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Molecular Mechanisms of the DYRK1A-regulated DNA Repair

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The functions of human Dual-specificity tyrosine (Y)-Regulated Kinase 1A, or DYRK1A, include cell cycle control and cell differentiation, for which the optimum dosage of DYRK1A is critical. DYRK1A is required for assembly of the DREAM complex (Dimerization Partner, Retinoblastoma-like, E2F, and MuvB) and repression of the cell cycle-dependent genes, such as BRCA1 and RAD51, during quiescence. Our laboratory previously reported that overexpression of DYRK1A inhibits the accumulation of a DNA repair protein 53BP1, at the DNA double-stranded breaks (DSB). Accumulation of 53BP1 is attributed to repair by the non-homologous end joining (NHEJ) as opposed to homologous recombination (HRR). The function of 53BP1 is opposed by RNF169, a ubiquitin-binding protein that also accumulates at the DSB sites and promotes HRR over NHEJ. It was found that DYRK1A interacts with RNF169 to regulate the displacement of 53BP1 from the DSB sites. The molecular mechanism of this regulation is not fully understood therefore the current study focuses specifically on RNF169 in order to better understand the role of DYRK1A in the DNA damage response pathway. Here, we used the Multi-Dimensional Protein Identification Technology (MudPIT) proteomic analysis to identify RNF169-interacting proteins. Human cancer U-2 OS cells stably expressing HA-tagged RNF169, as well as the control (parental) cells were used for immunoprecipitation with anti-HA antibody agarose beads. The sample quality was assessed by protein gel electrophoresis followed by silver staining and Western blot to confirm the pulldown and elution of RNF169. The samples were then sent to Stowers Institute for Medical Research for MudPIT proteomic analysis. Three repeats of this experiment were performed to identify high confidence RNF169 interacting proteins for further validation. This study is now in progress. In order to further understand the regulation of DNA repair by DYRK1A, the RNA sequencing dataset was obtained and analyzed out as part of other studies in the laboratory. The expression of the mRNA for repair factors RAD51 and BRCA1 was found to be regulated by DYRK1A. To determine the significance of this finding, an experiment was designed to assess BRCA1 and RAD51 protein levels in the normal U-2 OS cells and in the cells lacking DYRK1A (U-2 OS DYRK1A knockout cells) after inducing DNA damage by gamma irradiation. It was found that the levels of RAD51, BRCA1 and 53BP1 levels were increased with DYRK1A knockout. These results were consistent with the finding that DNA repair efficiency is increased with DYRK1A knockout. Further studies can help to understand the whether these effects are mediated by DYRK1A-regulated DREAM complex, or by other mechanisms. Overall, our studies characterize DYRK1A as multi-functional protein kinase involved in DNA repair and other processes.

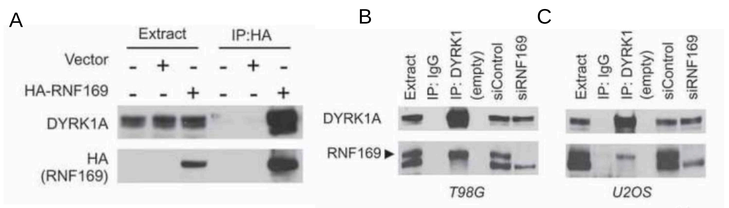

Figure 1: (a) IP/WB assay shows binding between transiently expressed HA-tagged RNF169 and endogenous DYRK1A in T98G cells. (b, c) IP/WB analyzes of the interaction between the endogenous RNF169 and DYRK1A in T98G and U-2 OS cells. RNF169-depleted cell extract (siRNF169) is included to identify the RNF169-specific protein band. IgG, negative control.

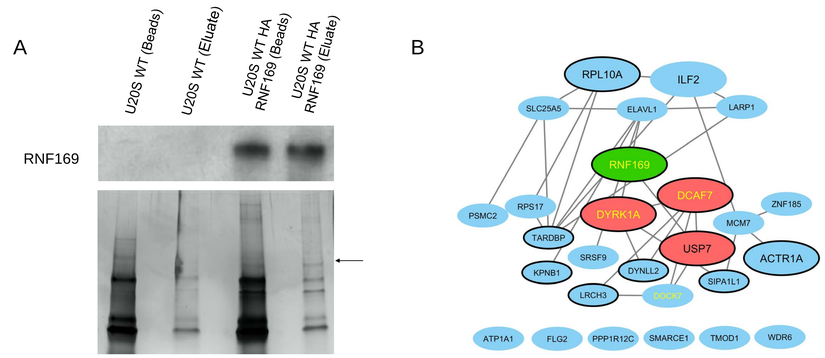

Figure 2: MudPIT proteomic analysis revealed the RNF169 interactome: (A) WB indicated levels of HA RNF169 in the cell lines used for MuPIT proteimic analysis (Top) and silver stained gel indicating 1/10th of the eluate used for proteomic analysis. **(B)** Analysis of the RNF169 - interacting protein network. Hierarchical network of interactions (CytoScape) involving RNF169 binding proteins in U-OS cells identified in this study, constructed using MetaScape analysis tool. Larger nodes correspond to proteins detected in all three replicates. Unconnected nodes are not known to interact with other factors. RNF169 is shown in green whereas red and blue circles correspond to either listed in the BioGrid database, or new-RNF169 binding proteins, respectively. Black outline indicates an overlap with our previous DYRK1A dataset. Yellow label indicates an overlap with our DCAF7 dataset.

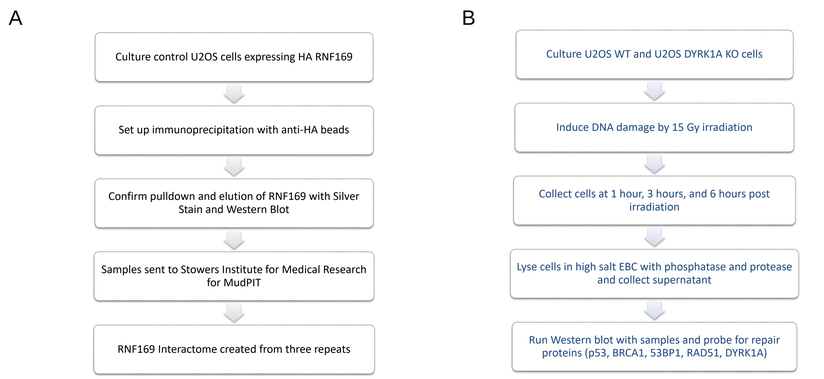

Figure 3: Flowchart of methods: (A) Methods for characterizing RNF169 interactome. **(B)** Methods for DNA damage experiment.

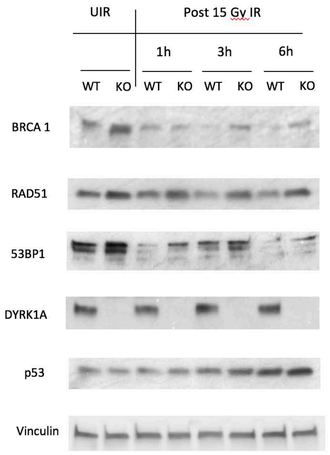

Figure 4: Western Blot carried out to probe for various repair proteins following 15 Gy Irradiation. 53BP1, p53, BRCA1, RAD51, DYRK1A, and loading control probed for in U2OS WT and U2OS DYRK1A KO cells collected 1 hour, 3 hours, and 6 hours post damage.

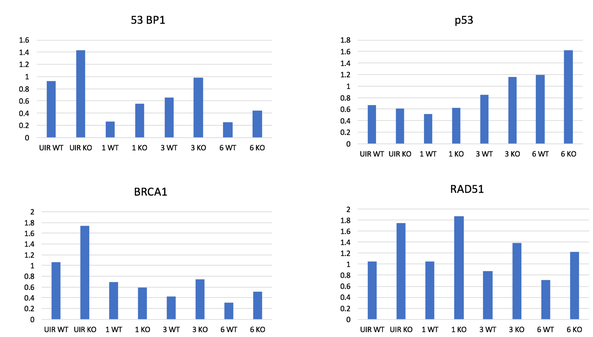

Figure 5: Quantification levels for proteins probed in WB. 53BP1, BRCA1, and RAD 51 show increased levels in KO cells. p53 shows increasing levels with higher time interval post damage.

Future directions:

Previously, it was determined that DYRK1A loss could increase HRR, but the mechanism was unknown (Menon et al. 2019). From this data, it can be seen that RAD51, BRCA1, and 53BP1 levels increase with DYRK1A knockout but further studies can help understand the unknown mechanisms by which these proteins are affected.

References:

Menon, Vijay R et al. “DYRK1A regulates the recruitment of 53BP1 to the sites of DNA damage in part through interaction with RNF169.” *Cell cycle (Georgetown, Tex.)* vol. 18,5 (2019): 531-551. doi:10.1080/15384101.2019.1577525

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