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HEMATOPOIESIS & STEM CELLS

Comment on Xiao et al, page 941

Stem cell maintenance: aMPLe splicing choices

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In this issue of *Blood*, Xiao et al provide a direct link between the OTT protein and regulation of splicing of the RNA for c-MPL, which encodes the thrombopoietin receptor.[1]

OTT was first identified as part of the recurrent t(1;22) translocation in acute megakaryoblastic leukemia (AMKL) and was called OTT for "one twenty-two." OTT1 is also known as RNA binding motif protein 15 (RBM15). Like other RNA binding motif proteins, RBM15 contains RNA recognition motifs (RRMs), 3 in total, which can bind to RNA and, at least for some RRMs, may also bind to DNA. Lifelong maintenance of hematopoietic stem cell (HSC) numbers is essential, and the genes for Ott and for Mpl are required for such maintenance. In prior studies from Dr Glen Raffel's laboratory at the University of Massachusetts and other laboratories, Rbm15 expression was shown to be critical for maintenance of HSCs in vivo in mice under stress.[2] Loss of Rbm15 led to increased cycling and subsequent exhaustion of HSCs under stress conditions. In the current work, Xiao et al[1] suggest a potential mechanism leading to this stem cell exhaustion.

Mpl, the receptor for thrombopoietin (Thpo), is expressed on hematopoietic stem and progenitor cells as well as on cells of the megakaryocyte lineage. Like Rbm15 knockout (KO) mice, mice lacking expression of either Mpl or Thpo develop exhaustion of the HSC pool. The full-length *Mpl* mRNA encodes the full-length transmembrane protein (Mpl-FL). However, a splice variant of Mpl that lacks exons 9 and 10 encodes a truncated Mpl protein (Mpl-TR), which lacks the transmembrane and intracellular domains.

In HSCs from Rbm15 KO mice, the ratio of Mpl-TR to Mpl-FL is greatly increased compared with control HSCs, suggesting that Rbm15 acts to promote the full-length *Mpl* transcript. To address whether expression of truncated Mpl affects the cellular response to Thpo, thereby explaining the stem cell defect in Rbm15 KO HSCs, Xiao et al[1] tested the ability of HSCs to engraft recipient mice in control conditions or conditions in which they overexpress the truncated form of the Mpl protein. As predicted, overexpression of Mpl-TR decreased HSC function in a dominant-negative manner, most likely due to decreased signaling from Thpo in these cells.

Because Rbm15 is known to be a component of the spliceosome, this study tested the hypothesis that Rbm15 may regulate Mpl splicing. Xiao et al[1] demonstrate that Rbm15 indeed binds *Mpl* pre-mRNA, suggesting that Rbm15 promotes exon retention in *Mpl* in HSCs. To dissect the mechanism by which Rbm15 affects exon inclusion, chromatin immunoprecipitation was performed. Confirming their hypothesis that cotranscriptional splicing (pre-mRNA being spliced during the process of transcription) underlies the splicing mechanisms conferred by Rbm15, the investigators found that in addition to binding Mpl pre-mRNA, Rbm15 also binds to regions of the Mpl genomic DNA in the vicinity of the spliced exons. An epigenetic (chromatin-modifying) function for Rbm15 had previously been shown, in that Rbm15 interacts with the Setd1b histone H3K4 methyltransferase (H3K4me).[3] Consistent with this association, H3K4me levels differed on the Mpl locus in Rbm15 wild-type (WT) vs KO cells: in the absence of Rbm15, trimethylated H3K4 (H3K4me3) was markedly reduced in the region shown to bind Rbm15 in WT cells. In contrast, H4 acetylation was increased in the absence of Rbm15. Both such chromatin modifications, H4 acetylation and H3K4me3, are associated with an increased rate of transcription and spliceosome recruitment.[4] The authors suggest that lack of H3K4me3 on the Mpl locus due to absence of Rbm15 results in faster transcription across the region of the alternatively spliced exons, resulting in their exclusion from the final transcript.

Conversely, in the presence of Rbm15 in WT cells, transcription across the Mpl gene is slower, and Mpl-FL, with inclusion of all the exons, is favored. The regulatory role of histone modifications on alternative splicing of Mpl RNA was corroborated by showing that addition of a histone deacetylase inhibitor to WT cells resulted in an increase in the Mpl-TR:Mpl-FL ratio. The study presented here reveals a novel level of regulation of stem cell function by linking transcription and splicing. This finding opens up new therapeutic avenues for stem cell regulation in vivo and in vitro, whereby epigenetic modifiers regulate alternative splicing events.

Now that Xiao et al[1] have shown that RBM15 affects differential splicing of a gene critical for HSC maintenance, the path is cleared for future studies focused on several questions: What other RNAs are differentially spliced in the presence/absence of RBM15? What is the mechanism by which RBM15

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regulates splicing? What is its interaction with the core spliceosome? How is RBM15's association with specific genes regulated? What is the dominant-negative mechanism by which Mpl-TR acts to decrease HSC maintenance? Answers to these questions not only will elucidate critical aspects of the splicing machinery, but will be relevant for our understanding of the role of the RBM15-MKL1 fusion protein in AMKL.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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Comment on Mir et al, page 992

Serum cytokines in follicular lymphoma

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In this issue of *Blood*, Mir et al present data on the prognostic role of cytokines, chemokines, and their ligands measured in serum in patients with follicular lymphoma (FL).[1] This lymphoma has a variable course, and reliable markers for predicting outcome are needed.

FL is the second most common type of non-Hodgkin lymphoma with a highly variable course and with several treatment options. Both clinical features and biological factors, such as properties of the tumor and its microenvironment, have been shown to influence outcome.[2-4] The tumor microenvironment consists of tumor-reactive T cells, follicular dendritic cells, and macrophages, and their activity might be mirrored in the serum. High numbers of $CD8^+$ T cells have been associated with good prognosis, whereas $CD4^+$ T cells (especially inside the follicles) are mostly associated with poor prognosis.[2-4] The entire composition of the microenvironment and the architectural pattern (and not individual subsets) might be best associated with outcome.[5,6] However, data on the prognostic importance of the tumor microenvironment are conflicting, mainly due to heterogeneity in study design and end points, patient selection, and technical aspects of immune cell quantification (flow cytometry or immunohistochemistry with manual or computer-assisted scoring),[7] and because patients are heterogeneously treated.[8] As an example, the addition of the monoclonal anti-CD20 antibody, rituximab, to chemotherapy, has been shown to abrogate the negative impact of macrophages.[9]

Serum markers have previously been suggested to contribute to or mirror the tumor microenvironment in FL.[10] Serum levels of IL-1R1, IL-6, IL-7, IL-10, IL-13, TNF-α, vascular endothelial growth factor (VEGF), and platelet-derived growth factor were increased in 60 FL patients compared with controls. Multivariate analysis identified early stage and high TGF-β levels as independent predictors of improved overall survival, while high lactate dehydrogenase and VEGF levels were independently associated with poorer progression-free survival.[10]

Mir et al now report data on serum levels of multiple cytokines and chemokines, and their receptors, in 2 large patient cohorts.[1] They have used a multiplex enzyme-linked immunosorbent assay and found that elevated levels of IL-2R, IL-1R1, and CXCL9 are associated with shorter event-free survival (EFS) in FL patients treated with chemotherapy or chemoimmunotherapy, whereas IL-1R1 and also IL-12 were associated with a shorter EFS in patients in a wait-and-watch cohort, as well as in patients treated with rituximab monotherapy. These serum factors seem independent of the FL international prognostic index, a validated prognosticator, and might be of great clinical impact, especially as they are easily accessible in blood.

The same research group has previously shown that soluble IL-2Rα facilitates IL-2–mediated immune responses and that elevated soluble IL-2R levels before treatment were associated with reduced survival in FL patients.[11] The prognostic relevance of these findings are now extended to more serum markers in 209 patients, prospectively enrolled on the University of Iowa/Mayo Clinic Specialized Program of Research Excellence Molecular Epidemiology Resource and confirmed in a meta-analysis, also including 183 patients from 3 South West Oncology Group trials.[1]

A problem with the interpretation of the data is that the endpoint used is EFS, which might not be solid enough, and no survival data are presented. The predictive effect of the level of serum markers on response to anti-CD20 antibodies, in this report rituximab, cannot be evaluated because the results are not presented in relation to rituximab administration. Patients with wait-and-watch are grouped together with those receiving rituximab monotherapy and no delineation is made between those with rituximab+chemotheraphy vs those few with chemotherapy alone.

The conclusion that IL-12 and IL-1R1 are predictive for poor outcome in FL patients who are initially observed (or getting rituximab monotherapy) is of interest, but prospective trials are needed to evaluate if early immunochemotherapy will affect the prognosis for patients with such high levels.

In newly diagnosed patients treated with more intensive regimens, Mir et al found that the elevated IL-2R is associated with short EFS.[1] The role of cytokines in lymphoma biology seems complex. A subset of lymphoma B cells express the IL-2R, and IL-2 is a critical homeostatic cytokine required for development, expansion, and activity of regulatory T cells. IL-2 is also necessary for the development of cytotoxic

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