International Journal of Radiation Oncology biology • physics

www.redjournal.org

Biology Contribution

# Intranuclear Delivery of a Novel Antibody-Derived Radiosensitizer Targeting the DNA-Dependent Protein Kinase Catalytic Subunit

**Hairong Xiong, M.D., Ph.D.,[*,†] Robert J. Lee, Ph.D.,[‡] Eric B. Haura, M.D.,[§] John G. Edwards, S.M.,[¶] William S. Dynan, Ph.D.,[*] and Shuyi Li, M.D., Ph.D.[*,¶]**

**Institute of Molecular Medicine and Genetics, Georgia Health Sciences University, Augusta, GA; †State Key Laboratory of Virology, Institute of Medical Virology, Wuhan University School of Medicine, Wuhan, China; ‡Division of Pharmaceutics, College of Pharmacy, Ohio State University, Columbus, OH; §Thoracic Oncology and Experimental Therapeutics Programs, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; and ¶Apeliotus Technologies, Inc., Atlanta, GA*

Received Jun 26, 2011, and in revised form Aug 11, 2011. Accepted for publication Aug 26, 2011

**Summary**

DNA repair enzymes are attractive targets for the development of cancer therapeutics. Previous studies have identified a single-chain antibody variable fragment (ScFv 18-2) that binds to DNA-PK, a key regulator of nonhomologous end-joining repair. Although the properties of ScFv 18-2 suggest that it might be useful as a radiosensitizer, a method for delivery to intracellular sites of DNA repair has been lacking. This study describes a delivery method based on folate receptor–mediated endocytosis. Folate-conjugated ScFv 18-2 is taken up by tumor cells

**Purpose:** To inhibit DNA double-strand break repair in tumor cells by delivery of a single-chain antibody variable region fragment (ScFv 18-2) to the cell nucleus. ScFv 18-2 binds to a regulatory region of the DNA-dependent protein kinase (DNA-PK), an essential enzyme in the nonhomologous end-joining pathway, and inhibits DNA end-joining in a cell-free system and when microinjected into single cells. Development as a radiosensitizer has been limited by the lack of a method for intranuclear delivery to target cells. We investigated a delivery method based on folate receptor–mediated endocytosis.

**Methods and Materials:** A recombinant ScFv 18-2 derivative was conjugated to folate via a scissile disulfide linker. Folate-ScFv 18-2 was characterized for its ability to be internalized by tumor cells and to influence the behavior of ionizing radiation–induced repair foci. Radiosensitization was measured in a clonogenic survival assay. Survival curves were fitted to a linear-quadratic model, and between-group differences were evaluated by an $F$ test. Sensitization ratios were determined based on mean inhibitory dose.

**Results:** Human KB and NCI-H292 lung cancer cells treated with folate-conjugated ScFv 18-2 showed significant radiosensitization ($p < 0.001$). Sensitization enhancement ratios were $1.92 \pm 0.42$ for KB cells and $1.63 \pm 0.13$ for NCI-H292 cells. Studies suggest that treatment inhibits repair of radiation-induced DSBs, as evidenced by the persistence of γ-H2AX-stained foci and by inhibition of staining with anti-DNA-PKcs phosphoserine 2056.

**Conclusions:** Folate-mediated endocytosis is an effective method for intranuclear delivery of an antibody-derived DNA repair inhibitor. © 2012 Elsevier Inc.

**Keywords:** Radiosensitization, scFv, DNA-dependent protein kinase, Folate-mediated delivery, Nonhomologous end joining, DNA repair

Reprint requests to: Dr. Shuyi Li, M.D., Ph.D., IMMAG CA-3010, Georgia Health Sciences University, 1120 15th Street, Augusta GA 30912. Tel: (706) 721-1671; Fax: (706) 721-8752; E-mail: sli@georgiahealth.edu

Supported by PHS grants CA 98239 and EY 018244, by a Georgia Research Alliance Venture Lab award, and by Apeliotus Technologies, Inc.

Conflict of interest: W.S.D. served as a consultant to Apeliotus Technologies, Inc. The authors report no other conflict of interest.

Int J Radiation Oncol Biol Phys, Vol. 83, No. 3, pp. 1023–1030, 2012
0360-3016/$ - see front matter 
doi:10.1016/j.ijrobp.2011.08.039

*in vitro*, localizes to the nucleus, and sensitizes cells to radiation in clonogenic survival assays. Further development of ScFv 18-2 as a radiosensitizer could affect the treatment of cancers where radiotherapy is a major treatment modality, including inoperable non−small cell lung cancer or head-and-neck squamous cell cancer. Folate-mediated delivery of single-chain antibodies is a general approach that could potentially be extended to other repair enzymes.

## Introduction

The DNA-dependent protein kinase (DNA-PK) regulates nonhomologous end joining, the main pathway for repair of DNA double-strand breaks (DSBs) induced by clinically relevant doses of ionizing radiation. Loss of DNA-PKcs function results in radiosensitization (1). Conversely, increased levels of DNA-PK activity correlate with tumor cell radioresistance (2). DNA-PK inhibitors are thus potential radiosensitizers (3).

We previously described a single-chain antibody variable fragment (ScFv 18-2) that binds to the DNA-PK catalytic subunit (DNA-PKcs) and inhibits DSB repair activity in a cell-free system (4). Microinjection of ScFv 18-2 into single human cells sensitizes them to an otherwise sublethal (1.5 Gy) dose of radiation (4).

The properties of ScFv 18-2 suggest that it might be useful as a radiosensitizer. Therapeutic agents based on antibodies and antibody fragments are widely used but are generally directed against molecules present on the exterior surface of tumor cells. By contrast, DNA-PKcs is separated from the extracellular milieu by the plasma membrane and the nuclear envelope. Prior work has shown that transferrin and folate receptors can be used to deliver proteins and nanoparticles into tumor cells (5, 6). Receptor-mediated delivery is attractive because of its demonstrated potential for clinical translation (6). We hypothesized that receptor-mediated endocytosis might serve as an effective means for delivery of ScFv 18-2 as a radiosensitizer. We describe here the synthesis and characterization of a folate-conjugated ScFv 18-2 derivative. Folate-ScFv 18-2 is internalized by human cancer cells, enters the nucleus, and sensitizes cells to ionizing radiation.

## Methods and Materials

### ScFv expression, purification, and conjugation

ScFv 18-2 was derived from the anti−DNA-PKcs monoclonal antibody, mAb 18-2 (7). The maltose binding protein (MBP)-ScFv 18-2 NLS LC2 derivative (8) or ovalbumin control protein was incubated with 10- to 20-fold molar excess of Traut's reagent (2-iminothilane HCl, Pierce Biotechnology, Rockford, IL) (9). Products were isolated by PD-10 desalting chromatography (GE Healthcare, Piscataway, NJ), reacted with a 10-fold excess of folate-hydrazido-(2-pyridyldithiopropionate) (folate-SS-Pyr) for 1 hour at room temperature (Fig. 1a), then desalted again.

Alternatively, folate-*N*-hydroxy succinimide (folate-NHS) (10) was coupled to the N-terminus of an endosome-disruptive hemagglutinin (HA) peptide (GLFGAIAGFIENGWEGMIDGC). The product was separated by Sephadex G-15 chromatography (GE Healthcare). ScFv or ovalbumin was activated with succinimidyl-3-(2-pyridyldithiopropionate) (SPDP, Thermo Scientific, Rockford, IL) and incubated with fourfold excess of folate-HA, and the conjugates were separated by Sephadex G-50 chromatography (GE Healthcare). Peptide enzyme-linked immunosorbent assay was performed as described (4) using Peptide A (DNA-PKcs residues 2001−2025, biotin-KKKYIEIRKEAREAANGDSDGPSYM), or Peptide C (DNA-PKcs residues 2031−2055, biotin-LADSTLSEEMSQFDFSTGVQSYSYS).

### Cell culture, flow cytometry, and imaging

KB cells (ATCC # CCL-17) were grown in folate-free RPMI 1640 medium with 10% fetal bovine serum. The KB cell line was originally thought to be derived from an oral carcinoma but was subsequently found to have been established via HeLa cell contamination. NCI-H292 (ATCC # CRL-1848) mucoid epidermoid non−small-cell lung cancer (NSCLC) cells were grown in RPMI 1640 medium with 10% fetal bovine serum. For flow cytometry, cells were fixed with 2% paraformaldehyde, blocked with 2% BSA, and incubated with anti−folate receptor mAb (Mov18/ZEL, 1:200, Alexis Biochemicals, San Diego, CA) and FITC-goat anti-mouse immunoglobulin $F(ab)_2$ (DAKO Cytomation Glostrup, Demark). FITC-ScFv 18-2 NLS was prepared by incubating ScFv 18-2 with fivefold excess FITC (Invitrogen) at pH 8.0 for 30 minutes at 37°C followed by PD-10 chromatography.