MOLECULAR ONCOLOGY 7 (2013) 944–954

available at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/molonc

Double-deleted vaccinia virus in virotherapy for refractory and metastatic pediatric solid tumors

Xueqing Lun[a,1], *Yibing Ruan*[a,1], *Aarthi Jayanthan*[a], *David J. Liu*[a], *Anjali Singh*[a], *Tanya Trippett*[a,b], *John Bell*[c], *Peter Forsyth*[d], *Randal N. Johnston*[e], *Aru Narendran*[a,*]

[a]*Pediatric Oncology Experimental Therapeutics Investigators Consortium (POETIC) Laboratory for Pre-Clinical and Drug Discovery Studies, University of Calgary, Canada and Division of Pediatric Oncology, Alberta Children's Hospital, Calgary, Alberta, Canada*

[b]*Memorial Sloan-Kettering Cancer Center, New York, NY, USA*

[c]*Ottawa Regional Cancer Centre Research Laboratories, Ottawa, Ontario, Canada*

[d]*Moffitt Cancer Center & Research Institute and University of Southern Florida, Tampa, FL, USA*

[e]*Department of Biochemistry & Molecular Biology, Faculty of Medicine, University of Calgary, Alberta, Canada*

ARTICLE INFO

Article history:
Received 14 May 2013
Received in revised form
27 May 2013
Accepted 28 May 2013
Available online 14 June 2013

Keywords:
Oncolytic virus
Double-deleted vaccinia virus
Pediatric tumors
Sarcoma
Neuroblastoma
Atypical teratoid rhabdoid tumors (AT/RT)

ABSTRACT

Background: Previous studies have shown successful antitumor effects of systemically delivered double-deleted vaccinia virus (vvDD) against a number of adult tumor models, including glioma, colon and ovarian cancers. The purpose of this study was to investigate the oncolytic potential of vvDD against a panel of cell lines representative of pediatric solid tumors that are currently difficult to cure.

Methods: Cell lines derived from central nervous system atypical teratoid rhabdoid tumor (AT/RT) (BT12, BT16 and KCCF1), sarcoma (143B, HOS, RD and RH30), and neuroblastoma (SKNAS, SKNBE2, IMR-5 and IMR-32) were examined for vvDD mediated cytotoxicity defined by virus expansion followed by loss of tumor cell viability. The normal human fibroblast cell line HS68 was used as a control. Next, relevant orthotopic, subcutaneous and lung metastasis xenograft models were treated with intravenous doses of live vvDD or killed virus controls (DV). Tumor growth inhibition and viral replication were quantified and survival outcomes of these animals were assessed.

Results: vvDD was able to infect and kill nine of eleven of the pediatric tumor cells (81.8%) *in vitro*. In xenograft models, intravenous administration of a single dose of vvDD significantly inhibited the growth of tumors and prolonged the survival of intracranial and metastatic tumors.

Conclusions: Oncolytic vvDD administered i.v. shows activity in preclinical models of pediatric malignancies that are resistant to many currently available treatments. Our data support further evaluation of vvDD virotherapy for refractory pediatric solid tumors.

* *Corresponding author*. Division of Pediatric Oncology, Alberta Children's Hospital, 2888 Shaganappi Trail NW, Calgary, Alberta, Canada T3B 6A8. Tel.: +1 403 210 6418; fax: +1 403 955 2645.
E-mail address: a.narendran@ucalgary.ca (A. Narendran).

[1] Contributed equally to the final manuscript.

1574-7891/$ – see front matter © 2013 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.molonc.2013.05.004

1. Introduction

Recent decades have seen significant advances in the overall survival rates of children with cancer. However, improving cure rates in several intractable pediatric solid tumors, particularly high risk neuroblastoma, infant brain tumors and metastatic sarcomas, still remains a challenge, with exceptionally high rates of failure under current treatment modalities. The escalation of conventional chemotherapeutic drugs and their combinations to induce clinical remission often leads to unacceptable toxicities. Consequently, such relapsed or refractory malignancies have become a leading cause of morbidity and mortality in children and young adults, requiring the search for differing therapeutic approaches (Macy et al., 2008).

A number of recent studies have provided evidence for the utility of oncolytic viruses (OVs) as means to deliver effective cytotoxic therapy to tumors that have become resistant to conventional treatment approaches (Russell et al., 2012; Bourke et al., 2011; Hammill et al., 2010). The strategy behind this approach is to develop viruses that can replicate specifically in cancer cells and cause cell lysis, while leaving non-malignant cells unaffected. The ability of OVs to effectively spread and reach distant invasive metastatic tumor cells may further aid in their application in patients with highly aggressive tumor phenotypes. Recently completed clinical trials of several different OVs (adenovirus, reovirus, measles, herpes simplex, Newcastle disease virus, vaccinia virus and parvovirus) have demonstrated acceptable safety and tolerability of OVs in patients (Hammill et al., 2010; Donnelly et al., 2012). However, there are no substantive preclinical data on the expediency of OVs in pediatric tumors (Morton et al., 2010; Friedman et al., 2009; Studebaker et al., 2010). Although limited results have been observed in preclinical models of pediatric cancers, their translation into clinical trials has not occurred. Hence, research studies are needed to address the applicability of OVs to the unmet needs in refractory pediatric tumors, while drawing on the rapid advances in the field of viral oncolytics in adult trials to achieve practical benefits for these children in a timely manner.

Previously, we have described preclinical data using distinct xenograft models to study the utility of OVs such as Myxoma Virus (MYXV) and vesicular stomatitis virus (VSV, deltaM51) for activity against brain tumors (Lun et al., 2005, 2009, 2010, 2009; Alain et al., 2010, 2010; Wu et al., 2008). However, the double deleted vaccinia virus (vvDD) provides unique benefits and has been explored in the current study for therapeutic potential against refractory pediatric solid tumors. Vaccinia virus (VV) is a double-stranded, enveloped, lytic DNA virus with several desirable attributes as a therapeutic agent over other OVs in development: 1. The clinical safety profile of the virus has been established from its accepted use as a vaccine in the smallpox eradication program. 2. Its contraindications and adverse reactions have been well identified and effective antivirals are available. 3. Previous studies have shown that VV is amenable for systemic (intravenous) delivery to distant tumors, making it an attractive agent for the treatment of pediatric tumors with metastases. Furthermore, there is significant evidence for its potential as an effective oncolytic agent from different preclinical studies in a number of adult tumor models (Lun et al., 2009, 2010; He et al., 2012; Haddad et al., 2012; Merrick et al., 2009). This has led to the initiation of early phase clinical trials using VV (JX-594) against adult patients (Donnelly et al., 2012; Park et al., 2008). The generation and use of a ''double-deleted'' version of the Western Reserve (WR) strain [double-deleted VV (vvDD)] with deletions of the thymidine kinase and vaccinia growth factor genes further enhances the safety profile of this agent (Park et al., 2008). In addition, vvDD was demonstrated to be nontoxic following i.v. delivery in nonhuman primates (Naik et al., 2006).

Based on the feasibility data provided by adult tumor studies (He et al., 2012; Haddad et al., 2012; Merrick et al., 2009; Park et al., 2008), we wanted to investigate the activity of vvDD against cell lines from refractory pediatric solid tumors. The objectives of this study were to determine the susceptibility and efficacy of vvDD against pediatric solid tumors *in vitro* and *in vivo*. Here we show for the first time that diverse pediatric solid tumor cell lines are susceptible to infection and cytotoxicity by vvDD *in vitro*, and single i.v. administration of the vvDD is capable of significantly inhibiting tumor growth in tumor xenograft models. We also demonstrate that systemic administration of vvDD in tumor bearing immunocompromised mice is well tolerated. The results of this study suggest that further investigation of this virus in the treatment of pediatric solid cancers is warranted.

2. Materials and methods

2.1. Cell lines and cell culture

BT12 and BT16 cell lines were established from infants with atypical teratoid/rhabdoid tumors or the central nervous system (CNS AT/RT) and generously provided by Drs. Peter Houghton (Nationwide Children's Hospital, Columbus, OH) and Jaclyn Biegel (Children's Hospital of Philadelphia, PA). The cell line KCCF1 was established in our laboratory from the cerebral spinal fluid (CSF) cells of a two-month-old male infant with AT/RT. Characterization of this cell line has been described previously (Narendran et al., 2008). The HS68 primary skin fibroblast cells were provided by Dr. Peter Forsyth's laboratory at the University of Calgary. The neuroblastoma cell lines were provided by Dr. Herman Yeger (The Hospital for Sick Children, Toronto, ON). The remaining cell lines were obtained from ATCC. All cell lines were routinely tested before animal studies for mycoplasma contamination.

Cell lines were cultured in Opti-MEM medium (Gibco, Invitrogen Corporation, Burlington, ON) containing 5% fetal bovine serum (FBS, Gibco), 100 units/ml penicillin and 100 units/ml streptomycin (Gibco). Cells were trypsinized with 0.25% Trypsin-EDTA in Ca^{2+} and Mg^{2+} free balanced salt solution (Gibco) every three to five days and maintained in incubators at 37 °C in a humidified atmosphere with 5% CO_2.

2.2. Double-deleted vaccinia virus (vvDD)

A mutant attenuated "double-deleted" version of the Western Reserve strain [double-deleted vaccinia virus (vvDD)] with