



MicroRNA master regulator of hematopoiesis may also hold the key to B-cell lymphoma pathogenesis and therapy

Charles H. Lawrie^{1,2,3}

¹Molecular Oncology Group, Biodonostia Research Institute, San Sebastián, Spain; ²IKERBASQUE, Basque Foundation for Science, Bilbao, Spain;

³Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Correspondence to: Charles H. Lawrie. Molecular Oncology Group, Biodonostia Research Institute, San Sebastián, Spain; IKERBASQUE, Basque Foundation for Science, Bilbao, Spain; Radcliffe Department of Medicine, University of Oxford, Oxford, UK. Email: charles.lawrie@biodonostia.org.

Comment on: Kozloski GA, Jiang X, Bhatt S, *et al.* miR-181a negatively regulates NF-κB signaling and affects activated B-cell-like diffuse large B-cell lymphoma pathogenesis. *Blood* 2016;127:2856-66.

Submitted Sep 01, 2016. Accepted for publication Sep 06, 2016.

doi: 10.21037/tcr.2016.10.35

View this article at: <http://dx.doi.org/10.21037/tcr.2016.10.35>

Lymphoma affects over one million people annually and is the fifth largest cause of cancer-associated death worldwide. Diffuse large B-cell lymphoma (DLBCL) accounts for almost half of all lymphomas, and despite great improvements in treatment over the last decade with the introduction of R-CHOP, most patients will eventually die of this malignancy.

DLBCL is a heterogeneous disease characterised by marked clinical, pathological and molecular differences. DLBCL consists of at least two distinct molecular subtypes reflecting the gene expression profile of their respective supposed cell-of-origin; the activated B-cell (ABC)-like and germinal B-cell (GCB)-like groups (1). These subtypes are functionally and genetically distinct; ABC-type DLBCL is characterised by constitutive NF-κB pathway activation, and recurrent *CARD11* mutations and *BLIMP1* inhibition, whereas GC-type DLBCL cases often display switched IgH classes and the presence of specific recurrent genetic lesions such as deletion of PTEN and p53 mutations (2). Importantly, these two groups have a very different clinical course, as typically ABC-type patients are more likely to relapse or display resistance to therapy and generally have a poor prognostic outcome compared to GC-type DLBCL patients (3). Consequently, there has been much interest in understanding the mechanistic basis behind this difference, with most studies focusing on the deregulation of NF-κB activity in ABC-type DLBCL as this represents a viable therapeutic target. Specifically, research has focused on recurrent activating mutations of this pathway including *CARD11*, *CD79B/A*, *MYD88* and *TNFAIP3* genes (4).

In contrast, the recent publication from Kozloski and colleagues describes how a microRNA (miRNA), *miR-181a* acts a negative regulator of the NF-κB signalling pathway in DLBCL leading to the possibility of a loss-of-function therapeutic approach based on over-expression of this miRNA (5).

Interestingly, *miR-181a* was one of the first miRNAs to be associated with haematological pathways and was formally identified by Chen *et al.* in 2004 who cloned this miRNA from murine bone marrow (6). They demonstrated the importance of *miR-181a* in lymphocyte development when they ectopically expressed this miRNA in hematopoietic stem cells (HSCs), and observed a dramatic increase in the proportion of B-lineage cells. Since this seminal research *miR-181a* has emerged as a master regulator of both B-cell and T-cell development, sensitivity and selectivity (7). Surprisingly, despite its key regulatory role in the later stages of B- and T-cell development, widely accepted as the cells of origin of lymphomas, until now the aberrant expression of *miR-181a* has more commonly been associated with leukaemia (8). Therefore, the publication by Kozloski *et al.* provides an interesting and novel insight as to how dysregulation of this miRNA might be a key player in B cell lymphomagenesis that has so far been overlooked.

The authors start by establishing that *miR-181a* is more highly expressed in the supposed cell of origin of GCB-like DLBCL, GC centroblasts, when compared with other B cell sub-populations. Consistent with these findings they establish that GCB-DLBCL cell lines as well as molecularly subtyped GCB-like clinical DLBCL cases express also

higher levels of *miR-181a* than ABC-DLBCL. These data suggest that *miR-181a* is not necessarily aberrantly expressed in GCB-like DLBCL but may simply represent elevated levels observed in the cell of origin, although a direct comparison is not made in the publication and it is a shame levels of the ABC-like counterpart, activated B cells were not included in this study.

As it is well known that NF- κ B activity is much lower in GCB-like DLBCL than ABC-like DLBCL the authors hypothesised that *miR-181a* may be acting as a negative regulator of this system. Using predictive algorithms they identified putative binding sites in four NF- κ B pathway genes; *CARD11*, *NFKB1A* (IKBA), *NFKB1* (P105/P50), *RELA/P65* (RELA) and *REL* (CREL). Using a combination of loss-of-function and gain-of-function approaches they demonstrated that altering *miR-181a* levels indeed affected the protein levels of these targets. Interestingly, when they examined the mRNA levels of these genes, they observed no associated changes in *RELA/P65* transcript levels suggesting that this gene was not a direct target of *miR-181a*. Luciferase assays were used to validate this observation. Next, they looked at the effect on NF- κ B pathway activity in response to *miR-181a* over-expression and demonstrated a significant reduction in activity in all DLBCL cell lines tested irrespective of molecular subtype or the presence of NF- κ B activating mutations. As REL/NF- κ B heterodimer cellular localisation is key to NF- κ B activity, the authors looked at the effect of *miR-181a* over-expression on this characteristic. Consistent with their other results they observed an overall decrease in CREL and P50 in both nuclear and cytoplasmic fractions, however for RELA there was no reduction in the nuclear fraction. This was validated by looking at the ability of NF- κ B proteins from *miR-181a* over-expressed cells to bind the DNA- κ B motif *in vitro* by ELISA, EMSA and super-shift analyses as well as measuring down-stream targets of the transcription factors. All techniques pointed to a decrease in the quantity and consequently function of CREL and P50 but not of RELA, consistent with their previous observations of transcript levels. The authors went further to show that this relation also exists in clinical samples of DLBCL. This provides further evidence that the regulation of RELA at the protein level by *miR-181a* is indirect and occurs by a mechanism yet to be determined. This is clearly an area that warrants a more detailed and thorough investigation particularly as RELA (p65) is such a promising therapeutic target for a number of cancer types (9).

In order to see if there was a subtype-specific effect of

miR-181a it was over-expressed in ABC-DLBCL cell lines (characterised by constitutive NF- κ B activity). This resulted in a marked decrease of proliferation rates and cell viability when compared to GCB-like cell lines suggesting that ABC-DLBCL is more sensitive to *miR-181a* over-expression. As it has been reported that *miR-181a* can directly target BCL2, an anti-apoptotic protein that is intimately associated with B-cell lymphomagenesis, the authors tested whether or not increased proliferation levels could be explained in a similar manner. They demonstrated that the phenotype was independent of direct targeting of BCL2 by *miR-181a*, although consistent with previous reports, they did observe decreased BCL2 levels. Finally, the authors extended their investigation *in vivo* using a xenograft model of *miR-181a* over-expression and demonstrated significantly improved survival of mice xenografted with ABC-DLBCL cell lines but a lesser effect with GCB-DLBCL cell lines.

Besides the clear clinical implications of the publication by Kozloski *et al.*, perhaps the most interesting point of the paper comes from what has not been said. The fact that high expression of *miR-181a* appears to be a feature of normal haematological development rather than lymphomagenesis *per se*, in GCB-like DLBCL, along with a reduced phenotypic effect when *miR-181a* is over-expressed either *in vitro* or *in vivo* in GCB-DLBCL cell lines, suggests that the most relevant aspect of *miR-181a* in the pathogenesis of DLBCL is why its expression is reduced in ABC-like DLBCL. Maybe this will be the subject of the authors' next publication?

Acknowledgments

Funding: CH Lawrie and his research is supported by grants from the IKERBASQUE foundation for science, the Starmer-Smith memorial fund, Ministerio de Economía y Competitividad of Spanish central government and FEDER funds (PI12/00663, PIE13/00048, DTS14/00109, PI15/00275), Departamento de Desarrollo Económico y Competitividad y Departamento de Sanidad of Basque government, Asociación Española Contra el Cancer (AECC), and the Diputación Foral de Guipuzcoa (DFG).

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Peipei Xu (Department of Hematology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China).

Conflicts of Interest: The author has completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2016.10.35>). The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

1. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000;403:503-11.
2. Lenz G, Staudt LM. Aggressive lymphomas. *N Engl J Med* 2010;362:1417-29.
3. Wilson WH, Dunleavy K, Pittaluga S, et al. Phase II study of dose-adjusted EPOCH and rituximab in untreated diffuse large B-cell lymphoma with analysis of germinal center and post-germinal center biomarkers. *J Clin Oncol* 2008;26:2717-24.
4. Roschewski M, Staudt LM, Wilson WH. Diffuse large B-cell lymphoma-treatment approaches in the molecular era. *Nat Rev Clin Oncol* 2014;11:12-23.
5. Kozloski GA, Jiang X, Bhatt S, et al. miR-181a negatively regulates NF- κ B signaling and affects activated B-cell-like diffuse large B-cell lymphoma pathogenesis. *Blood* 2016;127:2856-66.
6. Chen CZ, Li L, Lodish HF, et al. MicroRNAs modulate hematopoietic lineage differentiation. *Science* 2004;303:83-6.
7. Henao-Mejia J, Williams A, Goff LA, et al. The microRNA miR-181 is a critical cellular metabolic rheostat essential for NKT cell ontogenesis and lymphocyte development and homeostasis. *Immunity* 2013;38:984-97.
8. Weng H, Lal K, Yang FF, et al. The pathological role and prognostic impact of miR-181 in acute myeloid leukemia. *Cancer Genet* 2015;208:225-9.
9. Baldwin AS. Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. *J Clin Invest* 2001;107:241-6.

Cite this article as: Lawrie CH. MicroRNA master regulator of hematopoiesis may also hold the key to B-cell lymphoma pathogenesis and therapy. *Transl Cancer Res* 2016;5(Suppl 4):S913-S915. doi: 10.21037/tcr.2016.10.35