Oncogenic ER alpha (ERα), as an RNA-binding protein, controls mRNA stability and the epitranscriptome of breast cancer

Nimmy Mohan[1,2], Adrianna Dabrowska[1,2], Vishvak Subramanyam[1,2], Isabelle Liu[1,2], Lingru Xue[1,2], Duygu Kuzuoglu-Ozturk[1,2], and Davide Ruggero[1,2]

[1]Department of Urology, University of California, San Francisco, San Francisco, CA, USA,

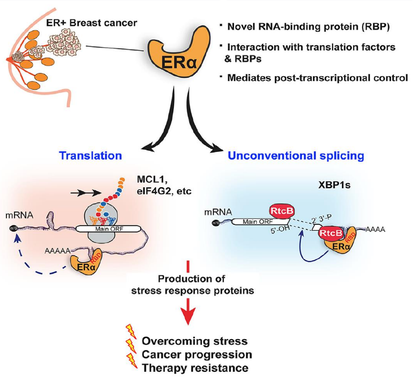
[2]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA

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Abstract

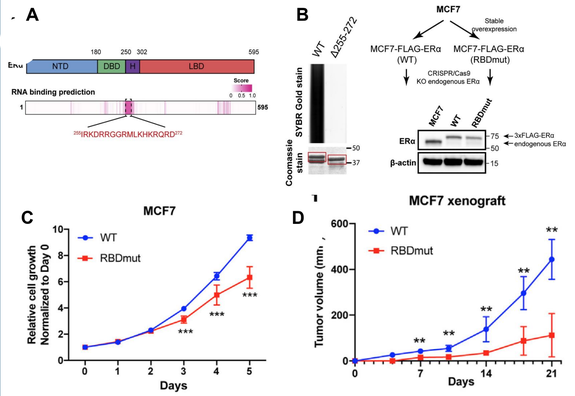
ERα, a key driver of breast cancer, has for decades been studied as a transcription factor, while it was recently discovered to act as an RNA-binding protein. The mechanism by which this new RNA function controls gene expression is poorly understood. Here, we surprisingly found that ERα controls the stability of a select mRNAs involved in cancer development. We mechanistically show an essential link for ERα in remodeling the cancer epitranscriptome. Specifically, the 5-methylcytosine (m^5C) writer NSUN2 becomes destabilized upon the loss of ERα RNA binding to its 3' UTR. Strikingly, m^5C mRNA methylation is significantly reduced in ERα RNA-binding mutant (RBD^M) cells, and this reduction is paralleled by changes in the expression of transcripts involved in breast cancer and cell cycle control. Importantly, NSUN2 overexpression enhances the tumorigenic potential of RBD^M cells in vivo, highlighting a novel mechanism for the selective control of the epitranscriptome in breast cancer.

Introduction and background of the study



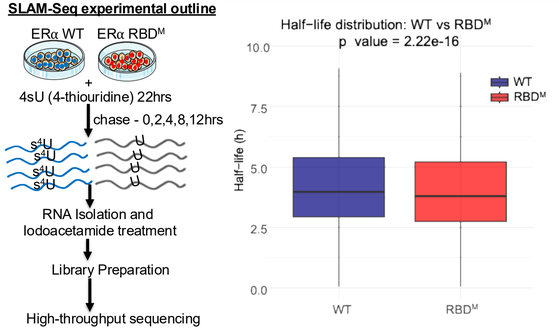
- ERα, a transcription factor critical for breast cancer, is an RNA-binding protein.
- ERα regulates post-transcriptional expression of stress response genes.
- ERα RNA binding activity is critical for tumor growth and therapeutic response.

ERα RNA-binding activity is a key determinant of its oncogenic role in breast cancer



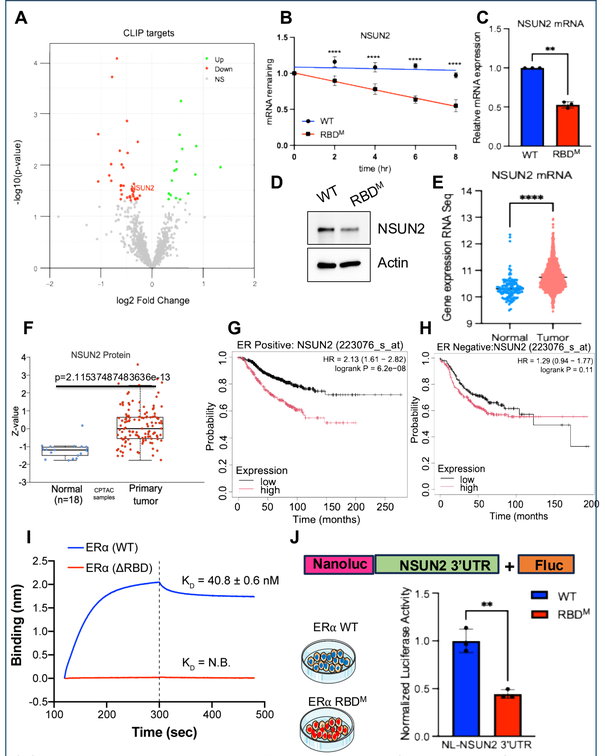
(A) RNA-binding domain (RBD) region of ERα protein. (B) A schematic of the strategy for generating MCF7 cells with WT and RBD^M. (C) Relative cell growth of MCF7 breast cancer cells with WT or RBD^M. (D) Tumor volumes of mouse xenografts implanted with MCF7 cells harboring WT or RBD^M.

Results : ERα regulates the stability of oncogenic mRNAs in breast cancer



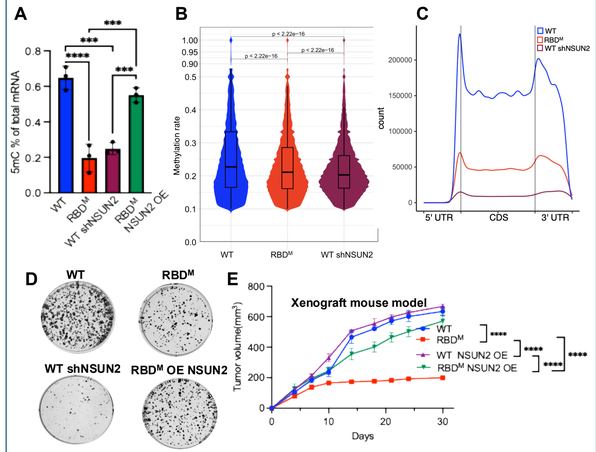
- Observed a significant reduction in the average transcript stability in the RBD^M cells genome-wide.
- Next our analysis focused on the half life of the transcripts directly bound by ERα: **NSUN2**

Results: Loss of ERα RNA binding activity results in less NSUN2 mRNA



(A)Volcano plot showing the ERα targets identified through SLAM-seq. (B) Proportion of NSUN2 mRNA remaining after transcription inhibition using Act. D (C & D) NSUN2 RNA and protein expression in WT and RBD^M cells (E) NSUN2 RNA expression in normal tissue and breast tumor patients (F) Protein expression of NSUN2 in normal tissue and breast tumor patients (G) Survival analysis correlation with NSUN2 RNA expression in ER-positive breast cancer patients (H) Survival analysis correlation with NSUN2 RNA expression in ER-negative breast cancer patients. (I) Biolayer interferometry experiment showing ERα-NSUN2 3'UTR binding in vitro. ERα WT strongly binds to NSUN2 3'UTR and in contrast, ERα RBD^M doesnot interact with the RNA. (J) Luciferase reporter assay using NanoLuc (NL) fused NSUN2 3'UTR together with firefly luciferase (Fluc) in WT and RBD^M cells.

Results: Loss of ERα-RNA binding leads to impaired global m5C methylation in breast cancer



(A) (C) The level of m^5C in purified total mRNA from ERα WT, ERα RBD^M, WT shNSUN2, NSUN2 OE RBD^M cells measured by m^5C ELISA. (B) Violin plot showing the overall m^5C methylation levels. (C) The m^5C distributions within different regions of mRNA. (D) Colony formation assay of ERα WT, ERα RBD^M, WT shNSUN2, and NSUN2 OE in ERα RBD^M cells. (E) Tumor volumes of mouse xenografts implanted with ERα WT, ERα RBD^M, NSUN2 OE in ERα WT and ERα RBD^M cells.

Conclusions

- ERα RBD^M cells show a decrease in NSUN2 mRNA and protein resulting in a global decrease in RNA m^5C marks.
- RBD^M cells fail to form tumors in vivo, whereas overexpression of NSUN2 restores ERα oncogenic potential.
- Thus, revealing a critical mechanism at the level of the epitranscriptome for breast cancer formation.
- ERα-NSUN2 axis is a driver of breast cancer.

Acknowledgement

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References:

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