transmembrane adaptor protein, binds the protein tyrosine kinase Csk and is involved in regulation of T cell activation. J Exp Med 2000;191:1591–604.
[2] Kawabuchi M, Satomi Y, Takao T, Shimonishi Y, Nada S, Nagai K, Tarakhovsky A, Okada M. Transmembrane phosphoprotein Cbp regulates the activities of Src-family tyrosine kinases. Nature 2000;404:999–1003.
[3] Davidson D, Bakinowski M, Thomas ML, Horejsi V, Veillette A. Phosphorylation-dependent regulation of T-cell activation by PAG/Cbp, a lipid raft-associated transmembrane adaptor. Mol Cell Biol 2003;23:2017–28.
[4] Horejsi V, Zhang W, Schraven B. Transmembrane adaptor proteins: organizers of immunoreceptor signalling. Nat Rev Immunol 2004;4:603–16.
[5] Mustelin T, Tasken K. Positive and negative regulation of T-cell activation through kinases and phosphatases. Biochem J 2003;371:15–27.
[6] Takeuchi S, Takazama Z, Ogawa A, Tamura K, Okada M. Transmembrane phosphoprotein Cbp positively regulates the activity of the carboxyl/terminal Src kinase Csk. J Biol Chem 2000;275:29183–6.
[7] Torgersen KM, Vang T, Abrahamsen H, Yaqub S, Horejsi V, Schraven B, Rolstad B, Mustelin T, Tasken K. Release from tonic inhibition of T cell activation through transient displacement of C-terminal Src kinase (Csk) from lipid rafts. J Biol Chem 2001;276:29313–8.
[8] Yasuda K, Nagafuku M, Shima T, Okada M, Yagi T, Yamada T, Minaki Y, Kato A, Tani-Ichi S, Hamaoka T, Kosugi A. Cutting edge: Fyn is essential for tyrosine phosphorylation of Csk-binding protein/phosphoprotein associated with glycolipid-enriched microdomains in lipid rafts in resting T cells. J Immunol 2002;169:2813–7.
[9] Baumgartner M, Angelisova P, Setterblad N, Mooney N, Werling D, Horejsi V, Langsley G. Constitutive exclusion of Csk from Hck-positive membrane microdomains permits Src kinase-dependent proliferation of *Theileria*-transformed B lymphocytes. Blood 2003;101:1874–81.
[10] Jaffe ES, Harris NL, Stein H, Vardiman JW. Pathology and genetics of tumors of haematopoetic and lymphoid tissues. WHO classification of tumors. Lyon: IARC Press; 2001.
[11] Kurosaki T. Regulation of B cell fates by BCR signaling components. Curr Opin Immunol 2002;14:341–7.
[12] Awashi-Kalia M, Schnetkamp PP, Deans JP. Differential effects of filipin and methyl-β-cyclodextrin on B cell receptor signalling. Biochem Biophys Res Commun 2001;287:77–82.
[13] Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI, Gascoyne RD, Muller-Hermelink HK, Smeland EB, Giltnane JM, Hurt EM, Zhao H, Averett L, Yang L, Wilson WH, Jaffe ES, Simon R, Klausner RD, Powell J, Duffey PL, Longo DL, Greiner TC, Weisenburger DD, Sanger WG, Dave BJ, Lynch JC, Vose J, Armitage JO, Montserrat E, Lopez-Guillermo A, Grogan TM, Miller TP, LeBlanc M, Ott G, Kvaloy S, Delabie J, Holte H, Krajci P, Stokke T, Staudt LM. Lymphoma/Leukemia Molecular Profiling Project. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. N Engl J Med 2002;346:1937–47.
[14] Borisch B, Yerly S, Bochet M, Hoessli DC. Raft signalling platforms in Hodgkin lymphoma (HL) and anaplastic large cell lymphoma. XII Meeting of the European Association for Haematopathology. Thessaloniki, Greece; 2004, p. 106 [Book of Abstracts].