2023

Investigation of the DYRK1A Regulation by LZTS2-SIPA1L1 Complex

Rebecca Gunnin
Virginia Commonwealth University

Austin Witt B.S.
Virginia Commonwealth University

Larisa Litovchick M.D.,Ph.D.
Virginia Commonwealth University

Investigation of the DYRK1A Regulation by LZTS2-SIPA1L1 Complex

Rebecca Gunnin, Austin Witt, Larisa Litovchick, MD-PhD

Department of Internal Medicine, Division of Hematology, Oncology, and Palliative Care and Massey Cancer Center

Introduction

A region on chromosome 21, the **Down Syndrome critical region (DSCR)**, is associated with major defects found in Down Syndrome, such as craniofacial malformations. ***DYRK1A*** is a gene found on chromosome 21 *within the DSCR* that encodes an enzyme, dual specificity tyrosine-phosphorylation-regulated kinase 1A. DYRK1A is known to phosphorylate many substrate proteins and is thought to be involved in tumor suppression, neurological development, cell cycle regulation, and aging. Recently, the Litovchick lab and others reported that DYRK1A also plays a role in the double-strand break repair of DNA, which could lead to mutations and tumorigenesis, if deregulated.[1]

The Litovchick lab is currently investigating novel interactions of DYRK1A, and their implications for cancer. One of these proteins, **DCAF7**, is already a well-known DYRK1A interacting partner. Another less characterized protein is **LZTS2**, also known as LAPSER1 or leucine-zipper tumor suppressor 2. LZTS2 is found to be deleted in many human tumors and is known to bind a signaling intermediate SIPA1L1.[2] Our preliminary data show that LZTS2 may promote DYRK1A phosphorylation, thereby regulating DYRK1A kinase activity. We suspect that SIPA1L1 may be involved in this interaction and, as this complex is known to be involved in the WNT pathway that plays a role in both cancer and orofacial formation, ***we hypothesize that the LZTS2-SIPA1L1 complex regulates DYRK1A in these processes.***

To test this hypothesis, we started characterization of the DYRK1A-LZTS2 interaction using ectopic expressions of the full-length LZTS2 and DYRK1A as well as their fragments expressed in human T98G cells. We will further perform experiments investigating the physical binding and functional interactions between DCAF7, SIPA1L1, and the DYRK1A-LZTS2 complex.

Methodology

The methods of this project include various protocols including **cell culture**, DNA transfection, gel electrophoresis and western blotting, **immunoprecipitation**, **cell staining and imaging**, and (non-radioactive) **kinase assays**. Stable T98G cell lines expressing Flag-HA tagged LZTS2, or F1 and F2 fragments have been already established and validated. To express GFP-tagged DYRK1A fragments lacking the N-terminus, C-terminus, or a kinase domain, transient DNA transfection will be used. To investigate the expression of proteins of interest, gel electrophoresis, western blotting, and immunoprecipitation protocols will be employed. Further, to visualize the localization of proteins, cell staining, and cell imaging will be performed.

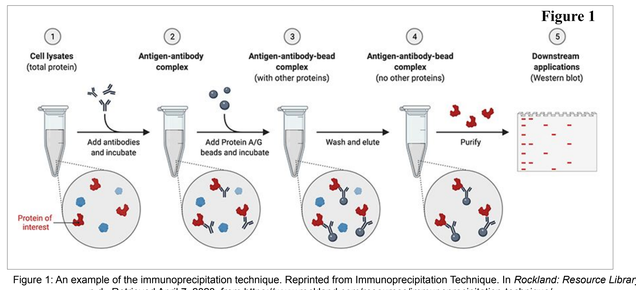

Figure 1: An example of the immunoprecipitation technique. Reprinted from Immunoprecipitation Technique. In *Rockland: Resource Library*, n.d., Retrieved April 7, 2023 from https://www.rockland.com/resources/immunoprecipitation-technique/.

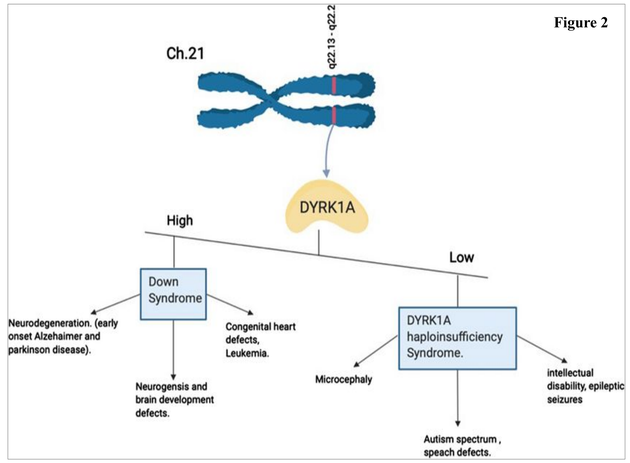

Figure 2: DYRK1A Dose Implications. Reprinted from ""DYRK1A: a down syndrome-related dual protein kinase with a versatile role in tumorigenesis" by A.J. Laham, M. Saber-Ayad, R. El-Awady. 2021. *Cellular and Molecular Life Sciences, 78*, 603-619.

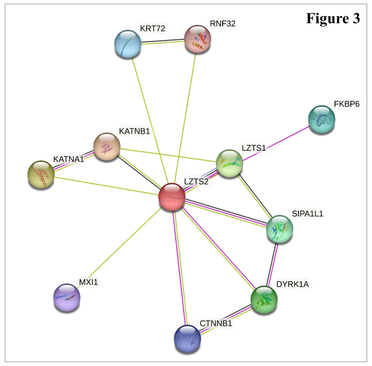

Figure 3: LZTS2 Protein Association Network. Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & von Mering, C. (2023). The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic acids research, 51(D1), D638–D646. https://doi.org/10.1093/nar/gkac1000.

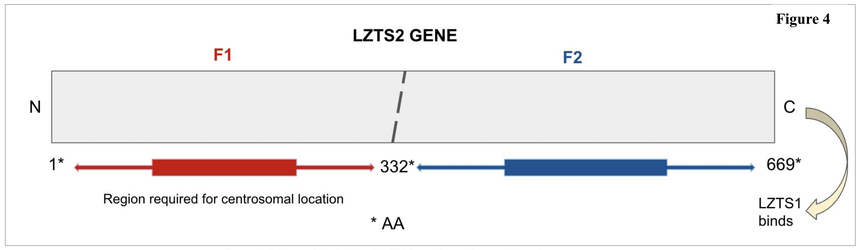

Figure 4: Schema of the full-length LZTS2 protein and fragment domains.

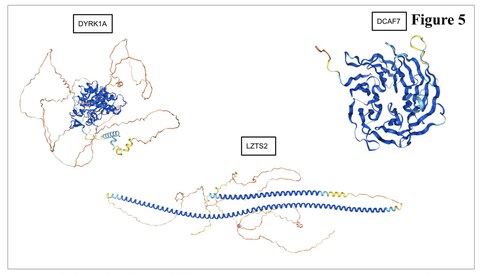

Figure 5: DYRK1A, DCAF7, and LZTS2 predicted protein structures. Image credit: Human Protein Atlas.

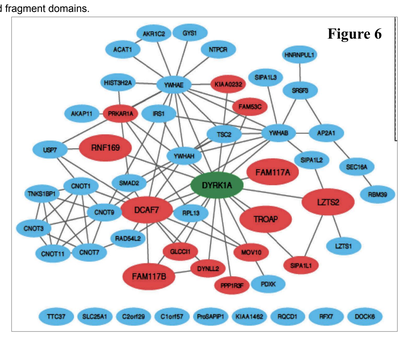

Figure 6: DYRK1A interaction map.VR Menon, V Ananthapadmanabhan, S Swanson, S Saini, F Sesay, V Yakovlev, L Florens, JA DeCaprio, MP Washburn, M Dozmorov, L Litovchick (2019) DYRK1A regulates the recruitment of 53BP1 to the sites of DNA damage in part through interaction with RNF169, Cell Cycle, 18:5, 531-551

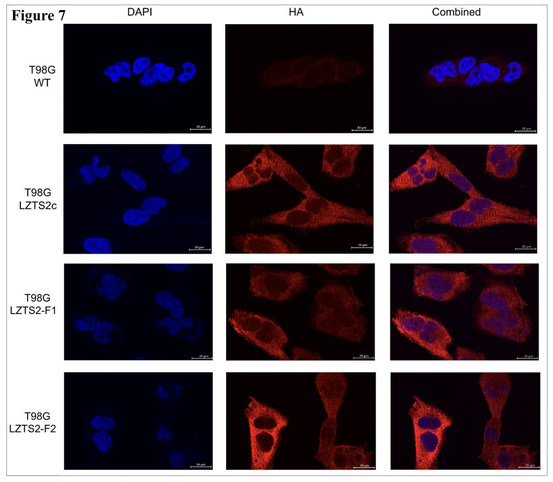

Figure 7: Cell staining of stable T98G cell lines with DAPI and anti-HA antibodies (in collaboration with Austin Witt).

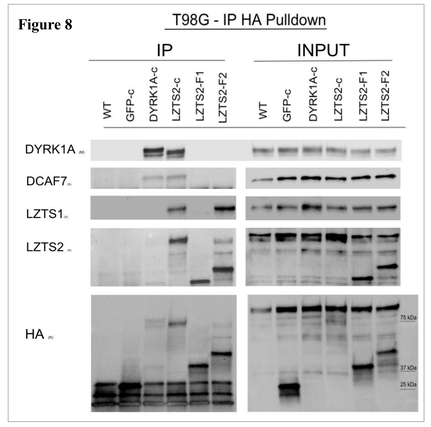

Figure 8: Immunoprecipitation of HA-tagged proteins using stable T98G cell lines, followed by detection of indicated proteins by Western blotting.

Preliminary Results/Discussion

Preliminary results of the **cell staining** (**Figure 7**) with anti-HA antibody and DAPI show the expected expression of HA in the T98G LZTS2, LZTS2-F1, and LZTS2-F2 cell lines. Further studies are needed to detect staining of the centrosomes of the T98G LZTS2-F1 cell line, which was expected as the LZTS2-F1 domain is required for the centrosomal localization of LZTS2, shown in **Figure 4.**

These cell lines were then used for the co-immunoprecipitation experiment. Preliminary results of **co-immunoprecipitation** experiment (**Figure 8**) show the lack of both DYRK1A and DCAF7 respective protein detection in the samples which have fragmented *LZTS2*, indicating that the full-length *LZTS2* is required for DYRK1A and DCAF7 to interact with LZTS2. To further investigate the relationship between the LZTS2-SIPA1L1 complex and DYRK1A, we will also check SIPA1L1 presence in these immunoprecipitated samples.

Future Directions

Since DYRK1A is known to suppress cell proliferation, the ability of LZTS2 or its fragments to regulate this DYRK1A function will be investigated using cell-based proliferation assays. I will also conduct a transwell migration assay, an assay that is used in cancer research to study the potential migration of cells toward a chemo-attractant, which can be relevant to the metastatic ability of tumor cells. This assay will be conducted with T98G cancer cells that have been modified to either overexpress full-length LZTS2 or express various fragments of LZTS2, such as LZTS2-F1 or LZTS2-F2.

Using in vitro kinase assays, I will determine if any specific domain of LZTS2 is responsible for the promotion of DYRK1A phosphorylation, as observed in preliminary studies. Additionally, these experiments will be performed in Kuramochi cancer cells to compare the findings between the two cell types and to further investigate this interaction in the context of the WNT pathway, which involves LZTS2.

Acknowledgements

I am very grateful for the opportunity to participate in this research project and thank Dr. Litovchick and Dr. Dickinson, as well as my lab partners Austin Witt, Angel Pajimola, Dr. Kathryn Shows, Dr. Anton Chestukhin, Dr. Eziafa Oduah, advisors, and professors for their continued advice and support.

This project is currently. funded by NIH 1R21HD105144 (MPI Litovchick, Dickinson) and VCU CHRI Collaboration Award (MPI: Dickinson, Litovchick),

In the future directions, the project will also be funded by the VCU Clinical and Translational Research Undergraduate Fellowship to R. Gunnin.

Works Cited

Citations:

1. Duchon A, Herault Y. DYRK1A, a Dosage-Sensitive Gene Involved in Neurodevelopmental Disorders, Is a Target for Drug Development in Down Syndrome. *Front Behav Neurosci.* 2016;10:104. doi:10.3389/fnbeh.2016.00104
2. Ishii, H., Baffa, R., Numata, S. I., Murakumo, Y., Rattan, S., Inoue, H., Mori, M., Fidanza, V., Alder, H., & Croce, C. M. (1999). The FEZ1 gene at chromosome 8p22 encodes a leucine-zipper protein, and its expression is altered in multiple human tumors. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(7), 3928–3933. https://doi.org/10.1073/pnas.96.7.392
3. Justus CR, Leffler N, Ruiz-Echevarria M, Yang LV. In vitro cell migration and invasion assays. *J Vis Exp.* 2014;(88):51046. doi:10.3791/51046

Figures:

Figure 5. DYRK1A structure image available from v22.proteinatlas.org. https://www.proteinatlas.org/ENSG00000157540-DYRK1A/structure. (Updated April 12, 2023)
Figure 5. DCAF7 structure image available from v22.proteinatlas.org. https://www.proteinatlas.org/ENSG00000136485-DCAF7/structure. (Updated April 12, 2023)
Figure 5. LZTS2 structure image available from v22.proteinatlas.org. https://www.proteinatlas.org/ENSG00000107816-LZTS2/structure. (Updated April 12, 2023)