Gynecologic Oncology 130 (2013) 579–587

Contents lists available at SciVerse ScienceDirect

Gynecologic Oncology

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

# Targeting CD133 in an in vivo ovarian cancer model reduces ovarian cancer progression

Amy P.N. Skubitz [a,*], Elizabeth P. Taras [b], Kristin L.M. Boylan [a], Nate N. Waldron [c], Seunguk Oh [b], Angela Panoskaltsis-Mortari [d], Daniel A. Vallera [b]

[a] Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA
[b] University of Minnesota Masonic Cancer Center, Section on Molecular Cancer Therapeutics, Department of Therapeutic Radiology-Radiation Oncology, University of Minnesota, Minneapolis, MN, USA
[c] Department of Pharmacology and Therapeutic Radiology, University of Minnesota, Minneapolis, MN, USA
[d] Department of Pediatrics, Hematology, Oncology, and Bone Marrow Transplantation, University of Minnesota, Minneapolis, MN, USA

## HIGHLIGHTS

- Cancer stem cells appear to be directly targeted by use of an antibody against CD133.
- An anti-CD133 targeted toxin, dCD133KDEL, shows promise for ovarian cancer therapy.
- dCD133KDEL inhibits growth of ovarian carcinoma in vitro and in vivo in a mouse model.

## ARTICLE INFO

*Article history:*
Received 13 February 2013
Accepted 17 May 2013
Available online 27 May 2013

*Keywords:*
Ovarian cancer
CD133
Xenograft model
Cancer stem cells
Targeted toxin

## ABSTRACT

*Objectives.* While most women with ovarian cancer will achieve complete remission after treatment, the majority will relapse within two years, highlighting the need for novel therapies. Cancer stem cells (CSC) have been identified in ovarian cancer and most other carcinomas as a small population of cells that can self-renew. CSC are more chemoresistant and radio-resistant than the bulk tumor cells; it is likely that CSC are responsible for relapse, the major problem in cancer treatment. CD133 has emerged as one of the most promising markers for CSC in ovarian cancer. The hypothesis driving this study is that despite their low numbers in ovarian cancer tumors, CSC can be eradicated using CD133 targeted therapy and tumor growth can be inhibited.

*Methods.* Ovarian cancer cell lines were evaluated using flow cytometry for expression of CD133. In vitro viability studies with an anti-CD133 targeted toxin were performed on one of the cell lines, NIH:OVCAR5. The drug was tested in vivo using a stably transfected luciferase-expressing NIH:OVCAR5 subline in nude mice, so that tumor growth could be monitored by digital imaging in real time.

*Results.* Ovarian cancer cell lines showed 5.6% to 16.0% CD133 expression. dCD133KDEL inhibited the in vitro growth of NIH:OVCAR5 cells. Despite low numbers of CD133-expressing cells in the tumor population, intraperitoneal drug therapy caused a selective decrease in tumor progression in intraperitoneal NIH:OVCAR5-luc tumors.

*Conclusions.* Directly targeting CSC that are a major cause of drug resistant tumor relapse with an anti-CD133 targeted toxin shows promise for ovarian cancer therapy.

© 2013 Elsevier Inc. All rights reserved.

## Introduction

Although most women with ovarian cancer achieve complete remission with current treatment regimens, the majority of women relapse with chemoresistant disease [1,2]. Cancer stem cells (CSC) have been identified in many types of solid tumors as cells that are relatively quiescent, can self-renew, can grow as spheroids, and maintain the tumor by generating differentiated cells which make up the tumor bulk [3,4]. These characteristics, and the observation that CSC are resistant to conventional chemotherapeutics, suggest that CSC

*Abbreviations:* dCD133KDEL, deimmunized pseudomonas exotoxin fused to anti-CD133 scFv with a KDEL terminus; aa, amino acid; Ab, antibody; CD19, cluster of differentiation 19; CD45, cluster of differentiation 45; ER, endoplasmic reticulum; FITC, fluorescein isothiocyanate; KDEL, Lys-Asp-Glu-Leu; mAb, monoclonal antibody; PE, pseudomonas exotoxin; photons/s/cm$^2$/sr, photons per second per square centimeter per steradian; scFv, recombinant single chain VH and VL domain.

* Corresponding author at: Department of Laboratory Medicine and Pathology, University of Minnesota, MMC 609, 420 Delaware Street, S.E., Minneapolis, MN 55455, USA. Fax: +1 612 625 5622.
*E-mail address:* skubi002@umn.edu (A.P.N. Skubitz).

0090-8258/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ygyno.2013.05.027

may be the primary source of tumor recurrence. Thus, identification of strategies aimed at eliminating CSC in ovarian cancer could impact disease survival.

CSC markers vary depending upon the type of cancer studied and include: CD133, CD44, ALDH1, ALDH2, CD117, CD24, and ABCG2 [3,4]. In general, CSC comprise from 0.1 to 20% of the tumor [3]. Very few cancer cells that express these CSC markers are needed for a tumor to grow in vivo in NOD-SCID mice compared to tumor cells lacking CSC markers [4]. The quiescent nature of CSC allows them to resist standard chemotherapies which target rapidly proliferating cells. Furthermore, CSC have upregulated drug resistance genes [5] and express drug transporters, which allow CSC to "pump out" the chemotherapy [6].

CD133, a pentaspan membrane glycoprotein, has been identified as a CSC marker for various cancers [7,8]. Also known as prominin-1, CD133 was originally found on neuroepithelial stem cells in mice and later in human tissues [9]. The biological function of CD133 remains unclear, but it may be involved in primitive cell differentiation and epithelial–mesenchymal interactions [10–12]. Expression of CD133 in cancer-initiating cells is well documented for brain, prostate, colon, and breast cancers [13–17], and is indicative of a poor prognosis in many tumors [13,18]. Ovarian cancer cell lines and primary tumors have been characterized for the expression of CSC markers [5,19–29], with CD133 emerging as the most promising.

Targeted toxins serve as enzymatic inhibitors of protein synthesis [30] and represent a compelling alternative to conventional therapies since they synergize with chemotherapy [31–33]. Recently, we developed a monoclonal antibody (mAb) to a CD133 fusion protein that recognizes a non-glycosylated region of CD133 [34–36]. A derivative of the mAb was made [36] coupling the scFV from the mAb to a deimmunized PE-toxin using the endoplasmic reticulum retention sequence KDEL [37–39]. The toxin moiety of dCD133KDEL has been genetically deimmunized to permit multiple treatments with drug minimizing an anti-toxin response [40–46]. On a molecule/cell basis, targeted toxins are among the best killers of cancer cells when internalized [47]; CD133 serves as a highly internalized receptor when bound by ligand. We have shown that dCD133KDEL is reactive with CD133 + cells, is cytotoxic to cancer cell lines, and inhibits tumor growth in a mouse model system for head and neck cancer as well as breast cancer [48,49]. dCD133KDEL is the first such anti-CSC agent that shows remarkable anti-cancer effects despite the expression of CD133 in only a minority of the cancer cells.

In this paper, we study for the first time the ability of a CSC-directed drug to inhibit the growth and metastasis of human intraperitoneal ovarian carcinoma in vitro and in vivo.

## Materials and methods

### *Cell lines*

Human ovarian cancer lines NIH:OVCAR5, SKOV3, and A2780-s were obtained from Dr. Barbara Vanderhyden (Ottawa) [50,51], and MA148 was obtained from Dr. Ramakrishnan (University of Minnesota, Minneapolis) [52].

NIH:OVCAR5 cells were stably transfected with a vector containing the firefly luciferase (luc) gene, and a blastocidin resistance gene (Clontech Laboratories, Mountain View, CA), as previously described [49]. Transfection was initiated with Lipofectamine (Invitrogen, Carlsbad, CA) and stable clones were isolated using a FACS Diva flow cytometer (University of Minnesota Flow Cytometry Core Facility of the Masonic Cancer Center). NIH:OVCAR5-luc cells retained identical morphological and biological properties to the specific parental cell line and was maintained with additional 10 μg of blastocidin (InvivoGen, San Diego, CA).

### *mAb against CD133 (clone 7)*

Clone 7 was the first mAb produced against the CD133 protein backbone, whereby mice were immunized with a fusion protein consisting of the two extracellular domains of CD133 and not the intracellular domain, as we have previously described [36]. In earlier studies, we had affirmed that mAb clone 7 was reactive with CD133 by transfecting cells that did not express CD133 with human CD133, and then performing flow cytometry binding studies [36].

### *Flow cytometry*

Flow cytometry was performed using a FACSCaliber (University of Minnesota Flow Cytometry Core Facility). Cells were incubated with mAbs against CD133 (clone 7) [36], EGFR [53], the white blood cell marker CD45 (clone AHN-12; provided by Dr. Keith Skubitz, University of MN), and the B cell marker CD19 (clone BU-12) [54]. mAbs that are specific for CD45 and CD19 are used interchangeably as negative controls when performing flow cytometry experiments on carcinoma cell lines, since they both yield the same results. Data was analyzed using FLOWJO software (Tree Star Inc., Ashland, OR).

### *Construction and purification of dCD133KDEL*

The construction of dCD133KDEL from the fusion of the scFV portion of the clone 7 mAb [36] and a deimmunized, truncated form of pseudomonas exotoxin 38, and its purification, have been previously described [48,49]. Briefly, the protein was expressed and purified from inclusion bodies using a Novagen pET expression system (Novagen, Madison, WI). Protein was refolded and then purified using ion exchange chromatography followed by size exclusion chromatography. Purity was determined by SDS-PAGE stained with Coomassie Brilliant Blue.

### *Time course viability assays*

Trypan blue viability assays were performed by plating 10,000 NIH:OVCAR5 cells/well into 24-well plates as previously described [48,49]. dCD133KDEL, dCD19KDEL, and media were added at 1 nM and replaced daily. Cells were harvested from triplicate wells on days 2, 4, and 7 using trypsin and counted on a hemocytometer via trypan blue staining. Untreated wells typically became confluent around day 7 permitting the assessment of drug activity over time.

### *Mouse studies*

Athymic nude mice (nu/nu) were purchased from the National Cancer Institute (Frederick Cancer Research and Development Center, Animal Production Area), housed in accredited, specific pathogen-free facilities at the University of Minnesota, and cared for by the Department of Research Animal Resources. Five to eight week old mice were injected intraperitoneally (i.p.) with 0.1 to 1 million NIH:OVCAR5-luc cells in PBS or saline, as indicated, on day 0 to initiate tumors. On day 3, mice were injected i.p. with dCD133KDEL, and then again multiple times during the week, as indicated. In Experiment 1, the drug was tested in male mice because of their larger size and the parameters for i.p. dosing of male mice had been optimized in our model of metastatic breast cancer [49]. In Experiment 2, the drug was tested for the first time in female mice.

Tumor growth was measured weekly in a minimally invasive manner using bioluminescent imaging. Mice were lightly anesthetized with isoflurane gas and received 100 μl of a 30 mg/ml D-luciferin aqueous solution (Gold Biotechnology, St. Louis MO) i.p. 10 min before imaging to provide substrate for the luciferase enzyme. Images were captured using the Xenogen IVIS 100 imaging system and analyzed with Living Image 2.5 software (Xenogen Corporation, Hopkington,

Download English Version:

https://daneshyari.com/en/article/6184527

Download Persian Version:

https://daneshyari.com/article/6184527

Daneshyari.com