The novel ABCG2 inhibitor induces ABCG2 internalization and degradation

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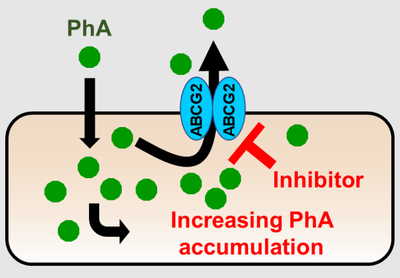
BACKGROUND

ABCG2 is a transmembrane efflux protein that utilizes ATP to pump substances out of the cell. ABCG2 is protective in the blood brain barrier but has a wide substrate range so also limits the penetration of chemotherapy drugs. It is used as a resistance mechanism by tumors and knockout of ABCG2 has limited leukemia progression in mice models (1).

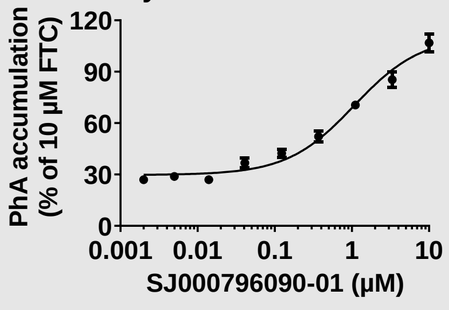
We recently identified the novel ABCG2 inhibitor SJ000796090-01 using a high throughput phenotypic screen (based upon inhibition of ABCG2 transport) for the purpose of improving leukemia therapy. SJ000796090-01 decreased the surface expression of ABCG2, however, the mechanism remains unknown.

By using a site specific biotinylation system (AviTag), we purified the ABCG2 protein using the streptavidin-biotin interaction. Using a proteomic approach with mass spectrometry, we identified FLOT-2 which increased interaction with ABCG2 upon SJ000796090-01 treatment. Based on the knowledge of FLOT-2, in our present study, we hypothesized that FLOT-2 internalizes ABCG2 upon SJ000796090-01 treatment.

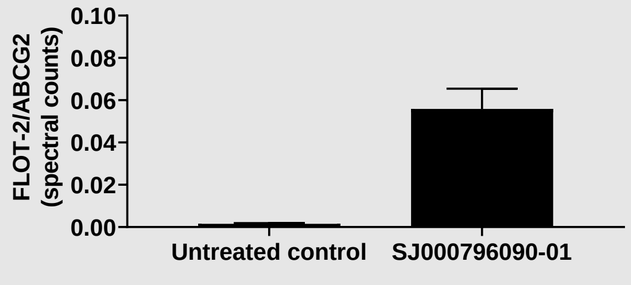
High throughput phenotypic screen
Specific ABCG2 fluorescent substrate pheophorbide (PhA)



Inhibition of ABCG2-mediated PhA transport by SJ000796090-01



FLOT-2 interacts with ABCG2



FLOT-2

- FLOT-2 is a ubiquitously expressed protein that homodimerizes with the homologous FLOT-1 to form discrete membrane microdomains known as lipid rafts.
- FLOT rafts induce membrane curvature and bud into the cell without the use of Clathrin.
- Dopamine transporter and GPI- anchored proteins utilize a FLOT dependent, Clathrin independent endocytic pathway (2).

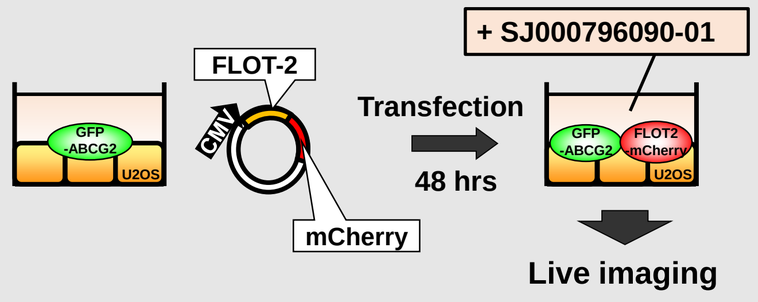
OBJECTIVES

To investigate whether FLOT-2 internalizes ABCG2 upon SJ000796090-01 treatment.

METHODS

Imaging of GFP-ABCG2 and mCherry-FLOT-2

U2OS stable cell lines expressing GFP-tagged ABCG2 were transiently transfected with mCherry-tagged FLOT-2. Cellular localization of fusion proteins of GFP-tagged ABCG2 and mCherry-tagged FLOT-2 were analyzed by confocal microscopy. Images were taken over 16 hours, 48 hours post transfection. 10 µM SJ000796090-01 was added to the cell media at the start of imaging.

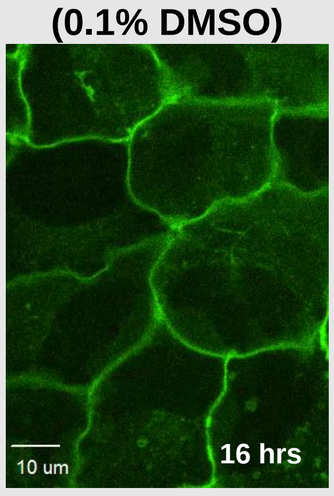


CONCLUSION

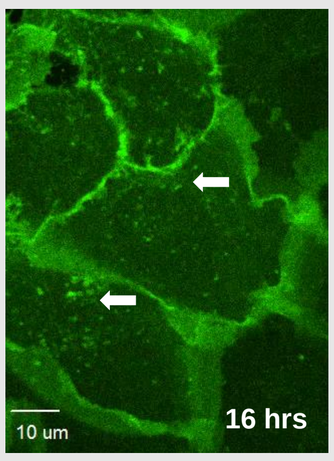
SJ000796090-01 causes ABCG2 internalization and degradation partially mediated by increased interaction with FLOT-2.

SJ000796090-01 causes ABCG2 internalization

Untreated control (0.1% DMSO)

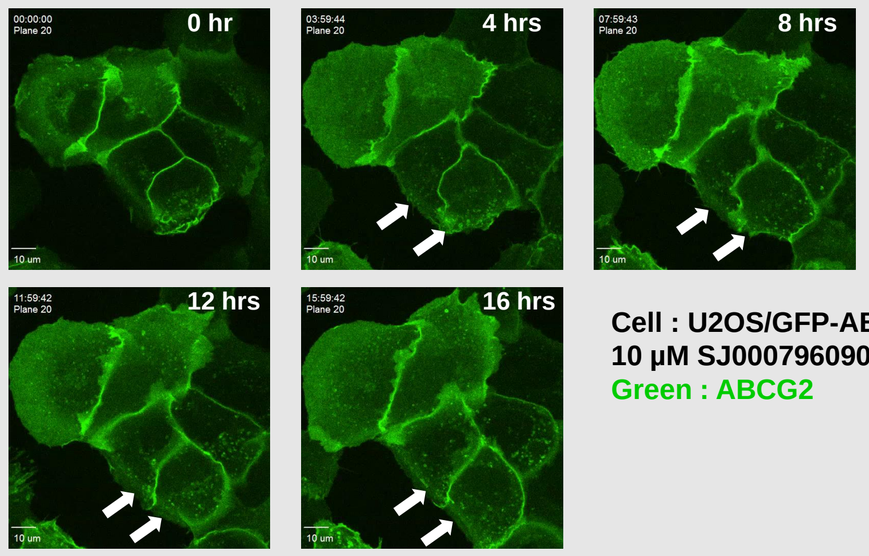


10 µM SJ000796090-01



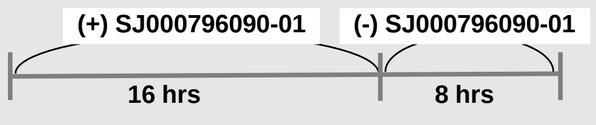
Cell : U2OS/GFP-ABCG2
Green : ABCG2

Time course of SJ000796090-01-mediated Internalization of ABCG2



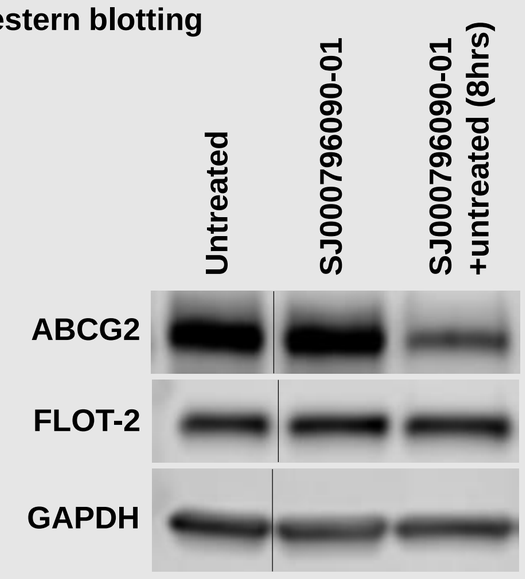
Cell : U2OS/GFP-ABCG2
10 µM SJ000796090-01
Green : ABCG2

SJ000796090-01 causes ABCG2 degradation



10 µM SJ000796090-01 for 16 hrs

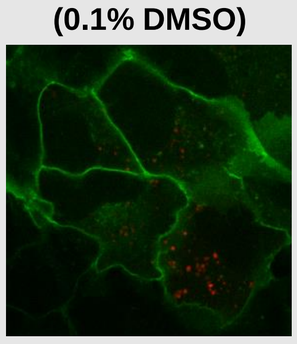
Western blotting

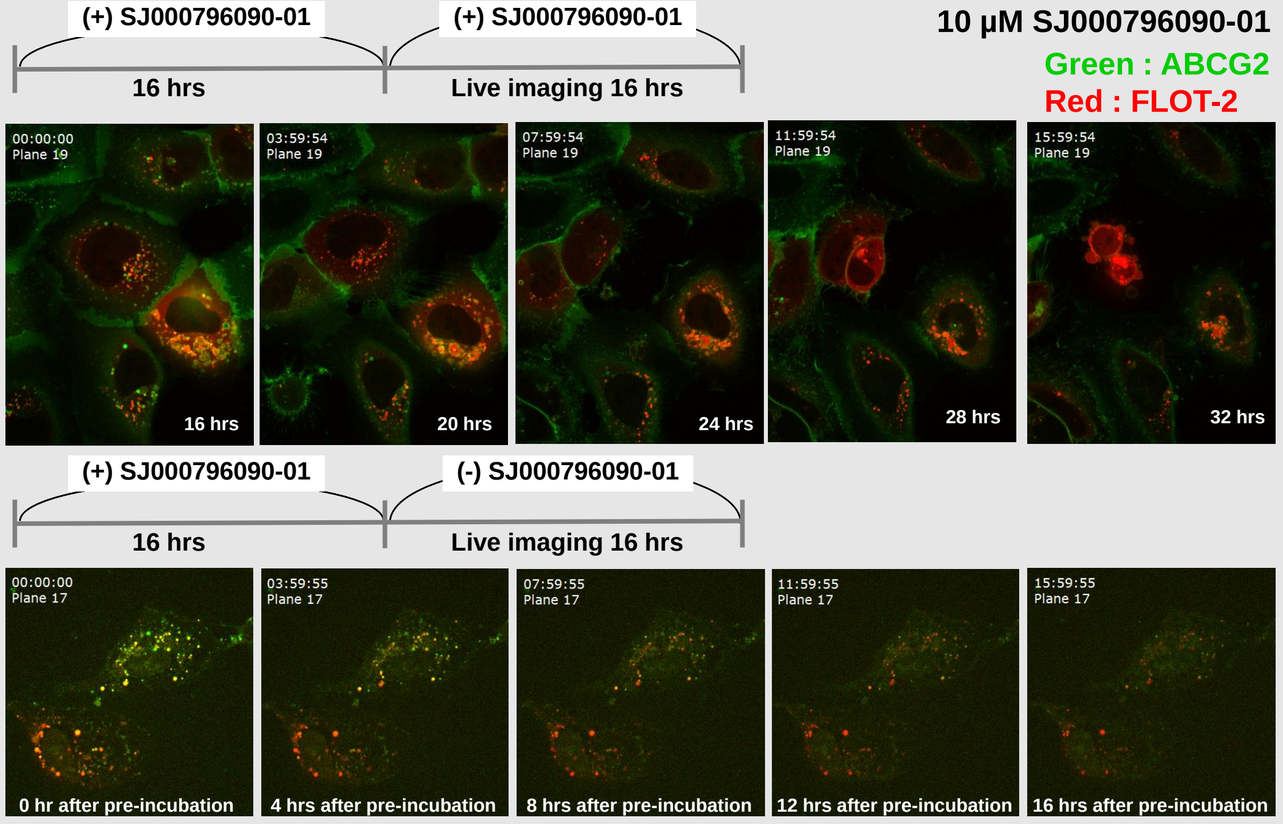


ABCG2 partially colocalizes with FLOT-2 and ABCG2 degrades with time under SJ000796090-01 treatment but FLOT-2 does not

Western blotting — U2OS/GFP-ABCG2 cells

Untreated control (0.1% DMSO)





(1) Fukuda, Y. *et al*., *JCI Insight*, **2**: e92409 (2017)
(2) Meister, M. *et al*., *Membranes*, **4**: 356–371 (2014)

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