NEOPLASIA
www.neoplasia.com

Volume 15 Number 7 July 2013 pp. 684–693 684

Targeting Cathepsin E in Pancreatic Cancer by a Small Molecule Allows *In Vivo* Detection[1,2]

Edmund J. Keliher*,[3], Thomas Reiner*,†,[3], Sarah Earley*, Jenna Klubnick*, Carlos Tassa*, Andrew J. Lee‡, Sridhar Ramaswamy‡, Nabeel Bardeesy‡, Douglas Hanahan§, Ronald A. DePinho¶, Cesar M. Castro*,‡ and Ralph Weissleder*,‡,#

*Center for Systems Biology, Massachusetts General Hospital, Boston, MA; †Department of Radiology, Memorial Sloan-Kettering Cancer Center, New York, NY; ‡Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA; §Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland; ¶MD Anderson Cancer Center, Houston, TX; #Department of Systems Biology, Harvard Medical School, Boston, MA

Abstract

When resectable, invasive pancreatic ductal adenocarcinoma (PDAC) is most commonly treated with surgery and radiochemotherapy. Given the intricate local anatomy and locoregional mode of dissemination, achieving clean surgical margins can be a significant challenge. On the basis of observations that cathepsin E (CTSE) is overexpressed in PDAC and that an United States Food and Drug Administration (FDA)-approved protease inhibitor has high affinity for CTSE, we have developed a CTSE optical imaging agent [ritonavir tetramethyl-BODIPY (RIT-TMB)] for potential intraoperative use. We show nanomolar affinity [half maximal inhibitory concentration (IC_{50}) of 39.9 ± 1.2 nM] against CTSE of the RIT-TMB in biochemical assays and intracellular accumulation and target-to-background ratios that allow specific delineation of individual cancer cells. This approach should be useful for more refined surgical staging, planning, and resection with curative intent.

Neoplasia (2013) 15, 684–693

Introduction

The incidence of pancreatic ductal adenocarcinoma (PDAC) has increased during the past several decades and now ranks as the fourth leading cause of cancer death in the United States. Patients usually present late in the disease process with symptoms of weight loss, pain, and/or jaundice. While improvements in imaging technology have generally increased the spatial resolution of scans as well as interventional capabilities, these technologies rarely allow detection of the small lesions without anatomic abnormalities or precursor lesions (pancreatic intraepithelial neoplasia). ^{18}F-fluorodeoxyglucose-positron emission tomography imaging is generally considered less sensitive since many cancers do not show increased glucose uptake [1,2] or are simply too small, i.e., below the detection threshold. As a result, patients often present with advanced stage disease when the decision to surgically resect is not straightforward. Moreover, the incidence of recurrent disease following surgical resection remains high, which underscores the need for improved detection of residual disease. Unfortunately, there are currently no reliable intraoperative imaging approaches to delineate microscopically invading cancers or to distinguish malignancy from inflammation for more accurate mapping and detection of cancer spread. In short, there is an urgent need for

Abbreviations: PDAC, pancreatic ductal adenocarcinoma; CTSE, cathepsin E; SPR, surface plasmon resonance; RIT, ritonavir; RIT-TMB, ritonavir tetramethyl-BODIPY

Address all correspondence to: Ralph Weissleder, MD, PhD, Center for Systems Biology, Massachusetts General Hospital, 185 Cambridge St, CPZN 5206, Boston, MA 02114. E-mail: rweissleder@mgh.harvard.edu

[1]This work was supported in part by a grant from the Lustgarten Foundation for Pancreatic Cancer Research and by grants from the National Institute of Health (2P01CA117969, 2P50CA086355, and PO1-AI54904).

[2]This article refers to supplementary materials, which are designated by Figures W1 to W5 and are available online at www.neoplasia.com.

[3]These authors contributed equally.

Received 18 January 2013; Revised 12 April 2013; Accepted 15 April 2013

DOI 10.1593/neo.13276

molecularly targeted imaging agents to improve the detection of this highly lethal disease.

Cathepsin E (CTSE) has emerged as a promising PDAC target from different lines of research. The Hanahan group demonstrated overexpression of CTSE in the ripTag model and subsequently confirmed high levels in the K-ras mouse model. Here, it was shown that 3.5% of the total protein within PDAC lysates consisted of CTSE protein (unpublished). Second, a meta-analysis by the Ramaswamy laboratory, employing whole-genome transcriptional arrays to analyze more than 500 samples (including primary human tumors, cell line screens, mouse model analysis, and metasearch), identified CTSE as a prime target. Finally, there are literature reports pointing to CTSE as a target both recently [3–6] and even dating back to the early 1990s [7–11]. There are several features that make CTSE a unique imaging target: 1) it exists only intracellularly and is not secreted, 2) it exists in a pro-form and is not proteolytically active (thus is not efficiently targeted by activity-based probes or substrates except for perhaps a small fraction of active enzyme), and 3) enzyme activity in live cells is not inhibitable by classic pan-cathepsin inhibitors. Furthermore, unlike other cathepsins (B, D, S, K) that can show high stromal levels [12–16], CTSE overexpression appears to be unique to PDAC tissue. Additionally, CTSE is expressed in the earliest forms of pancreatic intraepithelial neoplasia [3,6]. Interestingly, the United States Food and Drug Administration (FDA)-approved human immunodeficiency virus (HIV) protease inhibitor ritonavir (RIT; Figure 1*B*) has been shown to have a reasonable co-affinity for CTSE [17]. We hypothesized that this small molecule drug scaffold could be modified to yield high affinity imaging agents for intraoperative optical imaging and with appropriate pharmacokinetics for *in vivo* use.

In this research, we systemically analyzed different RIT variants and conjugation points, linkers to modulate hydrophilicity, imaging reporters, and labeling strategies. These compounds were screened against CTSE protein using surface plasmon resonance (SPR) analysis. We discovered that one particular compound [RIT–tetramethyl-BODIPY (TMB)] had low nM affinity, was cell permeable, and was fluorescent. Here, we show that this compound is selective for PDAC in human tissue samples and allows *in vivo* imaging of PDAC in mouse models.

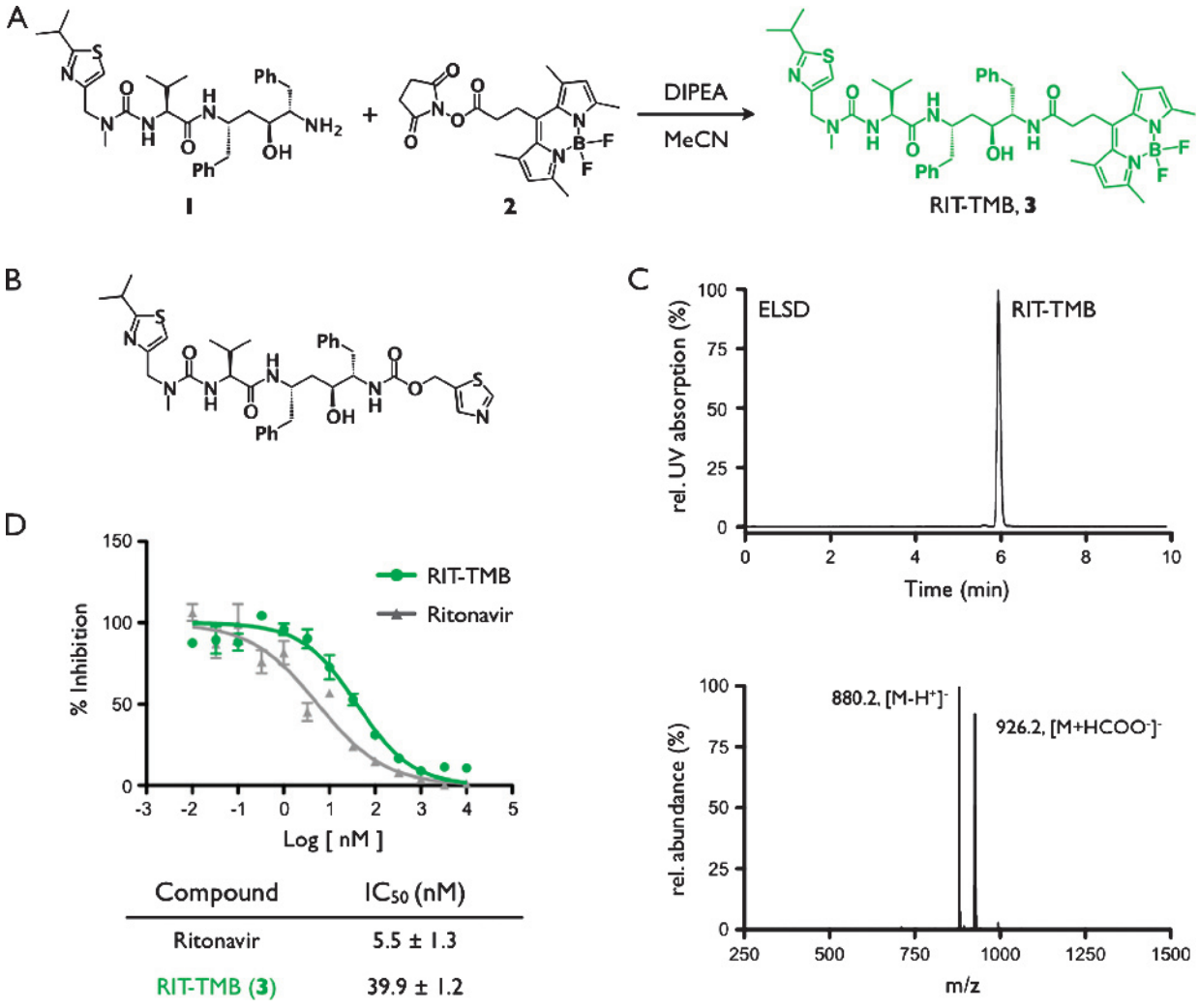

Figure 1. RIT-TMB synthesis and characterization. (A) Synthesis of RIT-TMB (**3**). (B) Chemical structure of RIT, P2′ thiazole on the right side of the molecule. (C) HPLC chromatogram and LC-MS analysis of purified RIT-TMB. (D) Binding affinity data for RIT parent compound and RIT-TMB against human CTSE.