the locus SubTAD by chromatin immunoprecipitation sequencing (ChIP-seq). Contact between the PU.1 gene and the URE occurred in parallel with an increase in PU.1 binding at the gene promoter and at the URE. The authors next engineered THP-1 cells with a doxycycline (dox)–inducible knockdown of PU.1. By reducing PU.1 expression either before or after induction of differentiation, they found that PU.1 was required to initiate the *PU.1*/URE gene contact but was dispensable after the contact was established.

This result raised the question of what was involved in maintaining the contact. LDB1 was investigated based on its role in enhancer looping in erythroid cells. LDB1 is a widely expressed nuclear protein that was originally identified as a cofactor for LIM-homeodomain and LIM-only proteins that have fundamental roles in development. LDB1-null mice die at embryonic day 8.5 with severe developmental defects in multiple systems and a lack of red blood cells.[5] In erythroid cells, LDB1 forms a complex with DNA-binding proteins SCL/TAL1 and GATA1 and bridging protein LMO2, an erythroid LIM-only protein. The complex links globin gene promoters to the β-globin locus control region enhancer by LDB1 self-interaction and functions as the primary mediator of global erythroid gene activation.[6,7]

Prior to Schuetzmann et al, the role of LDB1 in myeloid cells had been unclear. We now learn that LDB1 is expressed in THP-1 cells and is upregulated during differentiation. Furthermore, LDB1 is recruited to several positions within the PU.1 SubTAD, including the gene promoter and URE. Inducible knockdown of PU.1 resulted in substantial loss of LDB1 in the locus, indicating that PU.1 is required for LDB1 recruitment, although the effect may be indirect since PU.1 and LDB1 were not found to interact. Importantly, dox-inducible knockdown of PU.1 or LDB1 revealed distinctive functions. After differentiation-induced *PU.1*/URE contact was established, reduction of LDB1 resulted in lost contact, whereas PU.1 reduction had no effect. Therefore, contacts are maintained by LDB1. The figure depicts a speculative model incorporating these results.

This report affects several different lines of research. First, the work reinforces autoregulation of the master regulator of myelopoiesis PU.1, and we learn that its

long-range contacts to the URE enhancer correlate with its expression in normal and AML cells. To establish a causal role, it would be interesting to see if an LDB1 targeting approach, like that taken by Deng et al,[8] could reconnect PU.1 to its enhancer in AML cells and lead to a more normal cellular phenotype. Second, we learn that LDB1 both establishes and maintains PU.1 gene contact to the URE enhancer. This result nicely expands the mechanistic role of LDB1 in enhancer looping from erythroid cells to target genes characteristic of the myeloid lineage.

How is LDB1 recruited to the PU.1 gene and the URE? LDB1 requires DNA-binding partners and a LIM-domain cofactor to form an active complex and has a distinct E box/GATA compound motif recognized by SCL and GATA1 in erythroid cells.[7] What motif underlies its sites of occupancy in myeloid cells that result in targeting of myeloid-specific genes and enhancers? Proteomics would be a complementary approach to reveal the nature of specific differences between the erythroid and myeloid LDB1 complexes. The more we understand about how long-range enhancer contacts are engineered, the more we will be able to use the information for therapeutic interventions.

Conflict-of-interest disclosure: The author declares no competing financial interests. ∎

REFERENCES

1. Schuetzmann D, Walter C, van Riel B, et al. Temporal autoregulation during human PU.1 locus SubTAD formation. *Blood*. 2018;132(25):2643-2655.

2. Krumm A, Duan Z. Understanding the 3D genome: emerging impacts on human disease [published online ahead of print 12 July 2018]. *Semin Cell Dev Biol*. doi:10.1016/j.semcdb.2018.07.004.

3. Staber PB, Zhang P, Ye M, et al. Sustained PU.1 levels balance cell-cycle regulators to prevent exhaustion of adult hematopoietic stem cells. *Mol Cell*. 2013;49(5):934-946.

4. Phanstiel DH, Van Bortle K, Spacek D, et al. Static and dynamic DNA loops form AP-1-bound activation hubs during macrophage development. *Mol Cell*. 2017;67(6):1037-1048.e6.

5. Mukhopadhyay M, Teufel A, Yamashita T, et al. Functional ablation of the mouse Ldb1 gene results in severe patterning defects during gastrulation. *Development*. 2003;130(3):495-505.

6. Krivega I, Dale RK, Dean A. Role of LDB1 in the transition from chromatin looping to transcription activation. *Genes Dev*. 2014;28(12):1278-1290.

7. Li L, Freudenberg J, Cui K, et al. Ldb1-nucleated transcription complexes function as primary mediators of global erythroid gene activation. *Blood*. 2013;121(22):4575-4585.

8. Deng W, Rupon JW, Krivega I, et al. Reactivation of developmentally silenced globin genes by forced chromatin looping. *Cell*. 2014;158(4):849-860.

LYMPHOID NEOPLASIA

Comment on Kabrani et al, page 2670

Nuclear Fox(O1): not so fantastic

Marc A. Weniger | University of Duisburg-Essen

In this issue of *Blood*, Kabrani et al[1] report that the retained nuclear localization of the transcription factor forkhead box class O1 (FOXO1) is a lymphoma-promoting, oncogenic event in the pathogenesis of Burkitt lymphoma (BL). Recurrent mutations in FOXO1 have been reported in ~10% of diffuse large B-cell lymphoma (DLBCL) and BL cases,[2] but to date the functional consequences of these mutations in the context of germinal center (GC) B-cell lymphomagenesis remained incompletely understood.

FOXO1 plays an important role in early B-cell development as well as in the differentiation of GC B cells in the dark zone.[3,4] Several lines of evidence support a tumor suppressor role of FOXO1 in the

development of hematopoietic malignancies, for example, in classical Hodgkin lymphoma.[5] Mutations in FOXO1 in DLBCL however not only were reminiscent of oncogenic rather than tumor suppressor
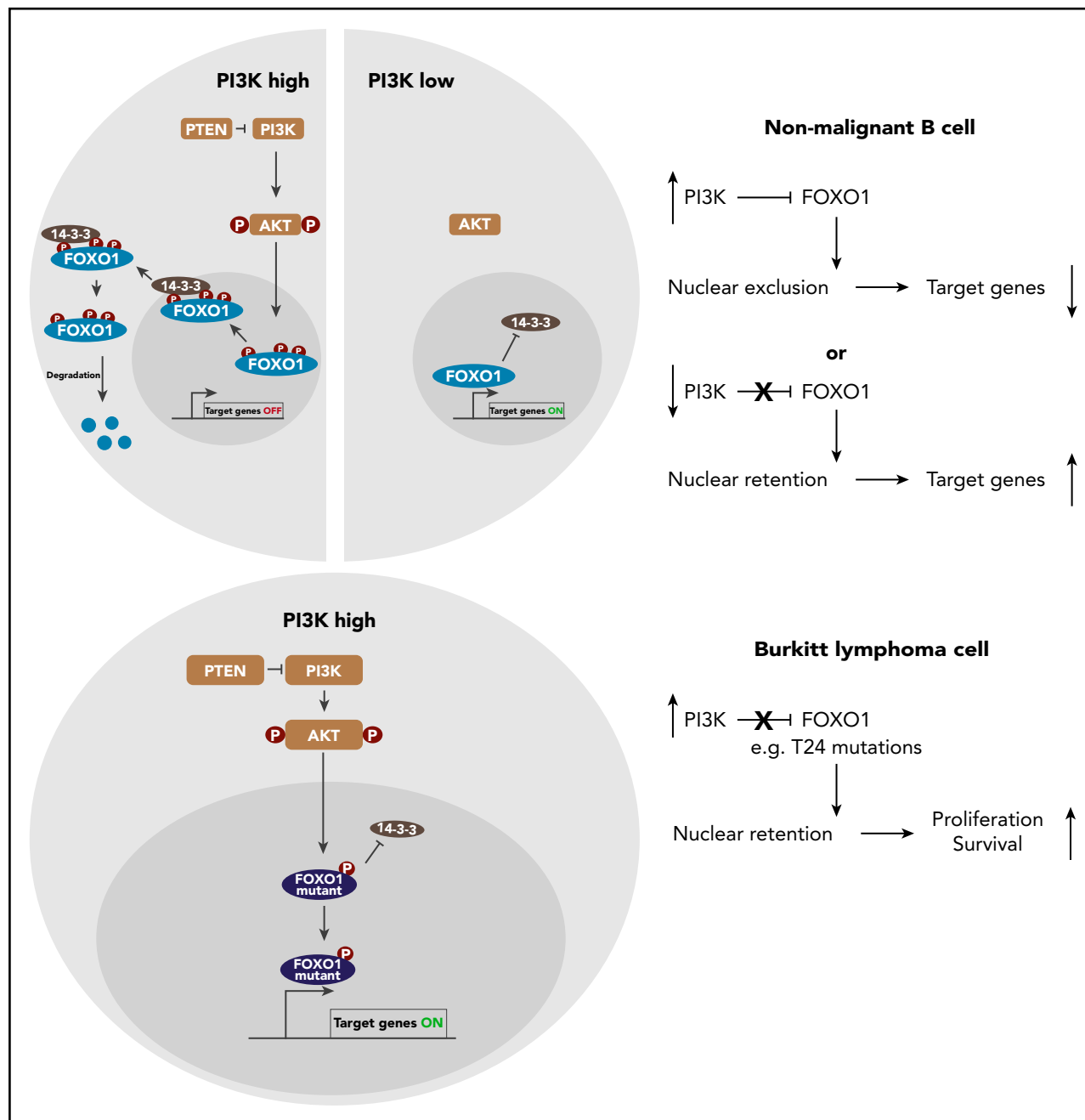
Activity of FOXO1 in nonmalignant and Burkitt lymphoma cells. High phosphoinositide-3-kinase (PI3K) kinase activity in nonmalignant cells leads to FOXO1 phosphorylation (P), followed by its 14-3-3 protein-associated nuclear export and proteasomal degradation, whereas FOXO1 regulates target genes in the absence of PI3K signaling. In Burkitt lymphoma cells, mutations in FOXO1 prevent its export from the nucleus in the presence of constitutive PI3K activity, promoting survival and proliferation.

mutations (few hotspots mutations that cause amino acid exchanges rather than a broad distribution of destructive mutations) but also were also shown to maintain nuclear localization and possibly transcription factor activity.[6]

Phosphorylation by the Akt kinase critically controls FOXO1 localization, as 14-3-3 proteins bind to the phosphorylated site of FOXO1 and assist its nuclear export. FOXO1 contains 3 Akt recognition motifs (consensus sequence RXRXXS/T), of which the most N-terminal one around threonine 24 (T24) and the one in the forkhead box domain around serine 256 (S256) are crucial for FOXO1 nuclear export (see figure).[7]

PI3K/Akt signaling downstream of constitutive B-cell receptor signaling, which is at least in part due to mutations in TCF3/ID3, cooperates with high MYC expression due to chromosomal translocations to promote BL development.[8,9] It is therefore expected that FOXO1 is continuously phosphorylated and exported from the nucleus. However, nuclear FOXO1 was detected in cells from human BL-derived cell lines and even in the lymphoma cells from a BL mouse model that is driven by constitutive MYC and P110 transgene expression.[9] Kabrani et al then identified recurrent mutations in FOXO1 in human BL-derived cell lines and in murine BL-like cells (similar to the ones in DLBCL) that prevented phosphorylation by Akt

including T24 amino acid exchanges. Consistent with an earlier study,[6] these mutations caused the predominantly nuclear localization of mutated FOXO1 also in BL cells, yet FOXO1 was detected in the nucleus of cells of the human BL cell lines independent of its mutation status.

Murine BL-like cells with T24 mutation but not with wild-type FOXO1 status showed increased cell death and impaired cell-cycle progression after Cas9/gRNA–mediated FOXO1 deletion. Of note, murine BL-like cells with mutated FOXO1 were still dependent on PI3K signaling as in-frame mutations following CRISPR/Cas9–mediated knockout of the P110 transgene showed preferential selection over time.

Gene expression profiling analysis of murine BL-like cells after Cas9-editing of FOXO1 revealed a "dominant effect" of mutated FOXO1 (independent of wild-type allele) on a subset of genes: cell cycle, cell division, and proliferation ontologies were enriched in BL cells with mutated FOXO1 and genes lower expressed in these cells included regulators of apoptosis and metabolism. These seem obvious functional targets, but a contribution of other processes that FOXO1 is involved in, such as metabolism, autophagy, and oxidative stress,[5] cannot be excluded. Mutant, nuclear FOXO1 likely maintains the expression of the target genes in BL cells that FOXO1 induces in dark zone GC B cells, suggesting that mutant and wild-type FOXO1 are transcriptionally and functionally similar. The T24 FOXO1 mutation did compromise S256 phosphorylation and 14-3-3 binding, but other posttranslation modifications (ie, lysine acetylation and O-glycosylation) were not different from those of wild-type FOXO1 in BL cells.

To determine whether nuclear, wild-type FOXO1 is sufficient to support lymphoma cell growth even in the presence of T24 phosphorylation, the authors created a FOXO1 mutant that eliminated the nuclear export signal, FOXO1-L375A. Expression of FOXO1-L375A in a FOXO1-knockout background of FOXO1-mutated murine BL cells (because they require nuclear FOXO1) was able to functionally compensate for T24-mutated FOXO1 and support lymphoma cell growth. Whether nuclear FOXO1 in primary human BL samples may be of clinical relevance and may serve as prognostic marker[6] remains to be clarified.

The findings by Kabrani and colleagues shed light on the functional role of FOXO1 in BL pathogenesis. Oncogenic FOXO1 activity in the presence of constitutive PI3/Akt signaling was mediated at least in part due to mutations that cause nuclear retention of the transcription factor (see figure). At the same time, the mechanism(s) that grant coexistence of nuclear FOXO1 and PI3K/Akt activity in the absence of FOXO1 mutations require further investigation; certainly, other signaling pathways affect FOXO1 shuttling.[5,7] With respect to the "2 faces" of FOXO1, it is tempting to speculate that similar to the situation in other cancers[10] the spatial-temporal activity and optimal balance of FOXO1 tip the balance toward either tumor promotion or tumor suppression.

Conflict-of-interest disclosure: The author declares no competing financial interests. ∎

REFERENCES

1. Kabrani E, Chu VT, Tasouri E, et al. Nuclear FOXO1 promotes lymphomagenesis in germinal center B cells. *Blood.* 2018;132(25):2670-2683.

2. Pasqualucci L, Dalla-Favera R. Genetics of diffuse large B-cell lymphoma. *Blood.* 2018;131(21):2307-2319.

3. Sander S, Chu VT, Yasuda T, et al. PI3 kinase and FOXO1 transcription factor activity differentially control B cells in the germinal center light and dark zones. *Immunity.* 2015;43(6):1075-1086.

4. Dominguez-Sola D, Kung J, Holmes AB, et al. The FOXO1 transcription factor instructs the germinal center dark zone program. *Immunity.* 2015;43(6):1064-1074.

5. Ushmorov A, Wirth T. FOXO in B-cell lymphopoiesis and B cell neoplasia. *Semin Cancer Biol.* 2018;50:132-141.

6. Trinh DL, Scott DW, Morin RD, et al. Analysis of FOXO1 mutations in diffuse large B-cell lymphoma. *Blood.* 2013;121(18):3666-3674.

7. Van Der Heide LP, Hoekman MF, Smidt MP. The ins and outs of FoxO shuttling: mechanisms of FoxO translocation and transcriptional regulation. *Biochem J.* 2004;380(Pt 2):297-309.

8. Schmitz R, Young RM, Ceribelli M, et al. Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. *Nature.* 2012;490(7418):116-120.

9. Sander S, Calado DP, Srinivasan L, et al. Synergy between PI3K signaling and MYC in Burkitt lymphomagenesis. *Cancer Cell.* 2012;22(2):167-179.

10. Hornsveld M, Smits LMM, Meerlo M, et al. FOXO transcription factors both suppress and support breast cancer progression. *Cancer Res.* 2018;78(9):2356-2369.