ACKNOWLEDGMENTS

The transcription-related work in the Kraus lab is supported by grants from the NIH/NIDDK (DK058110, DK069710), NIH/NCI (CA251943, CA229487), and the Cancer Prevention and Research Institute of Texas (CPRIT; RP190235, RP190236), and funds from the Cecil H. and Ida Green Center for Reproductive Biology Sciences Endowment to W.L.K.

REFERENCES

Baek, I., Friedman, L.J., Gelles, J., and Buratowski, S. (2021). Single molecule studies reveal branched pathways for activator-dependent assembly of RNA polymerase II pre-initiation complexes. Mol. Cell *81*, 3576–3588.

Chen, H., and Larson, D.R. (2016). What have single-molecule studies taught us about gene expression? Genes Dev. *30*, 1796–1810.

Hou, T.Y., and Kraus, W.L. (2021). Spirits in the Material World: Enhancer RNAs in Transcriptional Regulation. Trends Biochem. Sci. *46*, 138–153.

Koleske, A.J., and Young, R.A. (1994). An RNA polymerase II holoenzyme responsive to activators. Nature *368*, 466–469.

Nguyen, V.Q., Ranjan, A., Liu, S., Tang, X., Ling, Y.H., Wisniewski, J., Mizuguchi, G., Li, K.Y., Jou, V., Zheng, Q., et al. (2021). Spatio-temporal coordination of transcription preinitiation complex assembly in live cells. Mol. Cell *81*, 3560–3575.

Osman, S., and Cramer, P. (2020). Structural Biology of RNA Polymerase II Transcription: 20 Years On. Annu. Rev. Cell Dev. Biol. *36*, 1–34.

Roeder, R.G. (2019). 50+ years of eukaryotic transcription: an expanding universe of factors and mechanisms. Nat. Struct. Mol. Biol. *26*, 783–791.

Roeder, R.G., and Rutter, W.J. (1969). Multiple forms of DNA-dependent RNA polymerase in eukaryotic organisms. Nature *224*, 234–237.

Sabari, B.R. (2020). Biomolecular condensates and gene activation in development and disease. Dev. Cell *55*, 84–96.

Soutourina, J. (2018). Transcription regulation by the Mediator complex. Nat. Rev. Mol. Cell Biol. *19*, 262–274.

Exploiting a key transcriptional dependency: ZMYND8 and IRF8 in AML

Samuel J. Taylor,[1,2] Sriram Sundaravel,[1,2] and Ulrich Steidl[1,*]
[1]Departments of Cell Biology, and of Medicine, Blood Cancer Institute, Albert Einstein Cancer Center; and Ruth L. and David S. Gottesman Institute for Stem Cell Research and Regenerative Medicine, Albert Einstein College of Medicine, Montefiore Medical Center, Bronx, NY, USA
[2]These authors contributed equally
*Correspondence: ulrich.steidl@einsteinmed.org
https://doi.org/10.1016/j.molcel.2021.08.013

In this issue of *Molecular Cell*, Cao et al. (2021) report that AML cells are specifically addicted to an IRF8-MEF2D gene expression network. Furthermore, they identify a chromatin reader, ZMYND8, as the upstream regulator of the IRF8-MEF2D program whose activity is critical for AML cell survival.

Transcriptional addiction is a concept in which cancer cells display an absolute dependence on specific deregulated transcriptional networks to maintain the cancer state during disease progression (Bradner et al., 2017). Even though transcriptional addiction is a well-accepted paradigm, cell-type specific transcription factor (TF) addictions during tumorigenesis are poorly understood. In this issue, Cao et al. explore this gap in knowledge in the context of acute myeloid leukemia (AML) and report that AML cells exhibit an absolute and specific dependence on an IRF8-MEF2D transcriptional network. In addition, the authors uncovered the molecular underpinnings of the IRF8-MEF2D axis regulation. They demonstrate that chromatin reader ZMYND8 directly associates with IRF8 and MYC enhancers to regulate their transcriptional programs. The authors implicate that direct physical association between ZMYND8 and BRD4 is essential for the regulation of essential IRF8 and MYC transcriptional programs.

Extensive molecular studies carried out in the context of aggressive, heterogeneous hematopoietic stem cell malignancies such as AML, have identified hijacking of gene expression networks and alterations of TF levels and functionality as key hallmarks of the disease (Bradner et al., 2017; Tenen, 2003). With the overarching idea that identification of such cancer-specific transcriptional aberrations can pave the way for generation of novel therapeutic options (Thoms et al., 2019; Wheat and Steidl, 2021), the authors employed an elegant CRISPR-Cas9-based genetic screen to systematically uncover novel transcriptional addiction pathways in AML. Specifically, they identified a IRF8-MEF2D transcriptional network to be critical for the survival of AML cells. They found that ablation of IRF8 through genetic knockdown approaches, or targeted protein degradation using a dTAG system, resulted in impaired proliferation of a subset of AML cell lines indicating a strong dependency on an IRF8 transcriptional program. This is a highly interesting finding in the context of prior work implicating IRF8 in myelomonocytic differentiation and AML pathogenesis (Will et al., 2015). Intriguingly, acute IRF8 depletion resulted in the downregulation of MEF2D, which was also identified as an AML essential gene in their CRISPR-Cas9 genetic screen, and depletion of MEF2D resulted in downregulation of IRF8 suggesting a positive feedback loop between these two TFs. Furthermore, chromatin occupancy studies showed

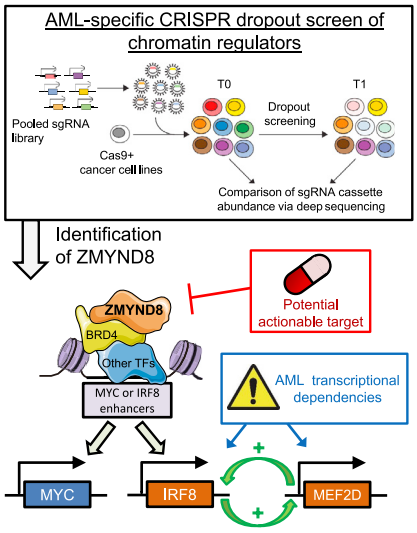

Figure 1. ZMYND8-IRF8 is a transcriptional dependency in AML

ZMYND8 was identified by an AML-specific CRISPR dropout screen to be a crucial chromatin regulator. ZMYND8, in concert with BRD4, maintains leukemia survival via regulation of its key transcriptional circuits (which include MYC and IRF8). The ZMYND8-downstream target, IRF8, is involved in a positive feedback loop with MEF2D, and both factors are AML-specific transcription factor dependencies.

that IRF8 bound to the MEF2D transcriptional start site (TSS), implying a direct role in MEF2D gene regulation by IRF8. Collectively, these findings reveal the IRF8-MEF2D network as a novel transcriptional addiction in AML.

Given that direct pharmacological targeting of TFs has been notoriously challenging (with exceptions emerging for select candidates [Antony-Debré et al., 2017; Shastri et al., 2018]), the authors attempted to identify easier-to-target, upstream chromatin regulators (CRs) of the IRF8-MEF2D transcriptional network. To this end, the authors conducted a CR-domain-focused screen on IRF8 high and low human cancer cell lines including AML. Interestingly, this experiment uncovered ZMYND8 (also known as RACK7) as an AML-specific dependency. Ablation of ZMYND8 resulted in abrogation of AML cell proliferation *in vitro* and *in vivo*, leading to a significant improvement in survival of mice in AML xenograft models. Mechanistically, ZMYND8 facilitated the maintenance of a leukemic state by activating the IRF8 and MYC transcriptional programs in parallel. Most importantly, genetic depletion of ZMYND8 in normal hematopoietic stem cells (HSCs) did not impact myeloid differentiation *in vitro*, suggesting a unique therapeutic vulnerability of AML cells by targeting ZMYND8.

One of the common mechanisms by which CRs regulate TFs is by binding to chromatin cis-elements (Sur and Taipale, 2016). Specifically, CRs can bind to enhancer regions of TFs alone or in association with co-binding proteins to regulate their transcription. In that regard, another important finding of this study pertains to the molecular mechanism by which ZMYND8 regulates IRF8 transcription. Upon careful examination of the non-coding regions flanking the IFR8 locus, the authors identified a potential IRF8 enhancer (IE), which displayed H3K27ac marks 23-86kb downstream of the IRF8 TSS. Within this 23-86kb region, the +81-86kb locus seemed to be a critical site of regulation because ablation of ZMYND8 binding to this site by CRISPR interference resulted in significant reduction of IRF8 expression and proliferation in AML cells. Likewise, Cao et al. found that ZMYND8 regulates MYC expression through similar mechanisms by associating with MYC enhancers. The authors identified BRD4 as a co-activator that is essential for MYC and IRF8 activation by ZMYND8. Through a series of co-immunoprecipitation experiments, they demonstrate that the PHD-BD-PWWP (Plant Homeodomain-Bromodomain-Pro Trp Trp Pro) reader cassette of ZMYND8 directly interacted with the ET (extra-terminal) domain of BRD4. This interaction was critical for both regulation of IRF8 and MYC, as disrupting the ZMYND8-BRD4 interaction by mutating the PHD-BD-PWWP domain of ZMYND8 resulted in abrogation of AML cell proliferation *in vitro* and *in vivo*. Overall, the authors propose this as a mechanism of broader importance as they also observed a similar regulation of IRF8 and MYC by ZMYND8 in primary AML samples.

In conclusion, this work by Cao et al. significantly advances our understanding of TF dependencies in cancer not only by uncovering IRF8-MEF2D as an AML-specific transcriptional network addiction, but also by uncovering ZMYND8 as a potentially actionable upstream regulatory candidate with translational potential (Figure 1). These advances are enabled by cutting-edge experimental tools including targeted *in vivo* CRISPR screens and systems to interrogate "fast TF biology" such as PROTAC technology (Stengel et al., 2021). While questions remain, including whether ZMYND8 inhibition may affect other healthy tissues, future investigations geared toward developing agents to directly target ZMYND8 and/or the essential ZMYND8-BRD4 interaction are warranted. Overall, the study by Cao and colleagues highlights the utility and power of identifying and targeting aberrant key transcriptional regulators in AML using state-of-the-art technologies, and serves as an excellent resource and road map to identify novel transcriptional dependencies in cancer in the future.

REFERENCES

Antony-Debré, I., Paul, A., Leite, J., Mitchell, K., Kim, H.M., Carvajal, L.A., Todorova, T.I., Huang, K., Kumar, A., Farahat, A.A., et al. (2017). Pharmacological inhibition of the transcription factor PU.1 in leukemia. J. Clin. Invest. *127*, 4297–4313.

Bradner, J.E., Hnisz, D., and Young, R.A. (2017). Transcriptional Addiction in Cancer. Cell *168*, 629–643.

Cao, Z., Budinich, K.A., Huang, H., Ren, D., Lu, B., Zhang, Z., Chen, Q., Zhou, Y., Huang, Y.-H., Alikarami, F., et al. (2021). ZMYND8-regulated IRF8 transcription axis is an acute myeloid leukemia dependency. Mol. Cell *81*, 3604–3622.

Shastri, A., Choudhary, G., Teixeira, M., Gordon-Mitchell, S., Ramachandra, N., Bernard, L., Bhattacharyya, S., Lopez, R., Pradhan, K., Giricz, O., et al. (2018). Antisense STAT3 inhibitor decreases viability of myelodysplastic and leukemic stem cells. J. Clin. Invest. *128*, 5479–5488.

Stengel, K.R., Ellis, J.D., Spielman, C.L., Bomber, M.L., and Hiebert, S.W. (2021). Definition of a small core transcriptional circuit regulated by AML1-ETO. Mol. Cell *81*, 530–545.e5.

Sur, I., and Taipale, J. (2016). The role of enhancers in cancer. Nat. Rev. Cancer *16*, 483–493.

Tenen, D.G. (2003). Disruption of differentiation in human cancer: AML shows the way. Nat. Rev. Cancer *3*, 89–101.

Thoms, J.A.I., Beck, D., and Pimanda, J.E. (2019). Transcriptional networks in acute myeloid leukemia. Genes Chromosomes Cancer *58*, 859–874.

Wheat, J.C., and Steidl, U. (2021). Gene Expression at a Single Molecule Level: Implications for MDS and AML. Blood. Published online June 24, 2021. https://doi.org/10.1182/blood.2019004261.

Will, B., Vogler, T.O., Narayanagari, S., Bartholdy, B., Todorova, T.I., da Silva Ferreira, M., Chen, J., Yu, Y., Mayer, J., Barreyro, L., et al. (2015). Minimal PU.1 reduction induces a preleukemic state and promotes development of acute myeloid leukemia. Nat. Med. *21*, 1172–1181.