A *nu* mouse model of diffuse large B-cell lymphoma in constitutional *Atm* loss

Laura K. Hilton

Center for Lymphoid Cancer, BC Cancer Research Center, Vancouver, British Columbia, Canada

Correspondence: L.K. Hilton
lhilton@bccrc.ca

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In this issue, Davies *et al*. present a new mouse model of B-cell lymphomagenesis in constitutional *Atm* loss.[1] ATM is a serine/threonine kinase that responds to DNA double stranded breaks by phosphorylating p53 and other targets to promote cell cycle arrest and/or apoptosis.[2] Biallelic loss of *ATM* in humans causes Ataxia Telangiectasia (A-T), a congenital disorder associated with high rates of cancer, most often diffuse large B-cell lymphoma (DLBCL).[2] Somatic mutations of ATM are also found in human B-cell lymphomas, especially mantle cell lymphoma, but occur at a lower frequency in sporadic DLBCL.[3,4] In mouse models, *Atm*$^{-/-}$ alone leads to the early development of thymomas, while the complete abrogation of T cells in an ATMKO.CD3εKO mouse leads to the development of DLBCL-like lymphomas.[5,6] By crossing *Atm*$^{-/-}$ mice with nude (*nu*$^{-/-}$) mice, which have incomplete loss of T cells and retain the ability to form germinal centers, Davies *et al*. were able to study B-cell lymphomas in a model system that more closely resembles the pathology of A-T in humans.

In order to understand the biology of DLBCL tumors arising in *Atm*$^{-/-}$ *nu*$^{-/-}$ mice, the authors first used RNA sequencing to show that most *Atm*$^{-/-}$ *nu*$^{-/-}$ DLBCL cluster with the activated B-cell-like (ABC) cell-of-origin (COO) subtype of sporadic human DLBCL, similar to the ATMKO.CD3EKO mouse model.[5] Cytogenetic analysis identified several rearrangements in each tumor, consistent with genomic instability expected in *Atm* loss. Differential expression and gene set enrichment analysis comparing DLBCL tumors to splenic B cells from *Atm*$^{-/-}$ *nu*$^{-/-}$ mice identified several key pathways upregulated in the lymphomas, including cell cycle regulation, MYC targets, and DNA repair. Although the *Myc* transcript itself was not differentially expressed, MYC protein was significantly elevated relative to control non-tumor B cells, suggesting a non-transcriptional mechanism of MYC overexpression leading to MYC pathway activation in these lymphomas.

Next, the authors performed a genome-wide CRISPR screen in two *Atm*$^{-/-}$ *nu*$^{-/-}$ lymphoma cell lines with the highest MYC expression. In total, 197 genes were essential in both cell lines, and these were enriched for pathways involved in nucleotide metabolism and DNA replication and repair. Having demonstrated that several such pathways are both upregulated and essential in *Atm*$^{-/-}$ *nu*$^{-/-}$ lymphomas, the authors went on to explore the effects of four drugs targeting these pathways: a MYC inhibitor (MYCi361); an inhibitor of *de novo* purine synthesis (MMF); a WEE1 inhibitor (AZD1775), which inhibits dNTP synthesis by promoting the degradation of the ribonucleotide reductase subunit RRM2; and a NEDD8 inhibitor (pevonedistat) that stabilizes RRM2. MMF and AZD1775 displayed dose-dependent synergistic cytotoxicity caused by replication fork stalling and apoptosis in *Atm*$^{-/-}$ *nu*$^{-/-}$ lymphomas (Figure 1), and this synergy was partially rescued by either MYCi, dNTP supplementation, or NEDD8i. This finding replicated in several human DLBCL cell lines, with the most potent cytotoxicity observed in the cell lines with the highest MYC expression levels.

MYC is a transcription factor that drives the expression of genes in several cell proliferation and growth pathways, many of which overlap with upregulated pathways identified in *Atm*$^{-/-}$ *nu*$^{-/-}$ lymphomas.[7] In normal germinal centers (GC), MYC is only transiently expressed at the initiation of GC formation and when GC B cells are transitioning from the GC light zone, where antigen selection takes place, to the dark zone, where surviving cells undergo rounds of proliferation and B-cell receptor hypermutation.[7] Enhancer hijacking rearrangements that place MYC adjacent to an immunoglobulin locus or other highly expressed genes occur in 100% of Burkitt lymphomas (BL) and 15-20% of sporadic DLBCL, where it is associated with more aggressive disease.[7] Although Davies *et al*. observed several rearrangements near the *Myc* gene in *Atm*$^{-/-}$ *nu*$^{-/-}$ lymphomas, none appeared to directly increase expression of the *Myc* transcript, alluding to a novel and still-unresolved mechanism underlying MYC dysregulation in these tumors.

Paradoxically, MYC also drives pro-apoptotic gene expression programs; therefore MYC rearrangements require cooperative

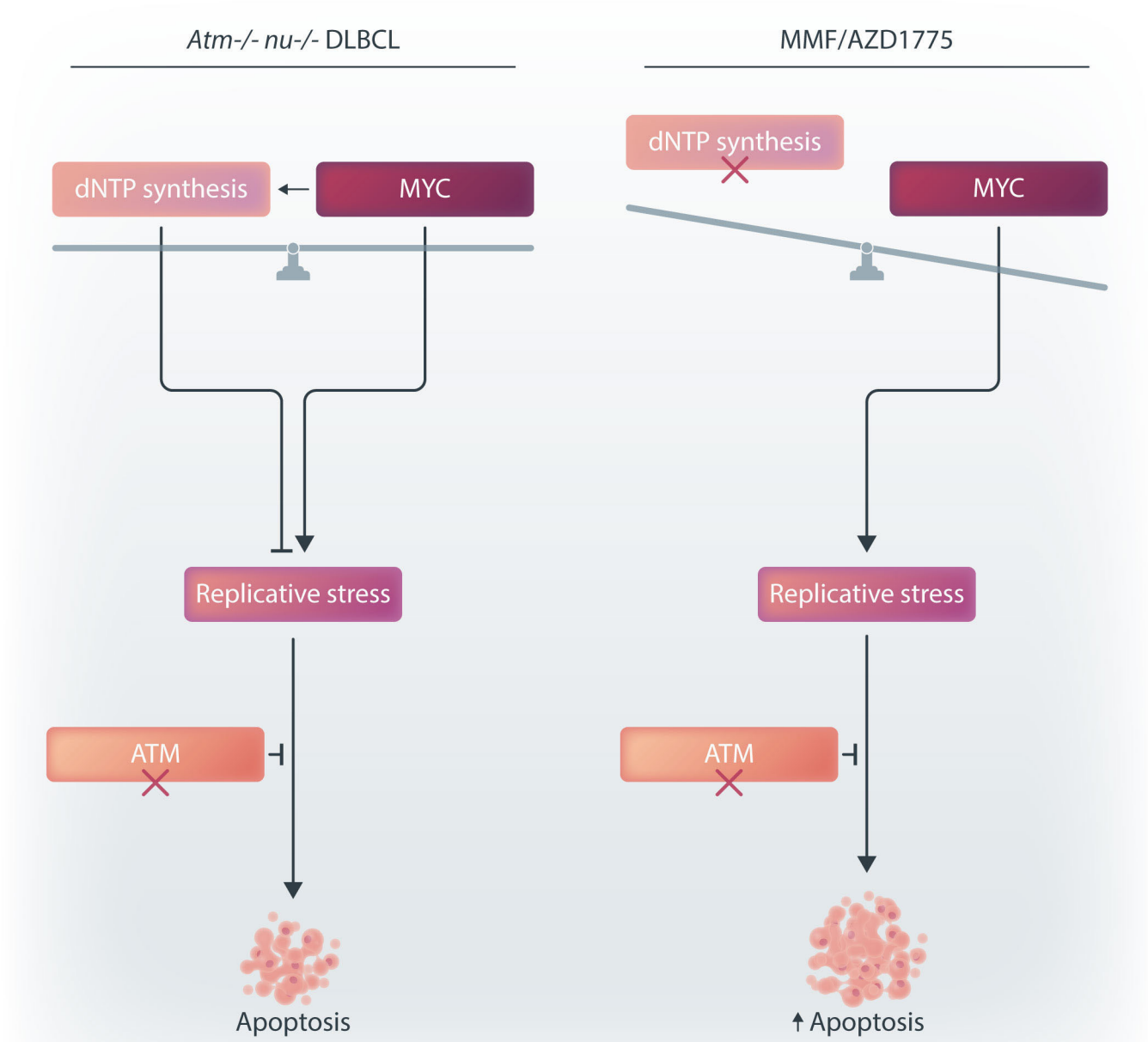

Figure 1. Effects of MYC overexpression in *Atm* loss. Left panel: *Atm* loss is permissive for high MYC expression and activity, which drives high rates of proliferation dependent on continued dNTP synthesis. Without ATM, lymphoma cells do not undergo apoptosis in response to the replicative stress induced by high MYC activity. Right panel: when dNTP synthesis is inhibited, cells can no longer compensate for replicative stress and undergo apoptosis. DLBCL: diffuse large B-cell lymphoma.

survival-promoting alterations such as *TCF3/ID3/CCND3* mutations in BL or *BCL2* rearrangement in high-grade B-cell lymphoma double hit with *MYC* and *BCL2* rearrangements (HGBCL-DH-*BCL2*).[7] Notably, *Atm* loss-of-function is another established anti-apoptotic event that is permissive for MYC overexpression,[8] therefore congenital *Atm* loss appears to enable excessive MYC activity and lymphomagenesis. Future studies could explore whether somatic *ATM* loss might similarly contribute to MYC activity in sporadic DLBCL, or whether *Atm*$^{-/-}$ *nu*$^{-/-}$ lymphomas remain dependent on *Atm* loss after transformation. In addition, genomic studies in DLBCL point to the existence of different founder mutations that lead to the formation of lymphomas representing distinct genetic subgroups.[9,10] Studying the mutational profile and evolutionary trajectory of *Atm*$^{-/-}$ *nu*$^{-/-}$ lymphomas through whole genome sequencing may provide new insights into the process of lymphomagenesis with *Atm* loss as a unique founder mutation.

A promising aspect of this study is the finding that nucleotide biosynthesis may be an underexplored vulnerability in A-T and MYC overexpressing lymphomas. Patients with A-T are uniquely sensitive to chemo- and radiotherapy due to the inherent genomic instability associated with ATM loss. In sporadic lymphoma, while patients with HGBCL-DH-*BCL2* have inferior outcomes to R-CHOP chemoimmunotherapy, no biologically targeted therapies for front line treatment have yet been developed. Targeted therapies (including AZD1775/adavosertib, which is under active clinical development) that exploit the synthetic lethality created by dNTP depletion with high MYC activity may be valuable tools for the treatment of the most aggressive and difficult to treat lymphomas.

Disclosures

No conflicts of interest to disclose.

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