Letter to the Editor

Cancer Research

Tumor-Initiating Cells in Childhood Neuroblastoma—Response

Loen M. Hansford and David R. Kaplan

We agree that the lines in question were contaminated by Epstein Barr Virus (EBV) and immediately withdrawn from circulation. In contrast to the note of caution expressed by Påhlman about using these 3 neuroblastoma tumor-initiating lines (NB12, NB88R2, and NB122R), we have recommended to the limited number of laboratories that were sent the lines or their derivatives that all stocks of them be destroyed. These lines were distributed under the condition that they are not to be circulated or provided to other laboratories, so we were confident that these lines no longer exist outside of our laboratory. Both Påhlman and we have noted neural characteristics in the lines, including widespread neural marker expression in tumor-initiating cells depleted of HIF-2α in xenografts (1) and neurosecretory granules by electron microscopy of NB88R2 xenografts (Fig. 1). Påhlman also published that downregulation of HIF-2α in the EBV-contaminated tumor-initiating cell lines resulted in a marked upregulation of neural and neuroblastoma-related gene expression in culture, indicating the persistence of neuroblastoma characteristics at later passages (1), at least in a subpopulation of the cells. Other neuroblastoma lines reported in the work of Hansford and colleagues (2) including NB04, NB05b, NB07, NB08, NB17, NB19, NB20, NB24, and NB32 were not contaminated with EBV as assessed by quantitative real-time PCR for EBNA1 (Table 1), whereas derivatives of NB12 as determined by short tandem repeat (STR) analysis including NB25, NB61, and NB67 expressed EBER1 (Table 1). NB88R2 and NB122R were

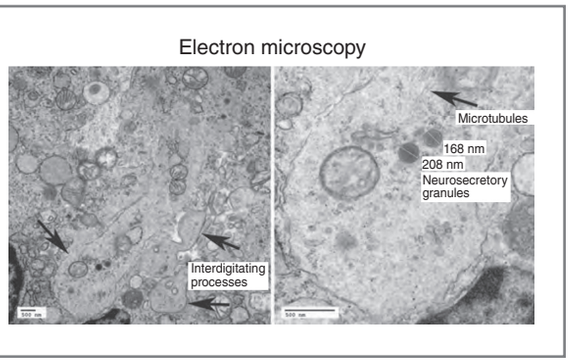

Figure 1. Analysis of tumors formed by injecting NB TICs supra-renally into immunocompromised mice illustrates that EBV-contaminated NB TIC tumors (NB88R2) show features typical of NB including interdigitating cell processes, marked with arrows in the left image (original magnification 15,000×), and a cell process with microtubules, marked by an arrow, and dense core neurosecretory granules (original magnification 50,000×) in the right image by electron microscopy.

Table 1. Summary of NB TIC lines published in Hansford and colleagues (2)

Sample name	EBV status	EBV test
NB04	Negative	RealStar EBV test (*EBER1*)
NB05b	Negative	*In situ* hybridization (*EBNA1*)
NB07	Negative	RealStar EBV test (*EBER1*)
NB08	Negative	RealStar EBV test (*EBER1*)
NB13	Insufficient material to test	
NB14	Insufficient material to test	
NB15	Insufficient material to test	
NB17	Negative	RealStar EBV test (*EBER1*)
NB19	Negative	RealStar EBV test (*EBER1*)
NB20	Negative	RealStar EBV test (*EBER1*)
NB24	Negative	RealStar EBV test (*EBER1*)
NB32	Negative	RealStar EBV test (*EBER1*)
NB12 and derivatives		
NB12	Positive	DNA PCR (*EBER1*, *EBNA2*); *In situ* hybridization (*EBNA1*)
NB25	Positive	DNA PCR (*EBER1*, *EBNA2*); *In situ* hybridization (*EBNA1*)
NB61	Positive	DNA PCR (*EBER1, EBNA2*); *In situ* hybridization (*EBNA1*)
NB67	Positive	DNA PCR (*EBER1*, *EBNA2*)

Authors' Affiliation: The Hospital for Sick Children, Toronto, Ontario, Canada

Corresponding Author: David R. Kaplan, The Hospital for Sick Children, Toronto Medical Discovery East Tower, 101 College St., 12th Floor, Room 12-314, Toronto, Ontario, Canada M5G 1L7. Phone: 416-813-7654, ext. 1433; Fax: 416-813-2212; E-mail: dkaplan@sickkids.ca

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first reported in the study by Pietras and colleagues, a publication from the Påhlman laboratory, with cell lines supplied by our laboratory (1).

Regarding the unpublished B-cell marker expression data in NB12, NB88R2, and NB122R that we reported at a meeting, we note that others have reported leukemia/lymphoma marker expression in neuroblastoma cell lines (3). The characterization of leukemia/lymphoma marker expression in non–EBV-contaminated neuroblastoma TIC lines is currently being examined and will be the subject of a future report.

We have reported that drugs that are effective on EBV-contaminated neuroblastoma TICs are effective on neuroblastoma established cell lines (4, 5) and we find that they are also effective on a non–EBV-contaminated primary neuroblastoma sphere-forming line (NB153). Our characterization of drug responses on NB153 and other neuroblastoma lines will also be the subject of a future report.

We thank Påhlman and colleagues for bringing this issue to our attention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interests were disclosed.

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References

1. Pietras A, Hansford LM, Johnsson S, Bridges E, Sjölund J, Gisselsson D, et al. HIF-2a protein expression is high at normoxia and maintains an undifferentiated state in neural crest-like human neuroblastoma tumor-initiating cells. Proc Natl Acad Sci U S A 2009;106:16805–10.
2. Hansford LM, McKee AE, Zhang L, George R, Gerstle JT, Thorner PT, et al. Neuroblastoma cells isolated from bone marrow metastases contain an enriched tumor-initiating cell. Cancer Res 2007;67:11234–43.
3. Sugimoto T, Tatsumi E, Kemshead JT, Helson L, Green AA, Minowada J. Determination of cell surface membrane antigens common to both human neuroblastoma and leukemia-lymphoma cell lines by a panel of 38 monoclonal antibodies. J Natl Cancer Inst 1984;73:51–7.
4. Smith KM, Datti A, Zhang L, Fujitani M, Morozova O, Blakely KM, et al. Selective targeting of neuroblastoma tumor-initiating cells by compounds identified in a stem cell-based small molecule screen. EMBO Mol Med 2010;2:371–84.
5. Grinshtein N, Datti A, Fujitani M, Uehling D, Prakesch M, Isaac M, et al. Small molecule kinase inhibitor screen identifies Polo-like Kinase 1 as a novel target for neuroblastoma tumor-initiating cells. Cancer Res 2011;71:1385–95.