Stem Cell Research (2014) 13, 164–171

Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/scr

SHORT REPORT

Deletion of *Ptp4a3* reduces clonogenicity and tumor-initiation ability of colitis-associated cancer cells in mice

Julie M. Cramer[a,b,1], Mark W. Zimmerman[c,1,2], Tim Thompson[d], Gregg E. Homanics[c,e], John S. Lazo[c,3], Eric Lagasse[a,b,*]

[a] *Department of Pathology, University of Pittsburgh School of Medicine, 200 Lothrop Street, Pittsburgh, PA 15261, USA*
[b] *McGowan Institute for Regenerative Medicine, Suite 300, University of Pittsburgh, 450 Technology Drive, Pittsburgh, PA 15219, USA*
[c] *Department of Pharmacology and Chemical Biology, University of Pittsburgh, 203 Lothrop Street, Pittsburgh, PA 15260, USA*
[d] *Department of Bioengineering, University of Pittsburgh, 3700 O'Hara St., Pittsburgh, PA 15261, USA*
[e] *Department of Anesthesiology, University of Pittsburgh, 3550 Terrace Street, Pittsburgh, PA 15261, USA*

Received 26 February 2014; received in revised form 9 May 2014; accepted 18 May 2014
Available online 27 May 2014

Abstract The PTP4A3 gene is highly expressed in human colon cancer and often associates with enhanced metastatic potential. Genetic disruption of the mouse *Ptp4a3* gene reduces the frequency of colon tumor formation in mice treated in a colitis-associated cancer model. In the current study, we have examined the role of *Ptp4a3* in the tumor-initiating cell population of mouse colon tumors using an *in vitro* culture system. Tumors generated *in vivo* following AOM/DSS treatment were isolated, dissociated, and expanded on a feeder layer resulting in a $CD133^{+}$ cell population, which expressed high levels of *Ptp4a3*. Tumor cells deficient for *Ptp4a3* exhibited reduced clonogenicity and growth potential relative to WT cells as determined by limiting dilution analysis. Importantly, expanded tumor cells from WT mice readily formed secondary tumors when transplanted into nude mice, while tumor cells without *Ptp4a3* expression failed to form secondary tumors and thus were not tumorigenic. These results demonstrate that *Ptp4a3* contributes to the malignant phenotype of tumor-initiating cells and supports its role as a potential therapeutic target to inhibit tumor self-renewal and metastasis.
© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

Abbreviations: PTP4A3, Protein tyrosine phosphatase 4A3; AOM, Azoxymethane; DSS, Dextran Sodium Sulfate; CFU, Colony Forming Unit; LDA, Limiting Dilution Analysis; FACS, Fluorescence Activated Cell Sorting; MUC2, Mucin2; WT, Wildtype; KO, Knockout; EpCAM, Epithelial Cell Adhesion Molecule.

* Corresponding author at: McGowan Institute for Regenerative Medicine, University of Pittsburgh, Suite 300, 450 Technology Drive, Pittsburgh, PA 15219, USA. Fax: +1 412 624 5363.
E-mail addresses: juc24@pitt.edu (J.M. Cramer), markw_zimmerman@dfci.harvard.edu (M.W. Zimmerman), tnt15pitt@gmail.com (T. Thompson), homanicsge@anes.upmc.edu (G.E. Homanics), lazo@virginia.edu (J.S. Lazo), lagasse@pitt.edu (E. Lagasse).
[1] Equal contribution.
[2] Present address: Department of Pediatric Oncology, 450 Brookline Avenue, Dana-Farber Cancer Institute, Boston, MA, 02215, USA.
[3] Present address: Department of Pharmacology, University of Virginia, P.O. Box 800793, Charlottesville, VA 22908, USA.

http://dx.doi.org/10.1016/j.scr.2014.05.004
1873-5061/© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

Introduction

Colon cancer is a complex disease in which normal epithelial cells abandon normal proliferative control and become malignant. These tumors comprise many different cell types that feature functional heterogeneity and unique roles in malignancy. The pathogenesis and recurrence of colon cancer following treatment likely depends on a specific subpopulation of tumor-initiating cells present within the tissue capable of self-renewal, multipotency and higher clonogenicity than differentiated cells (O'Brien et al., 2007; Ricci-Vitiani et al., 2007; Ashley, 2013). The process of metastasis in particular may depend on the ability of colon tumor-initiating cells to extravasate and clonally expand in secondary locations. This result is the consequence of a combination of genetic aberrations and environmental signals, which are often enhanced by inflammatory conditions such as ulcerative colitis. Although the 5-year survival rate for colorectal cancer is 90% when diagnosed at the local stage, the survival decreases to only 12% if metastases are found (Howlader et al.). Therefore, a better understanding of the genes that contribute to the metastatic process should elucidate opportunities for therapeutic intervention to reduce the lethality of this disease.

Protein tyrosine phosphatase 4A3 (PTP4A3) is highly expressed in human colon tumors and a potential driver of the metastatic cascade (Saha et al., 2001). We have recently reported that disruption of the mouse *Ptp4a3* gene reduces colon carcinogenesis in a colitis-associated cancer model (Zimmerman et al., 2013), although the manner in which PTP4A3 facilitates the malignant phenotype is not well understood. Several lines of evidence have emerged suggesting that PTP4A3 may have a role in the biology of tumor-initiating cells. We previously reported upregulation of *Ptp4a3* expression in pre-neoplastic colon tissue after acute exposure to azoxymethane (AOM). PTP4A3 has been reported to mediate both p53 and TGFβ signaling which are well described mediators of cell fate and tumorigenesis (Jiang et al., 2011; Basak et al., 2008). Additionally, a recent report observed that a PTP4A3 small molecule inhibitor prevents the tumorigenesis of human lung cancer stem cells and sensitizes them to combination chemotherapy (Xia et al., 2010). Therefore, it is possible that PTP4A3 has an important role in the tumorigenicity of tumor-initiating cells of colon cancer.

In the current study, we investigated the effects of *Ptp4a3* loss on the tumor-initiating cell population of colon tumors from wildtype (WT) or *Ptp4a3*-knockout (KO) mice using an *in vitro* culture system previously used to study human metastatic colon cancer stem cells (Odoux et al., 2008). The expanded tumor cells were ubiquitously CD133$^+$ and exhibited enhanced clonogenicity at higher passages. Interestingly, expanded cells derived from *Ptp4a3*-KO tumors demonstrated reduced clonogenicity as well as the inability to form secondary tumors compared to WT tumor cells expressing *Ptp4a3*. These findings provide the first strong evidence for the involvement of *Ptp4a3* in mediating the clonogenicity of colon tumor-initiating cells and suggest that this phosphatase could potentially be a target for cancer therapy directed at self-renewal as well as metastasis.

Methods

Genetically engineered mice and colitis-associated cancer model — Experimental *Ptp4a3* mutant mice were produced by mating heterozygous breeding pairs and offsprings were genotyped by Southern blot analysis. Colon tumors from the colitis-associated cancer model were generated following treatment with AOM/DSS (Dextran Sodium Sulfate) as previously described (Zimmerman et al., 2013). Briefly, mice were administered a single IP injection of AOM (12.5 mg/kg) followed by a 1 week treatment with DSS (2.5%) in drinking water and 2 weeks of normal water. The DSS cycle was repeated two additional times and mice were sacrificed at 16 weeks after the beginning of treatment. All animal experiments were performed in accordance with the guidelines of the University of Pittsburgh Animal Care and Use Committee.

Tumor cell culture and expansion — Single tumor cells were isolated from primary tissue and expanded as previously described (Odoux et al., 2008). Briefly, colon tumor tissue (n = 4 tumors/genotype) from AOM/DSS treated *Ptp4a3* WT (Floxed/Floxed) and KO (−/−) mice (n = 2 mice/genotype) was isolated, pooled and stored in Hank's Balanced Salt Solution (HBSS) on ice containing 10% FBS (Invitrogen). Tumors were transferred to EBSS/1 mM EGTA/1% HEPES (Life Technologies, NY/Sigma-Aldrich, MO/Mediatech, VA) and minced into small (<2 mm) pieces. Tissue was then transferred to a tube and incubated for 5 min at room temperature. After an EBSS wash, the tissue was treated three times with a cocktail containing 1 mg/mL collagenase II (Life Technologies, NY) and 20 mg/ml DNase I (Roche, IN) in HBSS/1% HEPES for 20 min. Tissue/cell suspensions were passed through a 100 μm cell strainer (Fisher Scientific, PA) to isolate single cells from undigested tissue. Trypsinization for 20 min was then performed to achieve complete dissociation of the tumor tissue. Cells were plated onto a confluent layer of previously irradiated LA7 (ATCC: CRL-2283) cells (feeder layer) at ~80,000 cells/cm^2 in DMEM/F12 supplemented with 0.5% FBS, 25 mg/mL gentamicin (Sigma-Aldrich, MO) and 1% Insulin-Transferrin Selenium (ITS) (Mediatech, VA). Initial cultures (P0) were passaged at 2–3 weeks post-plating (70% confluence) by incubating with EBSS/1 mM EGTA/1% HEPES followed by 0.25% trypsin/0.1% EDTA. Following initial expansion, cultures were maintained by passaging at ~70% confluence onto new feeder cells. For immunohistochemistry of expanded tumor cells, cells were plated onto 4-well chamber slides (Thermo Scientific, NY). The isolation procedure was performed three separate times for each genotype to eliminate variability associated with individual experiments and tumor samples.

Fluorescence Activated Cell Sorting and Cell Cycle Analysis — Flow cytometry and Fluorescence Activated Cell Sorting (FACS) were used to assess cell surface marker expression and colony-forming unit (CFU) frequency via Limiting Dilution Analyses (LDA). Single cell suspensions were immunolabeled with Epithelial Cell Adhesion Molecule (EpCAM), CD29, CD104 (BioLegend, Inc., CA), CD49f, CD13, CD44, CD81, CD24, CD9, CD54 (BD Bioscience, MA), and/or CD133 (eBioscience, Inc., CA) specific antibodies (200,000 cells/tube) and analyzed on the MACSQuant™ (Miltenyi Biotec, CA) or BD FACSAria II™ (BD Biosciences, MA). Dead

Download English Version:

https://daneshyari.com/en/article/2094272

Download Persian Version:

https://daneshyari.com/article/2094272

Daneshyari.com