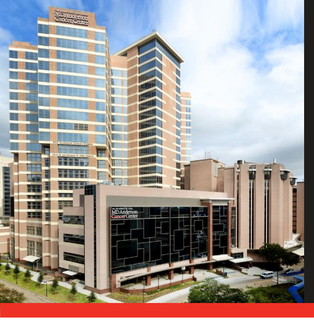

High-Throughput Profiling of Cancer Driving Fusions Implicated in Pan-cancer analysis

Sidharth Raghavan[1,2], Daniel J McGrail[3], Brandon S. Dunn[1], Xinxing Pan[4], Babita Kaundal[1], Deepa Bisht[1], Neha Damodar[1], Qing Li[1], Kara Cirillo[1], Collene Jeter[1], Gordon B. Mills[5], Joshua A Riback[6], Daniel Jarosz[7], S. Stephen Yi[4], and Nidhi Sahni[1]

[1]Department of Epigenetics and Molecular Carcinogenesis, MD Anderson Cancer Center, Houston, TX, [2]Department of Biomedical Engineering, Johns Hopkins University, Baltimore, MD, [3]Center for Immunotherapy and Precision Immuno-Oncology, Cleveland Clinic, Cleveland, OH, [4]Department of Biomedical Engineering, and Oden Institute for Computational Engineering and Sciences, The University of Texas at Austin, Austin, TX, [5]Department of Systems Biology, MD Anderson Cancer Center, Houston, TX, [6]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, [7]Department of Chemical and Systems Biology, Stanford University School of Medicine, Stanford, CA

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

Background

Phase separation is an emergent property of some proteins that results in their ability to spontaneously separate into a dense and dilute condensate phase (Figure 1).

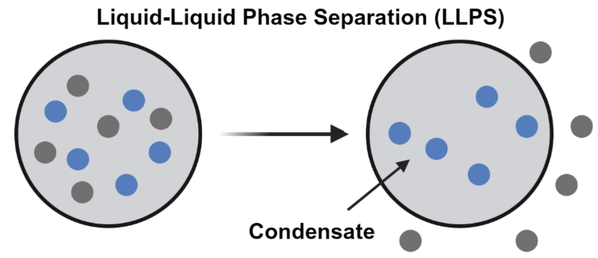

Figure 1: LLPS of proteins where a solution splits into two different phases.

Liquid-liquid phase separation is critical for maintaining homeostasis and allows for the spatiotemporal control over proteins [1]. However, many disease mutations and fusions can also be described by LLPS condensate formation.

The objective of this study is to identify LLPS phenotype and localization in various cancer-driving gene fusion and disease mutation candidates.

Candidate Selection Processes

(1) Gene fusions were selected through a pan-cancer analysis using the ChimerDB 3.0 fusion bank and various filtering algorithms (Figure 2).

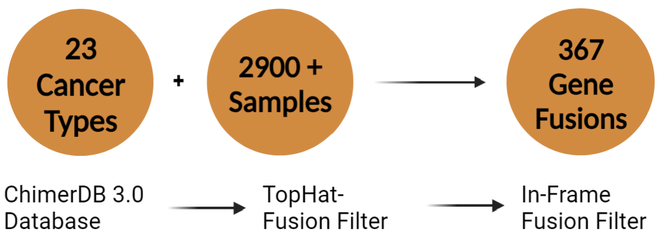

Figure 2: The ChimerDB 3.0 database of gene fusions was filtered to 367 fusion candidates.

Fusions were filtered by their ability to create a full-length fusion protein sequence. All possible combinations of isoforms at several breakpoints were also considered.

(2) Disease Mutations were screened as part of a project to identify condensate formation in wild-type (WT), mutant, and WT-mutant forms. Several thousand missense mutations have already been profiled through interaction assays [2]; however, condensate profiles were largely unknown.

The Human Gene Mutation Database list of human disease mutations was filtered to 3443 mutations by the following process (Figure 3).

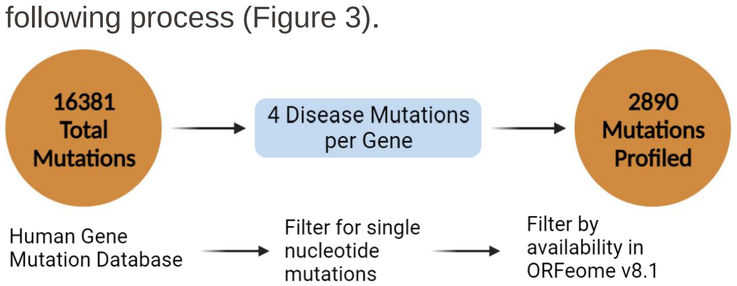

Figure 3: The Human Gene Mutation Database of disease mutations was filtered to 2890 mutation candidates that were profiled through interaction assays.

Methods

Fusion and disease mutation candidates were screened for condensate formation in HeLa and U-2 OS cells according to the following pipeline (Figure 4).

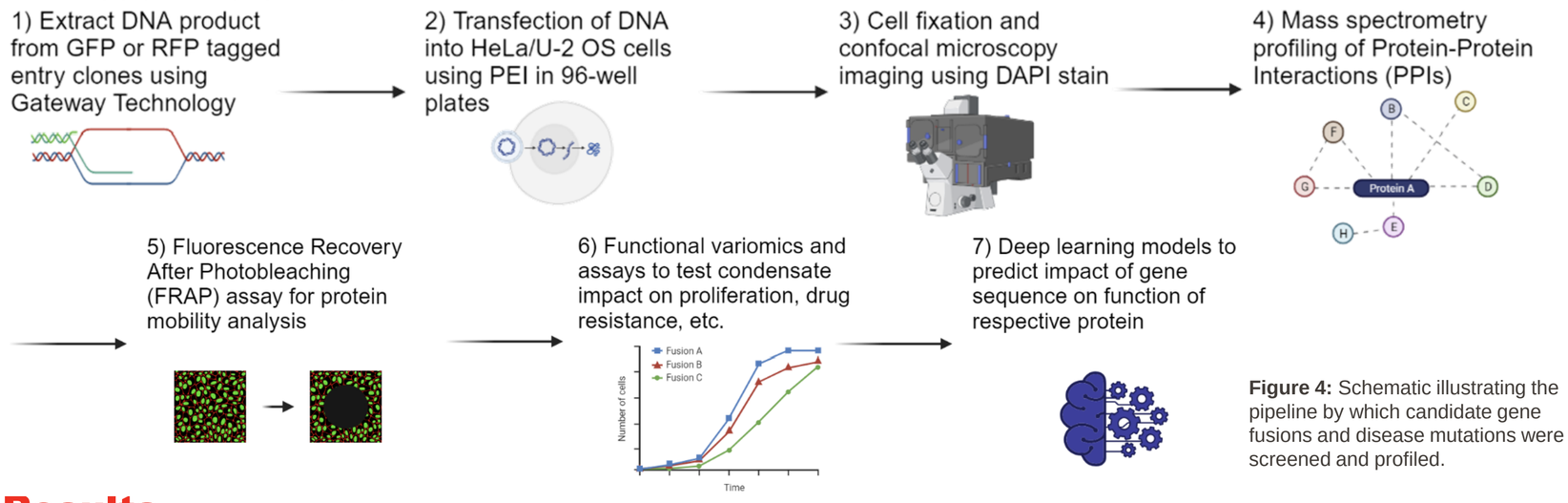

Figure 4: Schematic illustrating the pipeline by which candidate gene fusions and disease mutations were screened and profiled.

Results

Confocal Microscopy Imaging of Novel Fusion Candidates Demonstrates Condensate Formation

Cultured HeLa cells transfected with gene fusions were imaged showing formation of condensate with nuclear, cytosolic, or both nuclear and cytosolic localization in a series of replications (Table 1) (Figure 5).

Set	Transfection Efficiency	Phenotype Occurrence
1	50.8%	25%
2	69.16	52.5%

Table 1: The results from two replicate experiments of the complete set of 367 gene fusions. Set 1 was performed with HeLa cells; Set 2 was performed with U-2 OS cells.

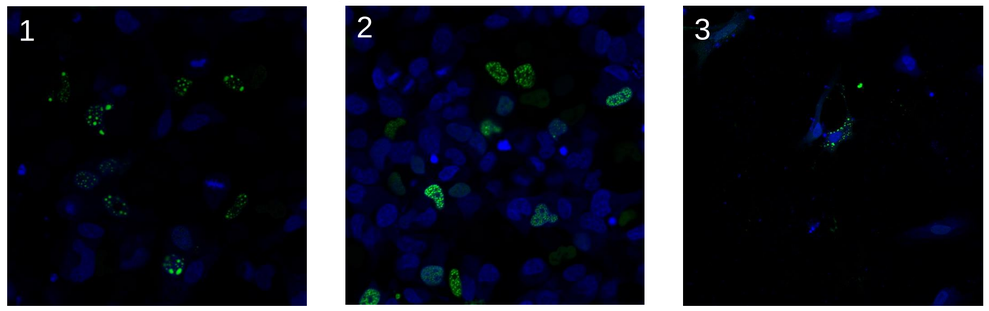

Figure 5: Performed in HeLa cells. (1) Confocal Microscopic Image of **LPP_HGMA2** gene fusion showing nuclear condensate. (2) Confocal microscopic image of **TCF3_PBX1** gene fusion showing nuclear condensate. (3) Confocal Microscopic Image of **KMT2A_MLLT3** gene fusion showing cytoplasmic condensate.

➡ **The fusions that showed a positive phenotype for condensate have important biological functions.**

KMT2A is a transcriptional co-activator and plays a crucial role in hematopoiesis; HGMA2 promotes entry to the cell cycle and is an inhibitor of apoptosis;
For candidates that regulate transcription, further analyses will study the affect of condensate on transcription factor DNA interactions.

➡ **KMT2A commonly appeared as a head/tail and consistently formed nuclear puncta in set 3.**

Confocal Microscopy Imaging of Disease Mutations Demonstrates Co-localization

Cultured HeLa cells transfected with disease mutations were imaged showing formation of co-localized condensates.

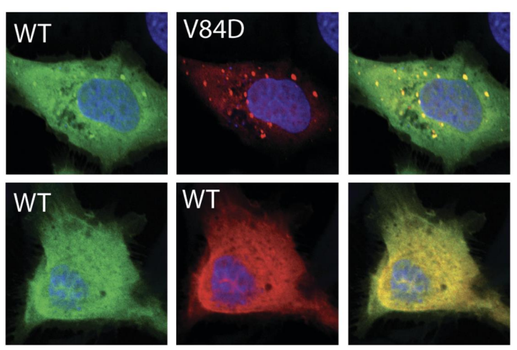

Figure 6: (Top) HeLa cells were transfected with a WT, Mutant, and WT-Mutant form of gene XYZ separately through a LR transformed GFP-tagged entry clone, RFP-tagged entry clone, and GFP-RFP-tagged entry clone. (Bottom) HeLa cells were transfected with WT form of gene XYZ in the same process.

➡ **Dominance of mutant cytoplasmic condensate suggests gain-of-function phenotype.**

Future Directions

Confocal microscopy imaging of gene fusions has provided candidates for further analysis

- Candidates must continue to be screened along the pathway outlined in Figure 2.
- For a candidate with a specific function, further analyses will study the affect of the fusion on the protein's function.
- A larger dataset of fusions will be profiled to expand the database to rare-occurring fusions.

References

[1] Jiang et.al. (2020) Protein phase separation and its role in tumorigenesis eLife 9:e60264
[2] Sahni et.al. (2015) Widespread macromolecular interaction perturbations in human genetic disorders. Cell, 161(3), 647-660

Acknowledgements

This work was supported by CPRIT RP 220292 to Nidhi Sahni. Laser scanning confocal microscopy and image processing were performed at the MD Anderson Cancer Center Epigenetics & Molecular Carcinogenesis Flow Cytometry and Cell Imaging Core (EMC-FCCIC) with funding support provided by the CPRIT core facility grant RP170628. S.R. was supported by the Summer Undergraduate Research Program (SURP) funded by US NCI grant R25CA181004.