Journal of Virological Methods 173 (2011) 266–270

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Removal of lactate dehydrogenase-elevating virus from human-in-mouse breast tumor xenografts by cell-sorting

Huiping Liu[a,b,*], Jessica Bockhorn[a], Rachel Dalton[a], Ya-Fang Chang[a,c], Dalong Qian[b], Lois A. Zitzow[d], Michael F. Clarke[b], Geoffrey L. Greene[a,*]

[a] The Ben May Department for Cancer Research, The University of Chicago, Chicago, IL 60637, USA
[b] The Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA 94305, USA
[c] The Department of Biomedical Imaging and Radiological Sciences, National Yang-Ming University, 112 Taiwan
[d] Animal Resources Center and Department of Surgery, The University of Chicago, Chicago, IL 60637, USA

Article history:
Received 9 December 2010
Received in revised form 10 February 2011
Accepted 15 February 2011
Available online 24 February 2011

Keywords:
LDV removal
Cell sorting
Human-in-mouse tumor models
Breast cancer

ABSTRACT

Lactate dehydrogenase-elevating virus (LDV) can infect transplantable mouse tumors or xenograft tumors in mice through LDV-contaminated mouse biological materials, such as Matrigel, or through mice infected with LDV. LDV infects specifically mouse macrophages and alters immune system and tumor phenotype. The traditional approaches to remove LDV from tumor cells, by transplanting tumors into rats or culturing tumor cells in vitro, are inefficient, labor-intensive and time-consuming. Furthermore, these approaches are not feasible for primary tumor cells that cannot survive tissue culture conditions or that may change phenotype in rats. This study reports that fluorescence-activated cell sorting (FACS) is a simple and efficient approach for purifying living primary human breast tumor cells from LDV$^+$ mouse stromal cells, which can be completed in a few hours. When purified from Matrigel contaminated LDV$^+$ tumors, sorted human breast tumor cells, as well as tumors grown from sorted cells, were shown to be LDV-free, as tested by PCR. The results demonstrate that cell sorting is effective, much faster and less likely to alter tumor cell phenotype than traditional methods for removing LDV from xenograft models. This approach may also be used to remove other rodent-specific viruses from models derived from distinct tissues or species with sortable markers, where virus does not replicate in the cells to be purified.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Current cancer research approaches take advantage of mouse models, including mouse tumors and human tumor xenografts in mice, to elucidate regulatory mechanisms of tumors through interactions between tumor cells and their microenvironment *in vivo*. In cancer stem cell (CSC) transplantation studies, mouse biological materials, such as the basement membrane matrix (Matrigel), are used to facilitate tumor initiation and progression in mice (Al-Hajj et al., 2003; Liu et al., 2010; Quintana et al., 2008). However, the species *lactate dehydrogenase-elevating virus* in the order Nidovirales, family Arteriviridae, genus Arterivirus (LDV, LDEV, or LDH virus), a natural mouse virus that infects macrophages, is one of the common organisms that contaminate mouse tumor models and cell byproducts like Matrigel (Peterson, 2008). LDV can significantly affect the immune system and tumor behavior (Ammann et al., 2009; Baker, 2003; Riley et al., 1978). It interferes with the mouse immune system by trapping macrophages and activating lymphocytes, which cause changes in immune response (Ammann et al., 2009; Riley et al., 1978). In addition, LDV alters tumor behavior by increasing or decreasing tumor incidence and growth rate, regulating oncogenic virus expression, and modifying responses to cancer therapies (Riley et al., 1978). In general, LDV is persistent and subclinical except for transient elevation of plasma lactate dehydrogenase levels, although certain laboratory mutants of LDV can induce secondary polioencephalomyelitis and flaccid paralysis in conditioned immunosuppressed mice (Anderson et al., 1995; Li et al., 1999). As macrophages and other immune cells play an important role in cancer development or progression, LDV contamination is a potential problem for mouse tumor studies or human cancer and cancer stem cell models in immune-compromised mice.

Detection of LDV in tumor models or mouse tissue can be achieved by polymerase chain reaction (PCR) with specific primers that recognize the single-strand virus RNA (Chen and Plagemann,

Abbreviations: LDV, lactate dehydrogenase-elevating virus; FACS, fluorescence-activated cell sorter; CSC, cancer stem cells; PCR, polymerase chain reaction; BCSC, breast cancer stem cells; NOD/SCID, non-obese diabetic/severe combined immunodeficiency; IACUC, Institutional Animal Care and Use Committee.

* Corresponding authors at: The Ben May Department for Cancer Research, The University of Chicago, 929 E 57th Street, Chicago, IL 60637, USA.
Tel.: +1 773 702 6966; fax: +1 773 702 3798.
E-mail addresses: hliu@uchicago.edu (H. Liu), ggreene@uchicago.edu (G.L. Greene).

0166-0934/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jviromet.2011.02.015

1997; Wagner et al., 2004). In some of laboratory-established human-in-mouse breast tumor models (Liu et al., 2010), LDV contamination was detected by PCR. However, there is currently no therapeutic approach to clear LDV from the host mice, where it produces lifelong persistent viremia (Infectious disease of mice and rats, 1991; Baker, 2003; Riley et al., 1978). Since primary tumor models do not survive in tissue culture or may change phenotype in rats, it is not realistic or appropriate to eliminate LDV from these tumors through prolonged selective culturing of tumor cells or by tumor transplantation into rats (Infectious disease of mice and rats, 1991; Baker, 2003; Riley et al., 1978). Based on the fact that LDV only infects a subpopulation of mouse macrophages (Kowalchyk and Plagemann, 1985; Plagemann et al., 1995; Ritzi et al., 1982), fluorescence-activated cell sorter (FACS)-based cell sorting was used as a fast and efficient approach to remove mouse stromal cells and LDV from tumor cells within a few hours, with minimal risk of tumor feature alterations.

2. Materials and methods

2.1. Materials and protocols

Antibodies (H2K^d-biotin, streptavidin-PE-Cy5, streptavidin-PE-Cy7, anti-hCD44-APC and lineage antibodies) and Matrigel were purchased from BD Biosciences (San Jose, CA, USA), the nucleic acid stain DAPI (the dilactate form), ACK lysis buffer and fetal bovine serum from Invitrogen (Carlsbad, CA, USA), Liberase Blendzymes from Roche (Palo Alto, CA, USA), Collagenase type III from Worthington Biomedical (Lakewood, NJ, USA), IgG blocking antibody, DNase I from Sigma (St. Louis, MO, USA). Research involving human tumor and mouse models was approved by the Institutional Review Board (IRB), Institutional Animal Care and Use Committee (IACUC) and Institutional Biosafety Committee (IBC) of the University of Chicago, in accordance with an assurance filed with and approved by the Office of Laboratory Animal Welfare, the Department of Health and Human Services.

2.2. Human breast tumor models

Bypassing cell culture *in vitro*, the human-in-mouse breast tumor models were generated from patient tumors mixed with Matrigel (purchased in 2007) and maintained by continuous passaging in non-obese, diabetic/severe combined immunodeficiency (NOD/SCID) mice *via* orthotopic implantation of tumor chunks or sorted CD44$^+$ breast cancer stem cells (BCSCs) with Matrigel (purchased in 2009) as described (Al-Hajj et al., 2003; Liu et al., 2010). Tumor chunks (fine minced pieces) or living tumor cells were stored in RPMI/10% DMSO/50% fetal bovine serum (FBS) at −135 °C or liquid nitrogen tank. To generate tdTomato or eGFP-labeled tumor models, BCSCs were transduced with optical reporters in a lentiviral vector pFU-tdTomato or pFU-eGFP (Liu et al., 2010). Mice bearing LDV$^+$ tumors or tumor cells isolated from these models were housed in a quarantined animal facility.

2.3. Tumor cell isolation and staining

Tumors were dissociated with Liberase Blendzyme 2 and 4 or Collagenase III as described (Al-Hajj et al., 2003; Liu et al., 2010). After red blood cell lysis with ACK buffer, single cell suspensions were filtered and re-suspended in HBSS/2% FBS for further staining. Following mouse IgG blocking (100 μg/ml) bulk tumor cells were stained with anti-H2K^d-biotin antibody and then the secondary Streptavidin-PE-Cy5 or Streptavidin-PE-Cy7 for labeling mouse stromal cells. In order to enrich human CD44$^+$ BCSCs, cells were stained with anti-CD44-APC antibody. DAPI was added into cell suspension at 1 nM (viability marker) prior to flow analysis and sorting. In addition, single color controls were prepared for color compensation settings on flow cytometer.

2.4. Tumor cell purification by double sorting on flow cytometer

A FACSAria I or II (BD) BD flow cytometer (BD, San Jose, CA, USA) was washed with 10% Contrad and then sterile H_2O, prior to and after each experiment. Single color controls (blank, DAPI, PE-Cy5/7, and APC, eGFP, tdTomato) were then analyzed for compensation settings. For sample sorting, all gates for human tumor cells were set up based on single DAPI-negative (viable) and H2K^d-negative tumor cells (P1 to P2, to P3, and then to P4), excluding debris, duplets or multiplets, dead cells (DAPI$^+$) or mouse stromal cells (H2K^{d+}) (P4). Additional markers for human tumor cells included the hCD44 surface marker, or eGFP or tdTomato as optical reporters. Sorted cells were collected in HBSS/10%FCS, concentrated by centrifugation and re-suspended in a small volume of HBSS/2%FCS prior to a second sorting. Double-sorted cells were used for LDV PCR tests and mouse injection. The double sorted cells were used for LDV PCR tests and mouse injection.

2.5. PCR test of LDV and murine viruses by RADIL

Tumor samples or sorted cells were prepared in 10 μl PBS solution and shipped on dry ice (overnight delivery) to the University of Missouri-Columbia Research Animal Diagnostic Laboratory (http://radil.missouri.edu/) (RADIL, Columbia, MO, USA) for PCR tests (the full list of IMPACT I mouse viruses or individual LDV tests). The initial screen for the full list of mouse viruses was carried out with pooled tumor samples and BD Matrigel (purchased after 2009). To identify or remove LDV, individual PCR tests were performed to examine the LDV status of Matrigel, unsorted bulk tumor cells from distinct tumor models, double sorted tumor cells for each tumor model, and tumors grown from these sorted or unsorted cells upon implantation in mice.

2.6. Statistical analysis

Two-tailed student's *t* tests were used to evaluate the significant difference or p values between samples. Standard deviations (SD) of mean values were calculated.

3. Results

3.1. Detection of LDV from human breast tumor models

Following IACUC requirements, and before passaging human-in-mouse tumors derived from clinical breast tumor specimens and old batches of Matrigel purchased in 2007, tumor samples and new Matrigel (purchased in 2009) were analyzed by PCR for the presence of 18 murine viruses and Mycoplasma (RADIL IMPACT I PCR panel). LDV was found in pooled samples containing tumors and Matrigel (2009) (Table 1). The new batch of Matrigel (2009) was excluded as the source of virus, and LDV contamination was confirmed in three breast tumors (unlabeled, eGFP-labeled and tdTomato-labeled, Table 1). Because these human-in-mouse tumor models are only maintained in mice *in vivo*, LDV appeared to transfer to the next series of tumor generations following orthotopic implantation, even after being frozen for a couple of years in liquid nitrogen (Table 2), suggesting that it persists in transplantable tumors unless it is removed. LDV specifically infects and propagates in mouse macrophages and may severely affect the immune system and tumor phenotype. Therefore, it was necessary to remove LDV from these tumors. Because tissue culture or rat transplantation were not appropriate for LDV removal from these human-in-mouse

Download English Version:

https://daneshyari.com/en/article/6135236

Download Persian Version:

https://daneshyari.com/article/6135236

Daneshyari.com