Manipulation of Abro1 Localization in U2OS Cells

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Background

When a replication fork stalls, that can lead to replicative stress which decreases genomic stability and can lead to cancer[1]. Abro1 has been found to be integral in protecting stalled replication forks.

Abro1 has been found to localize both in the nucleus and in the cytosol[2]. The purpose of this project is to generate Abro1 that only localizes to one region, which is the first step in identifying the differing Abro1 functions and determining whether they are related. The mutants were generated with either a Nuclear Localization Signal (NLS) or a Nuclear Export Signal (NES).

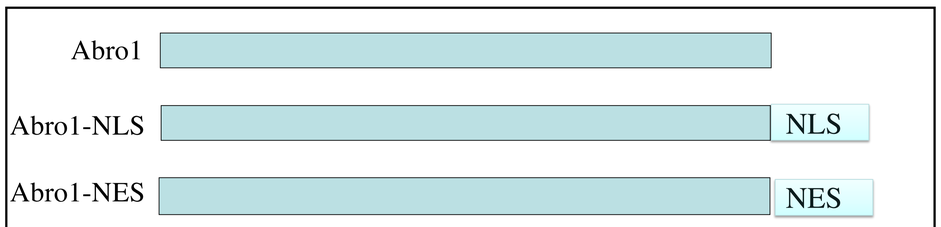

Fig. 1 Generation of Abro1-NLS and Abro1-NES mutants. Mutants were generated on the C-terminus region of Abro1.

Methods and Materials

Mutagenesis PCR	Abro1-NLS and Abro1-NES were generated on pENTR-Abro1 by mutagenesis PCR. DpnI was used to digest the DNA templates and the PCR products were then transfected to E. Coli. The E. Coli was then spread on Luria Broth (LB) plates and screened with antibiotics.
Sequencing	Mono-clones were sequenced to verify for correct DNA sequence
Gateway Recombination	Abro1, Abro1-NLS, and Abro1-NES were transferred from pENTR vectors to destination vectors which generated HA-Abro1, HA-Abro1-NLS, and HA-Abro1-NES
Transfection	HA-Abro1, HA-Abro1-NLS, and HA-Abro1-NES recombination products were transfected to E. Coli, spread on LB plates, and screened with antibiotics
Plasmid Extraction	Plasmids from HA-Abro1, HA-Abro1-NLS, and HA-Abro1-NES were extracted from mono-clones
Transient Expression	Transient expression of HA-Abro1, HA-Abro1-NLS, and HA-Abro1-NES occurred in U2OS cells for 48 hours
Western Blot	Western blot performed in order to verify expression
Fix Cells	Cells with fixed with 4% paraformaldehyde
Immunofluorescence	Immunofluorescence was performed using rabbit anti-HA antibody and Alexa Fluor™ Plus 488 conjugated anti-rabbit secondary antibody. A Confocal microscope was used to take a photo of the results

Results

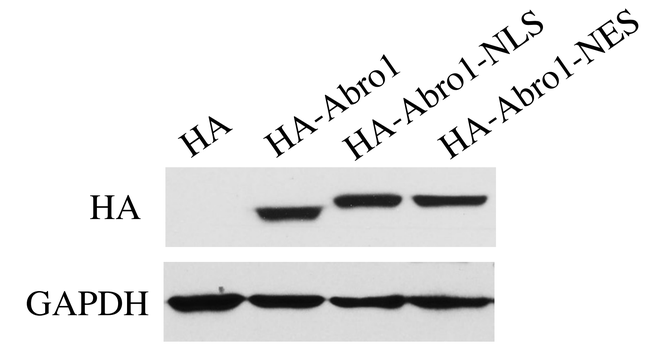

Fig. 2 Western Blot results indicating that the Abro1-NLS and Abro1-NES mutants were successfully generated with the localization signals.

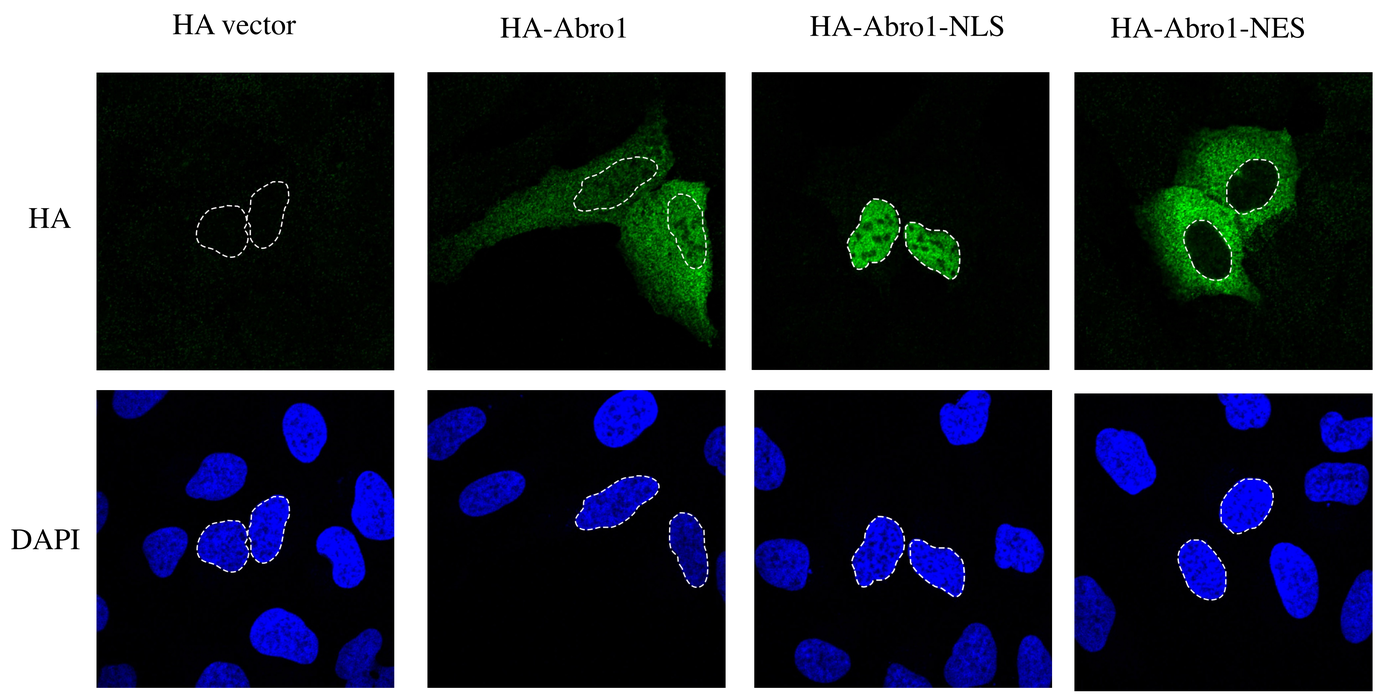

Fig. 3 Immunofluorescence results. The first column labeled HA serves as a negative control while the second column labeled WT serves as a positive control. The NLS column shows that the NLS signal successfully localized Abro1 inside of the nucleus while the NES column shows that the NES signal successfully localized Abro1 in the cytosol, outside of the nucleus.

Conclusions

The successful generation of the two Abro1 mutants is an important first step in the understanding of Abro1. In the future, these mutants could be used for further testing of the different functions of Abro1 based on its localization and how these functions interact. Thus, with a deeper understanding of Abro1, we can have an increased understanding of genome stability which can have implications in tumor suppression.

References

1. Xu S, Wu X, Wu L, et al. Abro1 maintains genome stability and limits replication stress by protecting replication fork stability. *Genes Dev*. 2017;31(14):1469-1482. doi:10.1101/gad.299172.117

2. Emam, A., Wu, X., Xu, S. *et al.* Stalled replication fork protection limits cGAS–STING and P-body-dependent innate immune signalling. *Nat Cell Biol* **24,** 1154–1164 (2022). https://doi.org/10.1038/s41556-022-00950-8

Acknowledgments

Thank you to The University of Texas MD Anderson Cancer Center and the Baylor University Pre-Health Department for partnering in the University Outreach Summer Program. Thank you to Dr. Tess Melancon, Dr. Chandra Bartholomeusz, Ms. Nancy Strange, Dr. Erika Abel, and the Department of Genetics at The University of Texas MD Anderson Cancer Center.