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Editorial

Editorial: **Feel the burn: blocking galectin-12 helps leukemic cells differentiate while staying lean**

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Neoplastic disease continues to represent one of the most formidable challenges in modern medicine. Many neoplastic lesions stem from the cumulative outcome of a variety of genetic mutations, allowing cells to rely on multiple and often redundant pathways to sustain undesirable growth [1]. In contrast, several forms of leukemia appear to result from unique translocation events that lead to the formation of a distinct gene product capable of driving neoplastic transformation. APL, a classic example of this phenomenon, results from a translocation event between chromosomes 15 and 17 that fuses the PML gene with RARα. The chimeric protein product of this translocation causes significant transcriptional repression, which reduces the expression of many genes needed for appropriate leukocyte differentiation [2]. These poorly differentiated cells accumulate, resulting in the increased number of immature promyelocytic blasts observed in patients with APL.

Although APL can be life threatening, the gene product of the 15:17 translocation (PML-RARα) can be targeted by ATRA, resulting in a 72–90% response rate in patients with APL [2]. ATRA serves as a ligand for the RARα domain of the chimeric PML-RARα protein. Engagement by ATRA allows de-repression of PML-RARα-inhibited genes and facilitates the expression of key factors required for APL differentiation [2]. Once differentiated, APL cells become sensitive to the normal turnover programs responsible for leukocyte homeostasis. Thus, ATRA removes the developmental block experienced by APL and in so doing, directly facilitates the very leukocyte differentiation needed to successfully treat this neoplastic disease. Unfortunately, although ATRA represents one of the most successful examples of targeted chemotherapy, not all patients respond to ATRA. This heterogenous response, in part, reflects 15:17 translocation variants that may produce slightly different translocation products, in addition to other types of translocations that generate entirely distinct RARα chimeric proteins [2]. However, even patients who appear to have acquired the same 15:17 translocation can display varied responses to ATRA treatment.

In the postgenomic era of modern medicine, understanding the molecular basis of resistance of APL to ATRA is necessary, not only to predict treatment outcome accurately but also, to tailor therapy to treat these patients most optimally. To address this challenge, Xue et al. examined the expression of galectin-12, a unique regulator of cell-cycle progression, in different types of acute myeloid leukemia [3, 4]. They found that galectin-12 displays significantly increased expression, specifically within the APL subtype of acute myeloid leukemia, implying a unique role for galectin-12 in APL pathogenesis. To examine this possibility, Xue et al. [3] performed a classic knockdown of galectin-12 in the APL cell line, NB4, to determine the fate of APL in the absence of this protein. Knockdown of galectin-12 alone did not impact APL differentiation or survival at baseline, strongly suggesting that galectin-12 does not appear to play a critical role in the PML-RARα-mediated developmental arrest of APL. However, knockdown of galectin-12 did render NB4 cells more sensitive to ATRA-induced differentiation, as evidenced by the increased development of neutrophil-like morphology, CD11b expression, and the assembly of key factors, such as P47, required for production of reactive oxygen species (**Fig. 1**). As a result, this study suggests that galectin-12 may serve as a unique inhibitor of ATRA therapy in patients experiencing APL and therefore, may serve as a useful additional target in patients with APL refractory to ATRA treatment.

In addition to providing important insight into the impact of galectin-12 on the sensitivity of APL to ATRA, these results lay an important framework for understanding fundamental biologic activities of galectin-12. Galectins originally received their name as a result of their unique ability to regulate biologic activity through recognition of distinct carbohydrate ligands [5]. Consequently, the vast majority of studies seeking to elucidate galectin function has focused on their

Abbreviations: APL = acute promyelocytic leukemia, ATRA = all-trans retinoic acid, PML = promyelocytic leukemia, RARα = retinoic acid receptor α

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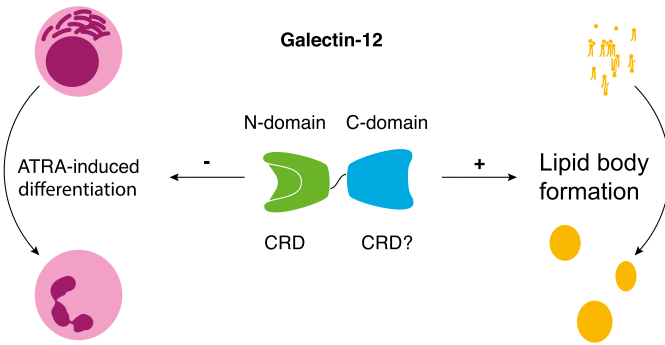

Figure 1. Galectin-12 negatively regulates ATRA-induced APL differentiation, while positively impacting the formation of lipid bodies. Galectin-12 is a tandem repeat galectin, with 2 distinct domains tethered by a peptide linker. Whereas the N-terminal domain contains canonical residues previously shown to be involved in carbohydrate recognition, the C-terminal domain does not possess many of these critical residues. This unique sequence organization of galectin-12 may be, in part, responsible for the distinct activities of this galectin family member in APL. CRD, Carbohydrate recognition domain.

carbohydrate-dependent activity. Although the potential role of carbohydrate recognition on galectin-12-mediated regulation of APL sensitivity to ATRA has not been formally examined, the results are highly consistent with previous studies suggesting that galectin-12 likely regulates a wide variety of signaling pathways through intracellular interactions [6–8]. Therefore, these data contribute to a substantial volume of studies by this group and others, demonstrating that galectins possess significant intracellular activities, nearly all of which occur independent of carbohydrate recognition [9]. Given their name, these intracellular, protein–protein interactions often represent an overlooked yet fundamental aspect of galectin biology [9].

Due to the historical focus on the carbohydrate-binding activity of galectins, much less is known at the biochemical level regarding the binding partners, domains, and overall mechanisms responsible for intracellular galectin-mediated regulation. Previous studies demonstrated that several members of the galectin family do possess key motifs required to engage and regulate intracellular targets [9]. The unique domain structure of galectin-12 may be, in part, responsible for some of its distinct intracellular activities. Similar to several other galectins, galectin-12 possesses 2 putative carbohydrate recognition domains (Fig. 1). Although 1 of these domains is clearly functional, based on the ability of galectin-12 to bind immobilized carbohydrate ligand, the sequence alignment of galectin-12 with the remaining galectin family members suggests that key residues required for carbohydrate recognition are not entirely conserved within the C-terminal domain. These results suggest that the carbohydrate-binding activity of galectin-12 may only reside within the N-terminal domain, whereas the unique C-terminal domain may be responsible for some of the distinct intracellular activities of galectin-12 (Fig. 1). However, it is clearly possible that both domains play key roles in mediating the intracellular functions of galectin-12.

As the direct mechanisms responsible for galectin-12-mediated regulation of APL and possibly leukocytes remain unknown, future studies will provide important insight into the individual requirement of each domain in this process, including the potential involvement of distinct motifs within each domain unique to galectin-12. As previous studies suggest that some galectins can interact directly with lipid molecules [5], galectin-12 may inhibit APL sensitivity to ATRA by directly binding and sequestering ATRA. In addition, given the ability of galectin-12 to localize to the nucleus [10], direct interactions with RARα may prevent ATRA engagement or enhance nuclear localization in a way that renders PML-RARα resistant to ATRA treatment. As galectin-12 regulates a variety of signaling pathways in APL and other cells, it may also regulate ATRA sensitivity by directly impacting cell signaling or ATRA metabolism [6, 7]. A combination of these or completely distinct pathways may ultimately be responsible for the impact of galectin-12 on APL; future studies will now be possible to elucidate how galectin-12 impacts APL sensitivity to ATRA and thus, provide the necessary insight to effectively target galectin-12 in ATRA-resistant APL.

In addition to regulating APL sensitivity to ATRA, galectin-12 appears to play a role in the formation of lipid bodies in cells responsive to ATRA-induced neutrophil differentiation (Fig. 1). These results are consistent with current literature that suggests a critical role for galectin-12 in lipid metabolism [6, 7, 10]. Indeed, the expression pattern of galectin-12 is one of the most restricted of all galectin family members, with significant expression observed in adipocytes and some leukocytes. Therefore, the highly unique C-terminal domain may not only regulate intracellular signaling events required for APL differentiation but might also directly facilitate the fundamental role of galectin-12 in lipid metabolism. In doing so, these results also suggest a common link between general lipid metabolism and the formation of lipid bodies in leukocytes. This is a unique finding, as lipid body formation is linked to the inflammatory function of leukocytes and may imply a previously unrecognized role for galectin-12 in linking general metabolism with the types of inflammation associated with chronic metabolic disorders. Although it remains to be tested whether galectin-12 is similarly required for the formation of lipid bodies in non-neoplastic neutrophils, recent results demonstrated that galectin-12 appears to impact M1 versus M2 macrophage polarization [11], suggesting that galectin-12 may likewise impact neutrophil function. Thus, this study provides fundamental understanding into the pathogenesis of ATRA resistance in APL, with important insight into the potential role of galectin-12 in overall leukocyte function.

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Editorial: **All that you can B: mirn23a regulates B versus myeloid fates**

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Since their discovery [1, 2], miRNAs (or mirns) have steadily gained appreciation as key regulators of gene expression in virtually all eukaryotic cells. These small, noncoding RNAs regulate their target genes through a variety of mechanisms, including transcript turnover and translational rates. In many cases, miRNAs control cellular differentiation programs and may be active in both early development and later, in affecting the function of differentiated cells. Not surprisingly, miRNAs play roles in lymphocyte biology, and within the B lineage, they have been implicated in development, maturation, and function. In this issue of *JLB*, the Dahl laboratory [3] reports results from a knockout mouse that show mirn23a is a key epigenetic regulator of the B versus myeloid fate decision during hematopoiesis (**Fig. 1**).

Whereas transcription factors governing B-lineage specification and commitment have been known for several decades, their complex interplay with epigenetic modifiers and miRNA networks are only now gaining appreciation [4]. For example, the transcription factors PU.1 and Ikaros are well-established players in early B lymphopoiesis. PU.1 is expressed in HSCs, as well as myeloid and lymphoid progenitors, and shifts in PU.1 expression yield altered developmental outcomes, generally favoring higher myeloid-to-lymphoid ratios. HSCs give rise to a CLP pool, which includes a BLP subset. These, in turn, yield the cells in which Ig gene rearrangement begins, termed prepro-B or early pro-B (Hardy fraction A). Ig heavy- and light-chain rearrangements continue through subsequent intermediate and late pro-B stages (Hardy fractions B and C), followed by large (cycling) and small pre-B stages (Hardy fractions C′ and D, respectively), and culminate in the immature B cell, characterized by surface expression of a complete BCR (Hardy fraction E). Transcription factors E2A, FoxO1, EBF1, and Pax5 orchestrate B cell commitment and development through these stages, and several miRNAs play regulatory roles. For example, the miR-17~92 cluster and miR-34a are key players in the pro- to pre-B cell transition [4], and the miR-212/132 cluster targets the Sox4 transcription factor, likely controlling pro-B cell survival and proliferation [5].

Another miRNA cluster, mirn23a (also known as MiRNA23a), was previously identified in a cell-line screen for miRNAs induced by PU.1 [6]. When overexpressed, mirn23a mimicked the ability of PU.1 to promote myeloid differentiation at the expense of B lymphopoiesis. Despite these tantalizing in vitro observations, the role of mirn23a in vivo

Abbreviations: BLP = B cell-biased lymphoid progenitor, CLP = common lymphoid progenitor, CMP = common myeloid progenitor, EBF1 = early B cell factor 1, FoxO1 = forkhead box O1, GMP = granulocyte/monocyte progenitor, HSC = hematopoietic stem cell, LMPP = lymphoid-primed multipotent progenitor, Mef2c = myocyte enhancer factor 2c, MEP = megakaryocyte/erythroid progenitor, miR/mirn/miRNA = microRNA, MPP = multipotent progenitor, MSCV = murine stem cell virus, Pax5 = paired box 5, Trib1/2/3 = Tribbles 1/2/3

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