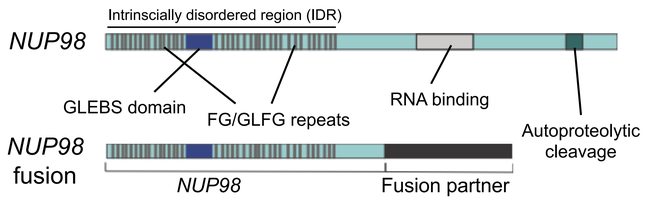
Phase Separation Mediates NUP98 Fusion Oncoprotein Leukemic Transformation

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BACKGROUND

Rearrangements of the nucleoporin 98 gene (*NUP98*) define a high-risk subset of childhood acute myeloid leukemia (AML). The resulting fusion oncoproteins (FO) involve the N-terminal, intrinsically disordered region of NUP98 and the C-terminal portion of one of more than thirty identified fusion partners. Approximately one third of fusion partners have DNA-binding homeodomains, and the remainder other domains involved in gene regulation[1].



NUP98 FOs have long been known to localize in nuclear puncta[2], and here we investigated whether these puncta form by liquid-liquid phase separation (LLPS) and how they might contribute to cell transformation.

RESULTS

1. NUP98::HOXA9 (NHA9) localizes in nuclear puncta in HEK293T cells and *in vitro*.

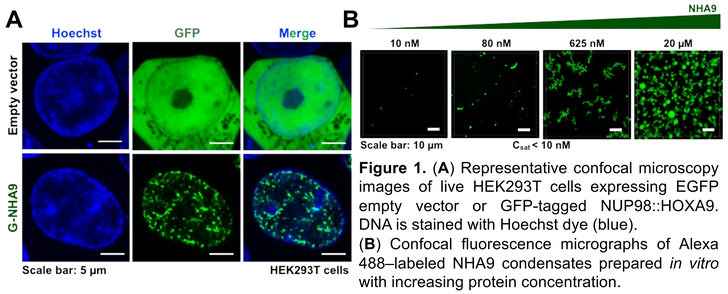


Figure 1. (**A**) Representative confocal microscopy images of live HEK293T cells expressing EGFP empty vector or GFP-tagged NUP98::HOXA9. DNA is stained with Hoechst dye (blue). (**B**) Confocal fluorescence micrographs of Alexa 488–labeled NHA9 condensates prepared *in vitro* with increasing protein concentration.

2. DNA binding by the HOXA9 homeodomain of NHA9 influences puncta morphology.

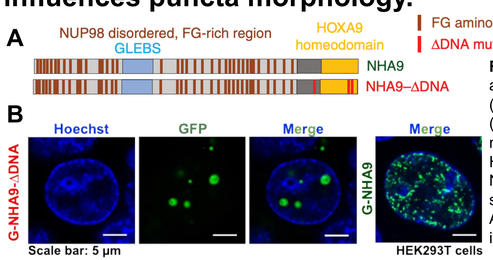


Figure 2. (**A**) Schema of NHA9 and DNA-binding mutant (NHA9-ΔDNA). (**B**) Representative confocal microscopy image of live HEK293T cells expressing G-NHA9–ΔDNA (green). DNA is stained with Hoechst dye (blue). A G-NHA9 overlay image is included for comparison.

RESULTS

3. Mutation of multiple F and FG residues in the IDR of NUP98 alters puncta formation.

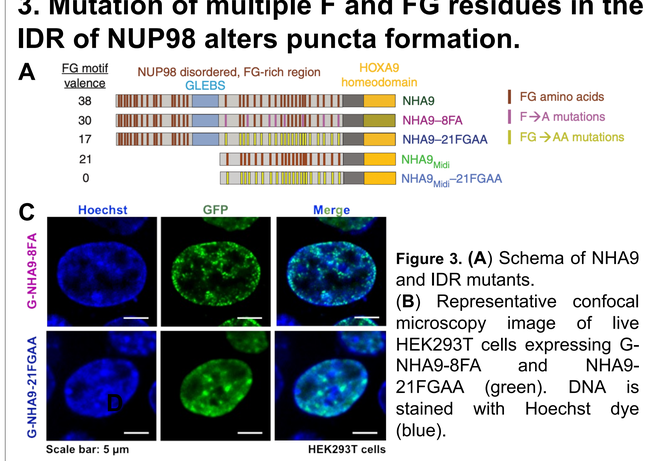


Figure 3. (**A**) Schema of NHA9 and IDR mutants. (**B**) Representative confocal microscopy image of live HEK293T cells expressing G-NHA9-8FA and NHA9-21FGAA (green). DNA is stained with Hoechst dye (blue).

4. NHA9 constructs form puncta in lineage negative hematopoietic stem and progenitor cells (lin- HSPCs).

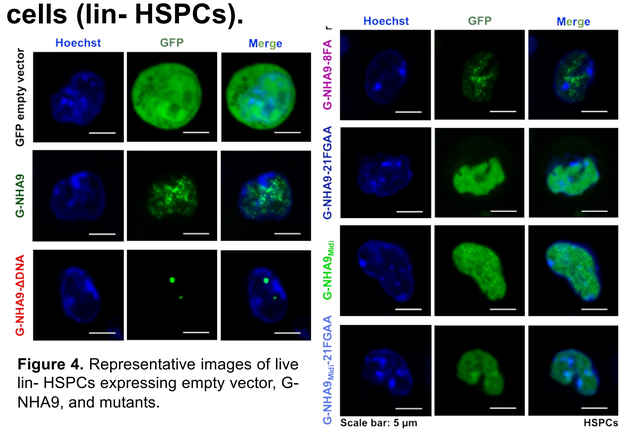


Figure 4. Representative images of live lin- HSPCs expressing empty vector, G-NHA9, and mutants.

5. NHA9 Transforms Lin− HSPCs and Alters Gene Expression

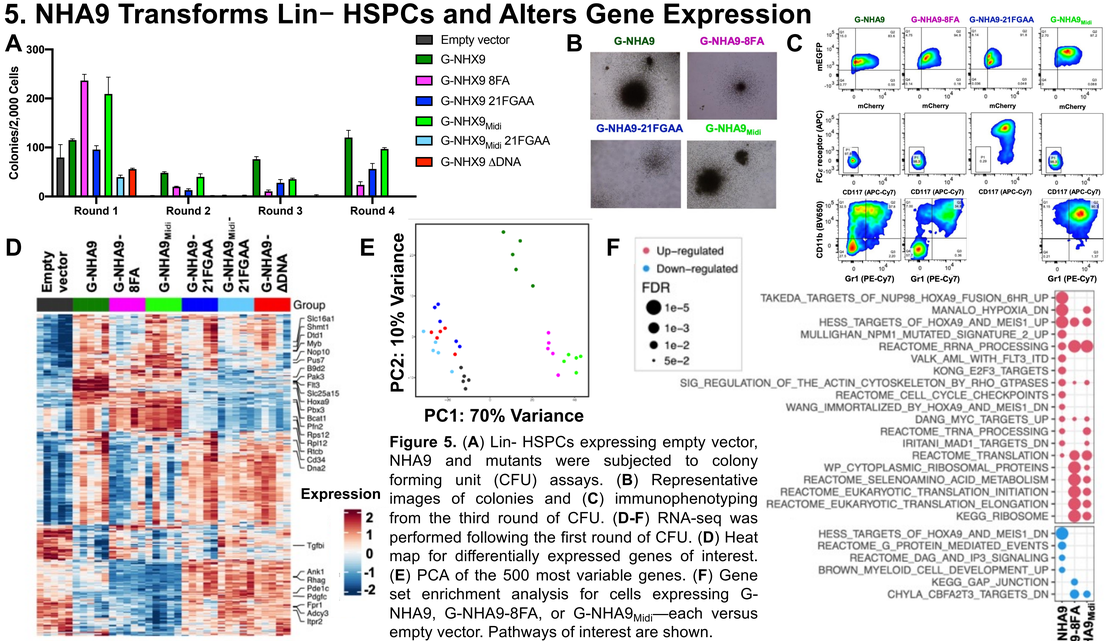


Figure 5. (**A**) Lin- HSPCs expressing empty vector, NHA9 and mutants were subjected to colony forming unit (CFU) assays. (**B**) Representative images of colonies and (**C**) immunophenotyping from the third round of CFU. (**D-F**) RNA-seq was performed following the first round of CFU. (**D**) Heat map for differentially expressed genes of interest. (**E**) PCA of the 500 most variable genes. (**F**) Gene set enrichment analysis for cells expressing G-NHA9, G-NHA9-8FA, or G-NHA9$_{Midi}$—each versus empty vector. Pathways of interest are shown.

6. Additional NUP98 FOs form nuclear puncta and transform lin- HSPCs.

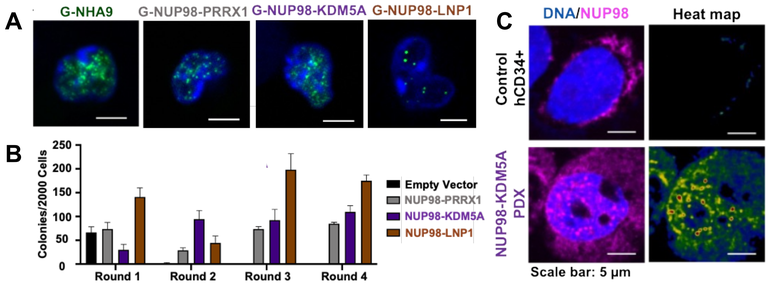
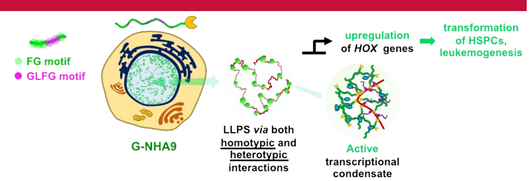


Figure 6. (**A**) Representative images of live lin- HSPCs expressing G-NHA9, G-NUP98-PRRX1, G-NUP98-KDM5A, and G-NUP98-LNP1. (**B**) Lin- HSPCs expressing empty vector, G-NUP98-PRRX1, G-NUP98-KDM5A, and G-NUP98-LNP1 were subjected to CFU assays. (**C**) Representative images of a fixed, nontransduced human CD34+ (hCD34+) cell and a NUP98–KDM5A patient-derived xenograft (PDX) cell stained with an antibody against NUP98. NUP98 is magenta, and DNA is blue. The heat map is a normalized representation of NUP98 fluorescence intensity across the PDX cell.

CONCLUSIONS

- The NHA9 fusion oncoprotein undergoes liquid-liquid phase separation through homotypic (fusion-fusion) and heterotypic (fusion-protein and fusion-DNA) interactions.
- The formation of nuclear puncta by NHA9 mediates cell transformation and deregulated gene expression.
- Other NUP98 fusions (with and without homeodomains) form nuclear puncta and drive cell transformation similar to NHA9, suggesting that phase separation is a common feature of NUP98 fusion oncoproteins.



References [1]Michmerhuizen, Klco, Mullighan. Blood 2020 (Review) [2]Kasper et al. Mol Cell Biol 1999