Cancer Research

Tumor-Initiating Cells in Childhood Neuroblastoma—Letter

Sofie Mohlin[1], Alexander Pietras[1], Caroline Wigerup[1], Ingrid Øra[2], Michael Andäng[5], Kenneth Nilsson[6], Tor Olofsson[3], David Gisselsson[4], and Sven Påhlman[1]

We report here that the neuroblastoma tumor-initiating cell (TIC) cultures (NB12, NB88, and NB122R) isolated from neuroblastoma patients and established in culture by Dr. David Kaplan and colleagues [reported by Hansford et al. (1) and Pietras et al. (2)] are severely overgrown by Epstein-Barr virus (EBV)–infected B lymphocytes. Our findings advise precaution when using the TIC lines as models for neuroblastoma development, even at modest passage numbers.

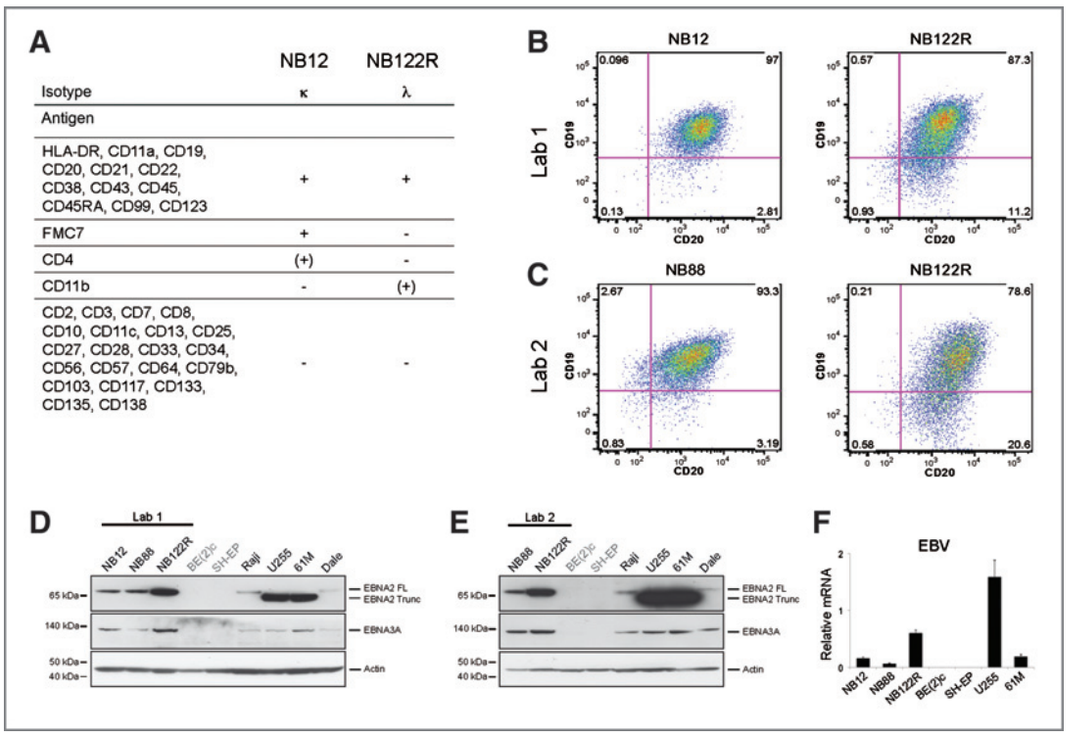

Figure 1. TIC cultures derived from bone marrow aspirates of patients with neuroblastoma are contaminated by EBV-infected lymphoblastoid cells. A, flow cytometric analysis of hematopoietic cell surface antigens on NB12 and NB122R TIC cultures. B and C, TICs from laboratory 1 (Påhlman; B) and 2 (Andäng; C) were analyzed for expression of CD19 and CD20 by flow cytometry. Quadrant gating shows percentages of positive and negative cells in respective quadrant. D and E, EBNA2 and EBNA3A Western blot analysis of TICs from laboratory 1 (D) and laboratory 2 (E). FL, full-length; Trunc, Truncated. F, quantitative PCR analysis of EBV-specific mRNA. Neuroblastoma cell lines SK-N-BE(2)c and SH-EP were used as negative and Raji and EBV-immortalized cell lines (U255, 61M, and Dale) as positive controls.

Authors' Affiliations: [1]Center for Molecular Pathology, Department of Laboratory Medicine, Malmö, CREATE Health, Lund University, Skåne University Hospital; Departments of [2]Pediatric Oncology and Hematology, [3]Laboratory Medicine, and [4]Clinical Genetics and Pathology, Lund University, Malmö; [5]Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Stockholm; and [6]Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

Note: S. Mohlin, A. Pietras, and C. Wigerup contributed equally to the work.

Corresponding Author: Sven Påhlman, Lund University, University Hospital MAS, Entrance 78 III Floor, Malmo S-20502, Sweden. Phone: 4640337403; Fax: 4640337063; E-mail: sven.pahlman@med.lu.se

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Hansford and colleagues have reported the successful retrieval of metastatic TICs from bone marrow aspirates of patients with non-*MYCN* amplified, high-stage aggressive neuroblastoma (1). These cells were suggested to be putative neuroblastoma stem cells on the basis of their immature neuronal phenotype (2). The TICs have been distributed to more than 20 laboratories worldwide and recently, several reports have identified drugs selectively targeting these TICs. At the 2010 Advances in Neuroblastoma Research Meeting in Stockholm, we reported that the TIC cultures (NB12, NB88, and NB122R) from 3 individual patients have a principally normal genotype [single-nucleotide polymorphism (SNP) analyses]. Kaplan and

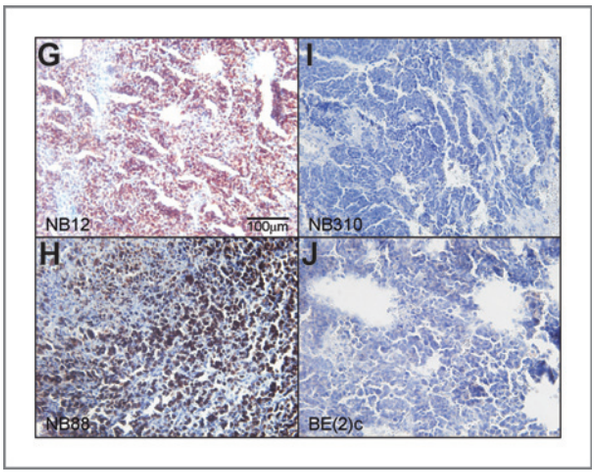

Figure 1. (*Continued*) G–J, immunohistochemical staining of tissue sections from xenografts established from short term cultured TIC lines NB12 (G) and NB88 (H) for EBV-antigen EBNA2. Negative controls are sections of a neuroblastoma specimen (I) and a xenograft tumor of SK-N-BE(2)c cells (J).

colleagues also reported the expression of B-cell markers and VDJ gene rearrangements in TIC cultures. The observations prompted us to investigate the phenotype of these TICs in more detail.

We found that the neuroblastoma patient–derived TIC cultures are contaminated by EBV-infected B-lymphoblastoid cells. They express B-lymphocyte antigens while lacking markers of immature B-, myeloid, and stem cells as analyzed by extensive flow cytometry (Fig. 1A). At fairly low passages (7 to 15), 78% to 97% of cells in the cultures were positive for CD19 and CD20, two well-characterized B-cell markers (Fig. 1B and C). Furthermore, the TIC cultures expressed the EBNA2 and EBNA3A proteins and EBV-specific mRNA (Fig. 1D–F). We carried out flow cytometric and Western blot analyses on these TICs obtained from a second independent laboratory with consistent results, excluding the possibility that the contamination arose in our laboratory (Fig. 1A–F).

TIC xenograft tumors stained positive for EBNA2 as compared with completely negative patient-derived tumors and xenograft specimens of the neuroblastoma cell line SK-N-BE (2)c (Fig. 1G–J). Most cells in TIC xenografts exhibited prominent cytoplasm occasionally with immunoblast/plasma cell–like features suggestive of a B-cell phenotype. There were no neurofibrillary matrix, rosette formation, or dispersed lumped chromatin pattern typical of neuroblastoma.

EBV infects most humans, normally beginning during childhood, and establishes a latent infection in a fraction of B lymphocytes distributed mainly in blood, peripheral lymphoid tissue, and bone marrow. As the 3 TICs analyzed by us were from 3 different individuals (SNP-data), it is likely that the retrieved bone marrow aspirates with neuroblastoma cells were contaminated with patient-derived EBV-carrying lymphoblastoid cells. Our observations explain the normal SNP profile, the B-cell phenotype, and the VDJ gene rearrangementsi n TIC cultures.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Hansford LM, McKee AE, Zhang L, George RE, Gerstle JT, Thorner PS, et al. Neuroblastoma cells isolated from bone marrow metastases contain a naturally enriched tumor-initiating cell. Cancer Res 2007; 67:11234–43.

2. Pietras A, Hansford LM, Johnsson AS, Bridges E, Sjölund J, Gisselsson D, et al. HIF-2alpha maintains an undifferentiated state in neural crest-like human neuroblastoma tumor-initiating cells. Proc Natl Acad Sci U S A 2009;106:16805–10.