NEOPLASIA
www.neoplasia.com

Volume 20 Number xx pp. 524–532 524

Efficacy of ONC201 in Desmoplastic Small Round Cell Tumor[1]

Andrea A. Hayes-Jordan[*], Xiao Ma[*], Brian A. Menegaz[†], Salah-Eddine Lamhamedi-Cherradi[†], Charles V. Kingsley[‡], Jalen A. Benson[*], Pamela E. Camacho[§], Joseph A. Ludwig[¶], Cynthia R. Lockworth[#], Gloria E. Garcia[#] and Suzanne L. Craig[#]

[*]Division of Surgical Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit, 1484, Houston, TX, USA; [†]Division of Sarcoma Medical Oncology-Research, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit, 1952, Houston, TX, USA; [‡]Department of Imaging Physics-Research, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit, 1902, Houston, TX, USA; [§]Department of Pediatrics-Patient Care, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit, 0087, Houston, TX, USA; [¶]Department of Sarcoma Medical Oncology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit, 0450, Houston, TX, USA; [#]Department of Veterinary Medicine & Surgery, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit, 0063, Houston, TX, USA

Abstract

Desmoplastic Small Round Cell Tumor (DSRCT) is a rare sarcoma tumor of adolescence and young adulthood, which harbors a recurrent chromosomal translocation between the Ewing's sarcoma gene (EWSR1) and the Wilms' tumor suppressor gene (WT1). Patients usually develop multiple abdominal tumors with liver and lymph node metastasis developing later. Survival is poor using a multimodal therapy that includes chemotherapy, radiation and surgical resection, new therapies are needed for better management of DSRCT. Triggering cell apoptosis is the scientific rationale of many cancer therapies. Here, we characterized for the first time the expression of pro-apoptotic receptors, tumor necrosis-related apoptosis-inducing ligand receptors (TRAILR1-4) within an established human DSRCT cell line and clinical samples. The molecular induction of TRAIL-mediated apoptosis using agonistic small molecule, ONC201 in vitro cell-based proliferation assay and in vivo novel orthotopic xenograft animal models of DSRCT, was able to inhibit cell proliferation that was associated with caspase activation, and tumor growth, indicating that a cell-based delivery of an apoptosis-inducing factor could be relevant therapeutic agent to control DSRCT.

Neoplasia (2018) 20, 524–532

Introduction

Desmoplastic Small Round Cell tumor (DSRCT) first described by Gerald and Rosai, is a rare poorly differentiated sarcoma cell of childhood and adolescence that has distinctive histologic, genetic and clinical features [1–3]. DSRCT is characterized as a small round blue cells, with fibrosclerotic stroma and abundant extracellular material co-expressing epithelial, mesenchymal, myogenic and neural bio-

Abbreviations: DR4/DR5, Death Receptors; DSRCT, Desmoplastic Small Round Cell Tumor; TRAIL, TNF-related apoptosis inducing ligand.

Address all correspondence to: Andrea A. Hayes-Jordan, University of Texas MD Anderson Cancer Center, Department of Surgical Oncology, 1400 Pressler St., Unit 1484, Houston, TX 77030. E-mail: ahjordan90@gmail.com

[1] This work was supported in part by MD Anderson's Cancer Center Support (Core) Grant, NIH/NCI P30 CA016672.
Received 24 August 2017; Revised 13 February 2018; Accepted 19 February 2018

1476-5586
https://doi.org/10.1016/j.neo.2018.02.006

markers [4]. This disease that harbors a t(11;22) translocation involving a fusion of the EWSR1 gene on chromosome 22 with the WT1 gene on chromosome 11, occurs primarily in male young patients as between 12 and 28 years and presents a pervasive peritoneal primary tumor spreading with metastases to the liver, lung and lymph nodes [1,4,5].

DSRCT patients were initially treated like Ewing sarcoma (ES) with similar multimodality intensive chemotherapy regimens and was considered to correlate with a good prognosis within a short following up [6–8]. However, relapses were acknowledged in the majority of patients after longer following up. The combination treatment strategies including this multimodal intensive chemotherapy, aggressive surgical resection, and radiation therapy that are now considered as the standard care for DSRCT patients with primary disease, can improve a survival benefit, but the overall prognosis remains poor [9–13].

Since this tumor is only recently described, and there are no commercial cell lines, little new information is available. Therefore, further insight into DSRCT tumor inhibition like triggering cell apoptosis may lead to the generation of beneficial specific targeted therapies.

Apoptosis can be induced by TNF-related apoptosis inducing ligand (TRAIL), a pro-apoptotic cytokine from the TNF superfamily. TRAIL can bind to five receptors, two death receptors (DR4 and DR5) triggering TRAIL-induced apoptosis, two decoy receptors that possibly inhibits this cell pathway, and one soluble inhibitor of RANK ligand (osteoprotegrin) at low affinity [12–14].

In the current investigation, we hypothesize that death receptor dependent apoptosis is a mechanism for cell death in DSRCT cells. Apoptosis through the activation of death receptor 4 and 5 has been found to be a promising candidate for the development of new therapeutic approach in the treatment of the most frequent malignant primary bone tumors [15–18], but has not been described for this type of sarcoma.

ONC201 is a novel drug not yet tested in sarcomas. ONC201 was discovered as a p53-independent inducer of TRAIL gene transcription and had an anti-proliferative and anti-apoptotic effects against a broad range of tumor cells [19–22]. The mechanism of action of ONC201 engages PERK-independent activation of the integrated stress pathway, leading to upregulation of DR5 and TRAIL in tumors [23,24]. The mechanisms of the stress response induced differ between hematological malignancies that prompt an atypical integrated stress responses (ISR) associated with p53-independent apoptosis and solid tumors that trigger an ISR dependent on ATF4 activation by specific eIF2a kinases[21,23,25]. A Synergistic anti-tumor effect was observed with the joined inhibition of Bcl-2 with ABT-263/ABT-199 and Mcl-1 with ONC201 (through Bag3/UspX inhibition) through the mitochondrial pathway of apoptosis involving caspase activation and PARP-cleavage [23,26].

Here, we demonstrate for the first time the efficacy of ONC201 and its anti-neoplastic mechanism in a new preclinical model of DSRCT, within in vitro cell line culture and in vivo xenograft animal model.

Materials and Methods

Cell lines and Antibodies

JN-DSRCT-1 and luciferase-transfected human JN-DSRCT-1 cell lines exhibiting a pathognomonic t(11;22)(p13;q12) translocation was generously provided from Dr. M Kikuchi's laboratory (Fukuoka University, Fukuoka, Japan) [27]. Cell line was banked in multiple aliquots on receipt to reduce risk of phenotypic drift and maintained in DMEM medium (Mediatech) containing 10% (v/v) FBS (Gemini Bio-Products) and antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin, Mediatech) in a humidified incubator at 37°C and in 5% CO2 atmosphere. Antibodies used were PARP, caspase 3 and β-actin from Cell Signaling Technology.

Preparation of Drug Solutions

For in vitro utilization, ONC201 (Oncoceutics, Inc., 3624 Market Street, University City Science Center, Suite 5E, Philadelphia, PA 19104) stock solution (10 mmol/L) was prepared in DMSO and stored at -20°C. For in vivo application, the stock solution of ONC201 (25 mg/ml) was prepared for an oral administration with either low dose (50 mg/kg) or high dose (100 mg/kg) in 1X PBS.

In Vitro Cell Viability Assay

Cell viability was measured using an MTT assay. JN-DSRCT-1 cells were cultures until the log-phase and were subsequently seeded into a 96-well plate at a density of 10^4 cells/well overnight to treatment with different concentrations of ONC201 (0.625-20 µM) or DMSO. Following an incubation of 72 h, the cells were then incubated with medium containing MTT for 4 h and the formazan crystals were dissolved with 150 µl DMSO. The plates were incubated on a shaker for 15 minutes at room temperature and the absorbance was measured at 490nm using a microplate reader (DTX880; Beckman Coulter). The cytotoxicity of the ONC201 was expressed either as percentage cell viability or as ratio of treated/DMSO. IC_{50} values were calculated by sigmoidal dose-response curve fit using Prism GraphPad 6.0.

Colony Formation Assays

The colony formation assays were conducted in 6-well plates with 200 JN-DSRCT-1 cells seeded per well; and 24 hours later, cells were exposed to variable concentration of ONC201, followed by growth in media for 2 weeks, to allow colony growth. Colonies were fixed with methanol, stained with crystal violet, and counted.

Flow Cytometry

JN-DSRCT-1 cells were analyzed for their TRAIL-receptors cell surface expression. The cells were dissociated with dissociation buffer and stained with PE-DcR1 (BD-Biosciences), APC-DcR2 (BD-Biosciences), PE-DR4 (BD-Biosciences) and APC-DR5 (BD-Biosciences). Cell stained were acquired using a FACSCanto II flow cytometer (BD-Biosciences) and analyzed using FlowJo software program 10.0.6 (Tree Star).

Immunofluorescent Microscopy

JN-DSRCT-1 cells were cultured on glass coverslips for overnight, and permeabilized with 0.2% Triton X-100 in PBS for 30 min at room temperature. After washing with PBS, the cells were incubated overnight at 4°C with antibodies to DR5. After washing, the cells were with the secondary antibody for 2 hours at room in dark and humidified chamber. The immuno-stained cells were mounted in mounting medium containing 4', 6-diamidino-2-phenylindole (DAPI) for 5 minutes, and washed with PBS. The cells were then visualized under a fluorescence microscope equipped with camera.

Protein Isolation and Western Blot Analysis

The preparation of extract protein from cells for western blotting were prepared by using lysis buffer containing freshly added protease and phosphatase inhibitors via cold incubation. The total lysed proteins were collected after centrifugation, quantified using BCA protein assay kit (Thermo Fisher Scientific), and stored at -80°C until