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Previews

Don't shoot the messenger… shoot the reader

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Einstein et al. (2021) uncover a novel role for the RNA-binding protein YTHDF2, one of the m^{6}A reader proteins, in TNBC proliferation and survival. This study demonstrates the clinical potential of targeting a specific reader protein in the treatment of breast cancer.

The tumor environment presents a wide range of challenges for cancer cells: conditions of hypoxia and nutrient deprivation often arise as a consequence of cell overcrowding, while increases in global protein synthesis can result in ER stress. Therefore, the ability of the cancer cell to survive often relies on its hijacking of the cell stress response. In recent years, the regulation of RNA fate by RNA-binding proteins (RBPs) has emerged as an important aspect of the intersection between cancer development and the stress response (Harvey et al., 2018; Pizzinga et al., 2019). For example, a large scale CRISPR-Cas9 screen to identify genes implicated in cancer progression has identified several RBPs with known roles in the stress response as "cancer fitness genes" (Behan et al., 2019). However, it has been a challenge to define the molecular details of the involvement of specific RBPs in cancer development and progression. In this issue of *Molecular Cell*, Einstein and colleagues combine an impressive array of advanced high-throughput techniques in an ambitious effort to, first, identify RBPs that are important for breast cancer survival and, second, dissect the mechanism of action of one such RBP, the N6-methyladenosine (m^{6}A) reader protein YTHDF2 (Einstein et al., 2021).

The role of RBPs in MYC-driven breast cancer was investigated by using an RBP-specific CRISPR-Cas9 screen. The screen yielded 57 cancer-supportive RBPs, that mostly had roles in mRNA stability and translation, of which at least 40 had not previously been implicated in the MYC pathway. As MYC overexpression in HMECs is used as an experimental model system, Einstein et al. importantly extended the validity of their findings by cross-referencing with The Cancer Genome Atlas where, remarkably, 67% of their candidates were found to be highly expressed in triple-negative breast cancer (TNBC). YTHDF2 was among a small group of RBPs for which, in addition, decreased expression correlated with patient survival, highlighting that targeting this protein could provide new therapeutic avenues.

YTHDF2, which has been shown to target mRNAs for decay (Wang et al., 2014), has also been described as an oncogene in AML and glioblastoma (Paris et al., 2019; Dixit et al., 2021). Einstein and colleagues indeed find that depletion of YTHDF2 leads to cell death in their model system and reduces cell proliferation in other TNBC cell lines. Moreover, *in vivo* engraftment of YTHDF2-depleted cancer cells resulted in significantly smaller tumors in mice. Perhaps most importantly, in YTHDF2-deficient mice there was no adverse effect on non-cancerous somatic tissue, suggesting that a therapeutic approach of targeting of YTHDF2 is a viable option.

YTHDF2 depletion appeared to be associated with induction of the adaptive unfolded protein response (UPR), and to confirm these observations, Einstein et al. used an ER stress inhibitor to demonstrate that proteotoxicity is key in YTHDF2 depletion-triggered apoptosis. The serine protease 23 (PRSS23), which is downregulated in patients with TNBC, has been identified as an RNA target of YTHDF2 by the authors and in previous studies by CLIP (Wang et al., 2014). Crucially, co-depletion of PRSS23 reduced apoptosis and rescued the expression of the ER chaperone BiP. What emerges is a model in which YTHDF2 promotes the degradation of specific transcripts in TNBC to protect and permit the cancer cell to adapt to ER stress (Figure 1).

The authors then extended the study by applying ribosome-STAMP, a newly developed technique whereby an enzyme fused to the 40S ribosome directs base editing of C to U on ribosome-bound transcripts (Brannan et al., 2021) to *in vivo* YTHDF2-depleted tumors. In line with their previous observations, they found that YTHDF2-depleted tumor cells have increased rates of mRNA translation and identified unique translatomes linked to tumor progression. These data represent an important application of ribosome-STAMP since, as tumors are inherently heterogeneous, the levels of mRNA translation in individual tumor cells have historically been challenging to assess.

In addition to the important contributions of this study, highlighted above, the identity of the protein investigated could have interesting further implications. In fact, m^{6}A is one of the most common RNA modifications, identified in almost all classes of RNA and with a key role in the regulation of RNA stability, splicing, export, and translation (Jiang et al., 2021). The finding that m^{6}A reader YTHDF2 is important for TNBC survival and proliferation supports the targeting of RNA modifications as a new cancer therapeutic avenue, an approach that will be aided by the recent advances in RNA sequencing. Moreover, now that YTHDF2 has been established as an attractive therapeutic target, one must assess how best this can be achieved. Inhibition with a small molecule or gene therapy depletion are both options;

however, an alternative approach, made possible in part by the work presented in this study, could be to target the downstream effectors of YTHDF2 by directly stabilizing the target mRNAs or overexpressing the proteins they encode.

In conclusion, this study represents an important step forward in the field, since, as well as highlighting the importance of RBPs in tumor progression, it identifies the molecular mechanisms by which YTHDF2 contributes to cancer survival, thus revealing this RBP and its RNA targets as therapeutically valid options in TNBC. Furthermore, these findings increase our understanding of how cancer cells can exploit the activity of specific RBPs to evade cell stress and will pave the way for future studies in this area.

REFERENCES

Behan, F.M., Iorio, F., Picco, G., Gonçalves, E., Beaver, C.M., Migliardi, G., Santos, R., Rao, Y., Sassi, F., Pinnelli, M., et al. (2019). Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. Nature *568*, 511–516.

Brannan, K.W., Chaim, I.A., Marina, R.J., Yee, B.A., Kofman, E.R., Lorenz, D.A., Jagannatha, P., Dong, K.D., Madrigal, A.A., Underwood, J.G., and Yeo, G.W. (2021). Robust single-cell discovery of RNA targets of RNA-binding proteins and ribosomes. Nat. Methods *18*, 507–519.

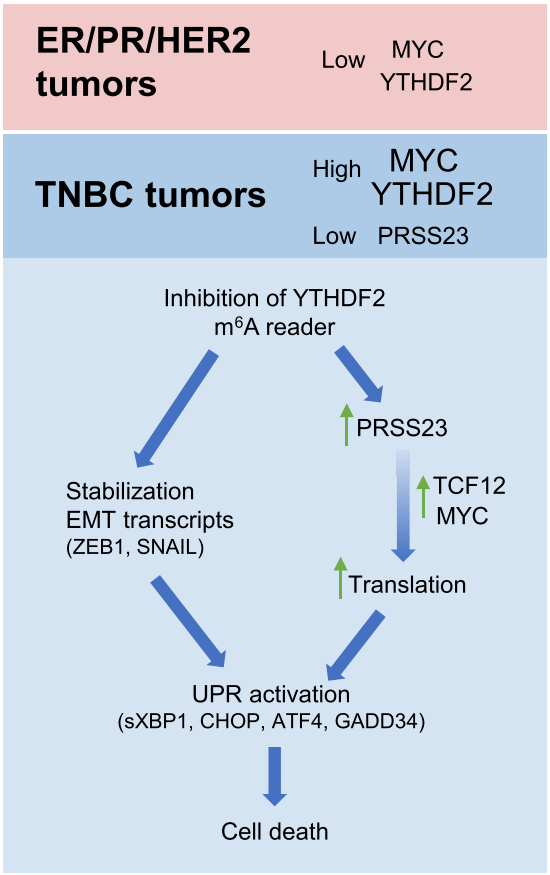

Figure 1. Novel role of YTHDF2 in triple negative breast cancer

High expression of the m^{6}A reader YTHDF2 in TNBC correlates with poor prognosis. The inhibition of YTHDF2 stabilizes its target mRNAs (PRSS23) and increases protein synthesis rates within cancer cells. Coupled with the stabilization of EMT promoting transcripts, increased mRNA translation places strain on the folding capacity of the ER, activating the UPR and culminating in cancer cell death.

Dixit, D., Prager, B.C., Gimple, R.C., Poh, H.X., Wang, Y., Wu, Q., Qiu, Z., Kidwell, R.L., Kim, L.J.Y., Xie, Q., et al. (2021). The rna m6a reader ythdf2 maintains oncogene expression and is a targetable dependency in glioblastoma stem cells. Cancer Discov. *11*, 480–499.

Einstein, J.M., Perelis, M., Chaim, I.A., Meena, J.K., Nussbacher, J.K., Tankka, A.T., Yee, B.A., Li, H., Madrigal, A.A., Neill, N.J., et al. (2021). Inhibition of YTHDF2 triggers proteotoxic cell death in MYC-driven breast cancer. Mol. Cell *81*, S1097-2765(21)00493-7.

Harvey, R.F., Smith, T.S., Mulroney, T., Queiroz, R.M.L., Pizzinga, M., Dezi, V., Villenueva, E., Ramakrishna, M., Lilley, K.S., and Willis, A.E. (2018). Trans-acting translational regulatory RNA binding proteins. Wiley Interdiscip. Rev. RNA *9*, e1465.

Jiang, X., Liu, B., Nie, Z., Duan, L., Xiong, Q., Jin, Z., Yang, C., and Chen, Y. (2021). The role of m6A modification in the biological functions and diseases. Signal Transduct. Target. Ther. *6*, 74.

Paris, J., Morgan, M., Campos, J., Spencer, G.J., Shmakova, A., Ivanova, I., Mapperley, C., Lawson, H., Wotherspoon, D.A., Sepulveda, C., et al. (2019). Targeting the RNA m^{6}A Reader YTHDF2 Selectively Compromises Cancer Stem Cells in Acute Myeloid Leukemia. Cell Stem Cell *25*, 137–148.e6.

Pizzinga, M., Harvey, R.F., Garland, G.D., Mordue, R., Dezi, V., Ramakrishna, M., Sfakianos, A., Monti, M., Mulroney, T.E., and Willis, A.E. (2019). The cell stress response: extreme times call for post-transcriptional measures. Wiley Interdiscip Rev RNA *11*, e1578.

Wang, X., Lu, Z., Gomez, A., Hon, G.C., Yue, Y., Han, D., Fu, Y., Parisien, M., Dai, Q., Jia, G., et al. (2014). N6-methyladenosine-dependent regulation of messenger RNA stability. Nature *505*, 117–120.