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Neuropilin-1 is expressed by chronic lymphocytic leukemia B cells

Grzegorz S. Nowakowski*,

Division of Hematology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA

Debabrata Mukhopadhyay,

Department of Biochemistry & Molecular Biology, Mayo Clinic, Rochester, MN, USA

Xiaosheng Wu, and

Department of Immunology, Mayo Clinic, Rochester, MN, USA

Neil E. Kay

Division of Hematology, Mayo Clinic, Rochester, MN, USA

To the Editor

Primary CLL B cells have been shown to secrete vascular endothelial growth factor (VEGF), and secreted VEGF was shown to increase the resistance of leukemic CLL B cells to spontaneous and drug induced apoptosis through what is believed to be an autocrine loop [1]. We and others have shown that CLL B cells express VEGF receptor 1 (VEGF-R1) and 2 (VEGF-R2) which provide these cells with a capacity for VEGF binding [2]. While these receptors were shown to be expressed on tumor cells and are likely to be involved in both autocrine survival as well as neovascularization in tumor models, there is increasing evidence that another VEGF receptor, neuropilin-1 (NRP-1), is critical in these tumor angiogenic features and most likely involved in VEGF mediated resistance to apoptosis [3]. Aberrant NRP-1 expression has been shown in several solid tumors and acute myeloid leukemia. In the latter, NRP-1 expression was associated with shortened overall survival of these patients [4]. Recently, we have investigated NRP-1 expression in CLL and normal B cells. All CLL patients had a confirmed diagnosis using the National Cancer Institute (NCI) Working Group definition. Patients' samples utilized in Western blot analysis were from all Rai stages where more than 90% of CD19+ and CD5+ CLL B cells were present as assessed by flow cytometry. Western blot was performed for 10 CLL patients using monoclonal anti-NRP-1 antibodies (Calbiochem, San Diego, CA) and showed NRP-1 protein expression in 7 of 10 patients (Fig. 1A). We also evaluated surface NRP-1 expression by flow cytometry ($n = 5$) (Fig. 1B). Patients in this cohort were from early Rai stages (0–2) and had a varying degree of lymphocytosis (median, $63,000 \times 10^9 \text{ L}^{-1}$; range, 6000–123,000).

Isolated mononuclear cells were stained with monoclonal allophycocyanin labeled anti-CD19 antibodies (BD Biosciences, San Jose, CA) and phycoerythrin labeled anti-NRP-1 antibodies (Miltényi Biotec, Auburn, CA) and then analyzed by flow cytometry gating on CD19+ cells. Isotype-specific antibodies were used as controls. NRP-1 expression by flow was seen in two of five patients, but NRP-1 expression was not detected in three of three normal B cells obtained from healthy controls. Our results demonstrate, for the first time,

*Corresponding author. Tel.: +1 507 284 2511; fax: +1 507 266 9277. nowakowski.grzegorz@mayo.edu (G.S. Nowakowski). All of the authors have contributed equally to this work.

Conflicts of interest

None.

that NRP-1 is expressed by a subset of CLL B cells. Importantly, normal B cells appear not to express NRP-1. Since we believe that VEGF supports an autocrine pathway that promotes CLL B cell survival, and NRP-1 expression is limited to a subset of CLL patients, it will be critical to investigate the relationship of NRP-1 expression with known prognostic factors. Thus, expression of NRP-1 on leukemic myeloid cells was linked to shortened overall survival in acute myeloid leukemia [5]. The exact prognostic role of NRP-1 expression in CLL warrants further studies in a larger cohort of patients which we are now actively pursuing. Blocking NRP-1 with VEGF-mimicking peptide and blocking-antibodies was shown to induce tumor apoptosis in breast cancer [6] and lung cancer xenografts [7]. NRP-1 expression on CLL B cells may offer a new relatively selective therapeutic target, particularly since we did not observe NRP-1 expression in normal B cells.

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References

1. Farahani M, Treweek AT, Toh CH, Till KJ, Harris RJ, Cawley JC, et al. Autocrine VEGF mediates the antiapoptotic effect of CD154 on CLL cells. *Leukemia*. 2005; 19:524–30. [PubMed: 15674425]
2. Lee YK, Shanafelt TD, Bone ND, Strege AK, Jelinek DF, Kay NE. VEGF receptors on chronic lymphocytic leukemia (CLL) B cells interact with STAT 1 and 3: implication for apoptosis resistance. *Leukemia*. 2005; 19:513–23. [PubMed: 15703780]
3. Ellis LM. The role of neuropilins in cancer. *Mol Cancer Ther*. 2006; 5:1099–107. [PubMed: 16731741]
4. Kreuter M, Woelke K, Bieker R, Schliemann C, Steins M, Buechner T, et al. Correlation of neuropilin-1 overexpression to survival in acute myeloid leukemia. *Leukemia*. 2006; 20:1950–4. [PubMed: 16990775]
5. Jelinek DF, Tschumper RC, Geyer SM, Bone ND, Dewald GW, Hanson CA, et al. Analysis of clonal B-cell CD38 and immunoglobulin variable region sequence status in relation to clinical outcome for B-chronic lymphocytic leukaemia. *Br J Haematol*. 2001; 115:854–61. [PubMed: 11843819]
6. Barr MP, Byrne AM, Duffy AM, Condron CM, Devocelle M, Harriott P, et al. A peptide corresponding to the neuropilin-1-binding site on VEGF(165) induces apoptosis of neuropilin-1-expressing breast tumour cells. *Br J Cancer*. 2005; 92:328–33. [PubMed: 15655556]
7. Pan Q, Chantry Y, Liang WC, Stawicki S, Mak J, Rathore N, et al. Blocking neuropilin-1 function has an additive effect with anti-VEGF to inhibit tumor growth. *Cancer Cell*. 2007; 11:53–67. [PubMed: 17222790]

Fig. 1. Neuropilin-1 expression in CLL. (A) Western blot. NRP-1 expression in CLL B-cells was detected by Western blot using specific monoclonal antibody. (B) Flow cytometry. NRP-1 expression was assessed gating on CD19 positive cells; representative histogram of a CLL patient expressing of NRP-1 is shown.