Biochemical and Biophysical Research Communications xxx (2018) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Met receptor inhibitor SU11274 localizes in the endoplasmic reticulum

Edwin J. Wiest [a, b], Heather Jensen Smith [b], Michael A. Hollingsworth [a, b, *]

[a] Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA
[b] Eppley Institute, Fred and Pamela Buffet Cancer Center, University of Nebraska Medical Center, Omaha, NE, USA

ARTICLE INFO

Article history:
Received 23 April 2018
Accepted 4 May 2018
Available online xxx

Keywords:
SU11274
Endoplasmic reticulum
Sulfonylurea receptor
Unfolded protein response

ABSTRACT

We discovered that SU11274, a class I c-Met inhibitor, fluoresces when excited by 488 nm laser light and showed rapid specific accumulation in distinct subcellular compartments. Given that SU11274 reduces cancer cell viability, we exploited these newly identified spectral properties to determine SU11274 intracellular distribution and accumulation in human pancreatic cancer cells. The aim of the studies reported here was to identify organelle(s) to which SU11274 is trafficked. We conclude that SU11274 rapidly and predominantly accumulates in the endoplasmic reticulum.

1. Introduction

In the course of examining activities of Met, a receptor tyrosine kinase that transduces signals from the extracellular space to the cell interior when its ligand, hepatocyte growth factor (HGF) binds to its extracellular domain [1], we noted that the small molecule inhibitor SU11274, a pyrrole indolinone compound first described in 2003 [2], showed unexpected properties of fluorescence and cellular uptake into specific organelles. SU11274 is reported to inhibit the Met receptor activation with an IC50 of 10 nM as determined in a cell-free assay [3], due to the its ability to bind tightly to the ATP pocket of the Met receptor [2]; however, rapid accumulation of this molecule in specific subcellular compartments has not been previously reported.

2. Materials and methods

2.1. Cell culture and treatment conditions

S2.013.MUC1F pancreatic cancer cells, derived from a liver metastasis, were cultured in DMEM containing 5% FBS at 37 °C and 5% CO_2 [4]. Cells were grown to 80% confluency prior to experimentation. For live cell experiments, SU11274 (Selleckchem) was applied to S2.013.MUC1F cells at 0.1, 1, and 10 μM (see below). For fixed cell experiments, 2 μM SU11274 was applied for 30 min before cells were fixed for organelle-specific labeling, as described below.

2.2. Organelle labeling in fixed and live cells

Cells were fixed (4% PFA in PBS) and subsequently permeabilized (0.15% Triton-X 100,1% BSA in PBS) prior to primary antibody staining (see below) for 2 h at 4 °C. Cells were subsequently stained with primary antibodies to detect specific intracellular organelles: anti-calreticulin Alexa Fluor 647 (endoplasmic reticulum, 1:100 v/v, Abcam), anti-Lamp1 (lysosomes, 1:1000 μg/μL, Abcam), anti-EEA1 Alexa Fluor 647 (early endosomes, 1:50 v/v, Abcam), and anti-giantin (Golgi apparatus, 1:300 v/v, Abcam). The antibody diluent was 1% BSA in PBS with 0.01% azide. For all non-directly conjugated primary antibodies, anti-rabbit Alexa Fluor 647 was applied at 1:3000 (μg/μL) for 1 h at room temperature. All samples were mounted with Duolink Mounting Medium containing DAPI (Sigma-Aldrich) and stored at 4 °C until imaged.

ER-Tracker™ Red (10 nM, ThermoFisher) was used to define the endoplasmic reticulum (ER) and to establish the time course of uptake and accumulation of SU11274 in live cells.

2.3. Confocal laser scanning microscopy

A Zeiss laser scanning microscope (LSM) 800 with Airyscan (Thornwood, NY) and a 40x/1.3 N.A. oil immersion objective lens was used to generate cell images. All imaging parameters (described below) were universally applied across experimental treatment groups. Briefly, images were acquired using sequential scanning of individual fluorophores with a final image size of

* Corresponding author. Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, NE, USA.
E-mail address: mahollin@unmc.edu (M.A. Hollingsworth).

https://doi.org/10.1016/j.bbrc.2018.05.034
0006-291X/

1024 × 1024 pixels and scaling of 0.156 μm per pixel. Images collected using bidirectional scanning were additionally averaged 2 to 4 times to optimize signal to noise ratios.

SU11274 was excited using a 488 nm laser line and the resultant emission was collected using a 495–570 nm filter. ER-Tracker™ Red was excited using a 561 nm laser line and the subsequent emission was collected with a 574–633 nm filter. Alexa Fluor 647, used to counterstain primary antibodies targeted to discrete organelles, was excited with a 640 nm laser line with the resultant emission collected using a 646–700 nm filter. DAPI and Hoechst dyes (for detection of fixed and living cell nuclei, respectively) were excited using 405 nm light with emission collected using a 411–488 nm filter.

Spectral profiling of SU11274 (at 32 μM) was performed using a Zeiss LSM 710 (Thornwood, NY). SU11274 was excited using 488 nm light and subsequent SU11274-associated fluorescence intensities were acquired in sequential 10 nm bins collected from 495 to 725 nm. Upon verification of SU11274's spectral properties, SU11274-specific emission was collected using a 495–570 nm filter set.

Given that SU11274 emission could contaminate the ER-Tracker™ Red signal (Fig. 1A), cells were sequentially imaged 3 min after the application of both compounds. We also observed that 488 nm excitation (used to excite SU11274) exhibits a 5% excitation efficiency for ER-Tracker™ Red and restricted ER-Tracker™ Red emission collection such that it was independent of the SU11274 fluorescence detection filter (Fig. 1A). As a final verification of independent imaging of SU11274 and ER-Tracker™ Red, we imaged SU11274 treated cells without ER-Tracker™ Red labeling and determined SU11274 contamination of the ER-Tracker™ Red channel to be 0.1% (±0.06) and therefore unlikely to generate image analysis artifacts. SU11274 is spectrally distinct from the excitation and emission of Alexa Fluor 647.

2.4. SU11274 cellular uptake assay

S2-013. MUC1F cells cultured on glass-bottom microwell dishes (MatTek) were treated with 100 nM ER-Tracker™ Red for 30 min and then rinsed before applying 2 μM SU11274. The cells were then imaged for 15 min at 20 s time intervals. Cells were maintained at 37 °C and 5% CO_2 during live cell imaging. Average, cell-specific SU11274 fluorescence intensities, were normalized to the highest fluorescence intensity observed and plotted as a function of time. The rate of uptake was calculated by dividing the change in fluorescence intensity from consecutive intensity measurements by the increment of time elapsed between measurements.

SU11274-associated mean fluorescence intensities in individual cells and cell-free areas were additionally analyzed to determine the relationship between SU11274 mean fluorescence intensities and SU11274 concentration. SU11274 cell medium concentration was plotted as a function of SU11274 mean fluorescence intensity measured in the cell-free areas.

2.5. Image analysis

For fixed cell analyses, different subcellular organelles were delineated as discrete regions of interest (ROIs). Raw image files were imported into NIH FIJI/Image J for subcellular organelle

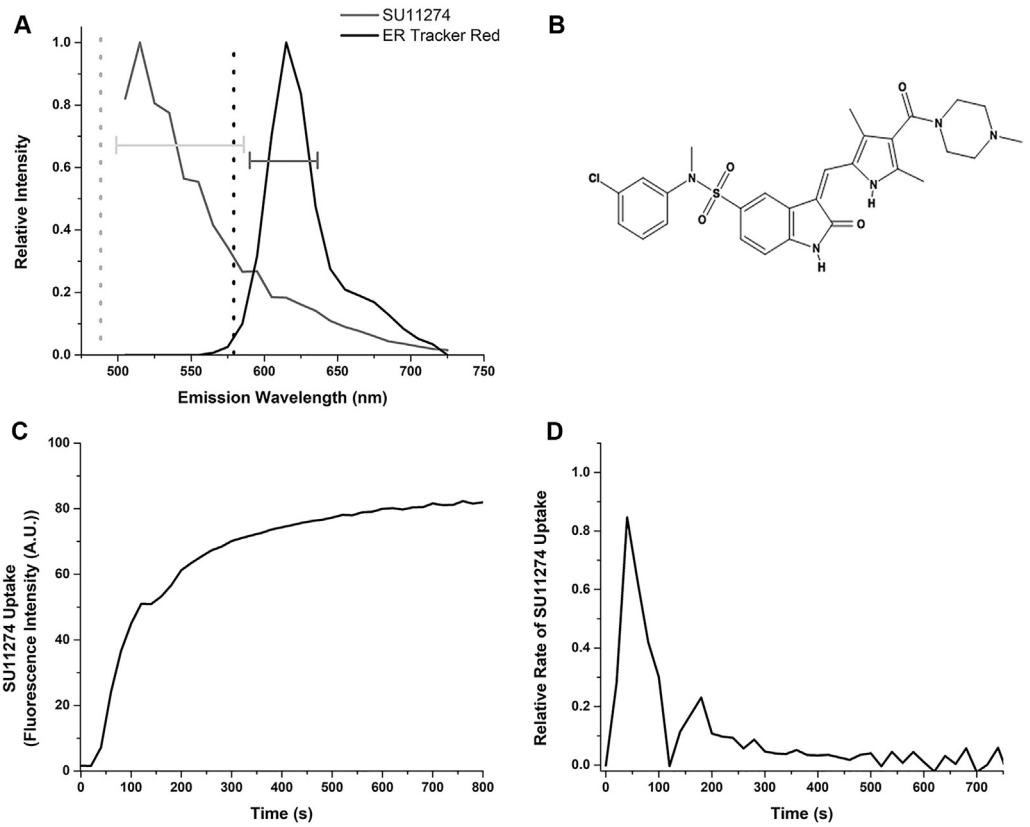

Fig. 1. The intrinsically fluorescent c-Met inhibitor, SU11274, rapidly accumulates in cells. (A) SU11274 excitation (488 nm, grey dotted line) and emission profile can be spectrally separated from ER-Tracker™ Red excitation (561 nm, black dotted line) and emission using SU11274- and ER-Tracker Red™-filter sets (grey, black bars, respectively). (B) Structure of SU11274 ($C_{28}H_{30}ClN_5O_4S$). (C) Cell-specific SU11274 uptake, measured by fluorescence intensity, is rapid in S2-013.MUC1F cells. (D) Relative rates of SU11274 uptake by S2-013.MUC1F are indicative of a biphasic uptake.

Please cite this article in press as: E.J. Wiest, et al., Met receptor inhibitor SU11274 localizes in the endoplasmic reticulum, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.034