UK CLL Forum Virtual Journal Club Review

***MicroRNA-155* influences B-cell receptor signalling and associates with aggressive disease in chronic lymphocytic leukemia.**

Cui B, Chen L, Zhang S, Mraz M, Fecteau JF, Yu J, Ghia EM, Zhang L, Bao L, Rassenti LZ, Messer K, Calin GA, Croce CM, Kipps TJ.

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It is now well accepted that signalling via the cell surface B-cell receptor (BCR) plays a major role in chronic lymphocytic leukaemia (CLL).[1] Overall, the balance between distinct BCR-induced signalling outcomes appears to determine variable clinical behaviour. Thus, anergy, a state of cellular lethargy induced following antigen engagement in the absence of T-cell help, is observed to a variable extent in all CLL, but predominates in M-CLL and is associated with indolent disease. Positive (ie, growth promoting) BCR signalling is most evident in U-CLL and is associated with poor prognosis. The kinases activated downstream of the BCR in CLL cells have been well studied and ibrutinib (BTK inhibitor) and idelalisib (PI3K inhibitor) are now approved for treatment in the US. By contrast, the regulation and function of phosphatases, the counterpoint to kinases, have been relatively little studied. In this interesting paper,[2] Thomas Kipps and colleagues investigated links between the SHIP1 phosphatase and miRNAs, small non-coding RNAs with important regulatory activity in normal and malignant cells. They demonstrate that increased *miR-155* expression is associated with relatively aggressive disease, potentially via effects on SHIP1 and BCR signalling.

SHIP1 is an inositol lipid phosphatase and is an important regulator of PI3K, a major mediator of pro-proliferation and survival signalling downstream of the BCR.[3] Following activation, PI3Ks catalyse the conversion of PI(4,5)P2 to PI(3,4,5)P3 resulting in recruitment and activation of signalling molecules, including AKT, BTK and PLCγ2. SHIP1 is one of several phosphatases which counter PI3K by decreasing levels of PI(3,4,5)P3, catalysing its conversion to PI(3,4)P3. Knock-out studies have clearly demonstrated an inhibitory function for SHIP1 since B-cell specific SHIP1 deficiency results in severe lupus-like disease.[4] SHIP1 deficient B cells are resistant to FcγRIIb-mediated repression of BCR signalling and are hyper-responsive following engagement of sIgM alone. SHIP1 also appears to play an important role in anergy; SHIP1 phosphorylation is enhanced in anergic B cells in mouse models and its deletion restores BCR signalling responsiveness.[4]

The second focus of the work is miRNAs. miRNAs reduce protein expression by inhibition of RNA translation and/or increased RNA degradation via base-pairing to sequence motifs in the 3' untranslated regions of target mRNAs. *miR-155* has emerged as an important player in CLL. Several studies have shown that *miR-155* is overexpressed in CLL cells compared to normal B cells, and that expression is particularly high in U-CLL/ZAP-70 cases.[5,6] *miR-155* has also been shown to be highly expressed in the proliferation centres of CLL/small lymphocytic lymphoma lymph nodes (LN).[7] However, the clinical significance and functional consequences of *miR-155* expression remain incompletely defined.

The first experiments described in the paper investigated the clinical significance of variable *miR-155* expression in an initial cohort of 86 patients, and an extension cohort of 181 patients. In the first cohort, mature *miR-155* was quantified directly using real-time PCR, whereas in the second, the *miR-155* precursor was quantified using array analysis. Consistent with previous studies, relatively high *miR-155* expression was significantly associated with reduced treatment-free and overall survival. Analysis of this relatively large number of samples allowed the authors to probe the relationship between *miR-155* expression and other prognostic markers. *miR-155* expression was generally higher in U-CLL compared to M-CLL, and in ZAP-70 positive compared to ZAP-70 negative samples.

However, there was substantial discordance between *miR-155* expression and these variables, and, in multivariate analysis, *miR-155* expression was an independent predictor of treatment-free survival.

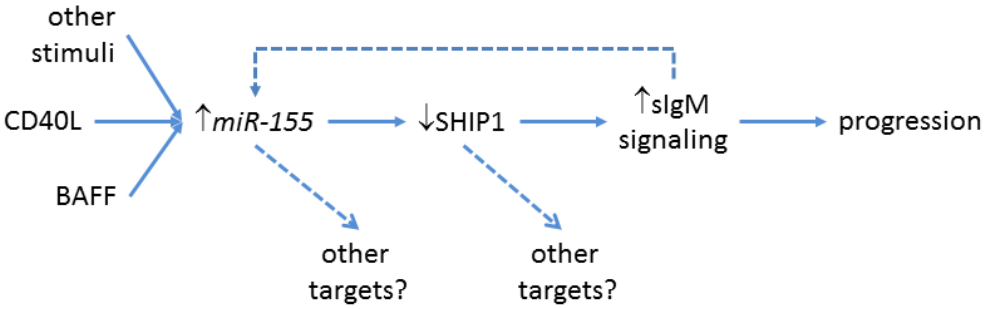
The second series of experiments investigated functional consequences of *miR-155* expression. Although any individual miRNA is likely to have many potential target mRNAs, the study focused on SHIP1, a well characterised *miR-155* target. Using flow cytometry to detect SHIP1 protein, the authors showed that there was an inverse relationship between expression of *miR-155* and SHIP1, consistent with the idea that *miR-155* negatively regulates SHIP1 expression. The authors then used transfection studies to demonstrate directly that *miR-155* negatively regulated SHIP1 in primary CLL cells; overexpression of a *miR-155* precursor decreased SHIP1 protein expression whereas overexpression of *miR-155* inhibitor (*anti-miR-155*) increased expression.

Since SHIP1 is an important regulator of BCR signalling, the authors went on to investigate effects of *miR-155* on responses to anti-IgM, using intracellular Ca^{2+} (iCa^{2+}) mobilisation as a readout of signalling. Relatively high expression of *miR-155* was associated with increased iCa^{2+} mobilisation. Transfection studies with *miR-155* precursor or *miR-155* inhibitor directly demonstrated that modulation of *miR-155* expression altered sIgM signalling.

The authors moved on from *in vitro* studies to probe potential regulation of *miR-155* and SHIP1 *in vivo*. The first approach was to compare expression between blood and LN derived CLL cells by analysing a publically available gene expression array dataset.[8] This demonstrated that *miR-155* expression was higher in LN cells, whereas *SHIP1* RNA has higher in blood cells. This suggested that microenvironmental factors may up-regulate *miR-155* expression *in vivo*. Consistent with this, stimulation of CLL cells *in vitro*, using CD40L or BAFF, increased *miR-155* expression, reduced SHIP1 protein expression, and enhanced anti-IgM-induced iCa^{2+} mobilisation. For CD40L, stimulatory effects on anti-IgM-induced iCa^{2+} mobilisation were suppressed in cells transfected with the *miR-155* inhibitor, demonstrating that effects of CD40L on signalling are at least partly mediated via *miR-155* induction.

The authors also studied intraclonal heterogeneity within CLL blood cells as an indirect approach to probe consequences of tissue interactions. CLL cells enter the blood stream following tissue-based stimulation *in vivo* and therefore carry a temporary imprint of their prior signalling responses.[1] This imprint then decays as the cells circulate. One way to track these events is through analysis of CXCR4 and CD5; previous work by Nicholas Chiorazzi and colleagues has shown that $CXCR4^{dim}CD5^{bright}$ cells have most recently entered the circulation, whereas $CXCR4^{bright}CD5^{dim}$ have been in the circulation for longer periods.[9] The authors sorted cells into $CXCR4^{dim}CD5^{bright}$ and $CXCR4^{bright}CD5^{dim}$ fractions and analysed expression of *miR-155* and SHIP1, and anti-IgM signalling responses. Consistent with the idea that stimulation in tissues leads to reversible *miR-155* induction, this miRNA was expressed more highly in the more recently released $CXCR4^{dim}CD5^{bright}$ subpopulation. These cells also had lower expression of SHIP1 and stronger anti-IgM-induced iCa^{2+} mobilisation.

Overall the study shows that elevated *miR-155* expression has important functional consequences for CLL cells, promoting BCR signalling via down-modulation of SHIP1 (**Figure**). Increased *miR-155* may be a result of stimulation of CLL cells within the LN microenvironment. Enhanced BCR signalling may provide at least a partial explanation for the association between increased *miR-155* expression and poor clinical outcome.



The study raises several interesting additional questions.

- What other stimuli might influence *miR-155* expression? In particular, *miR-155* is itself induced following sIgM stimulation of CLL cells,[10] suggesting the potential presence of a positive, "feed-forward" regulatory circuit.
- What are the other consequences of *miR-155*/SHIP1 alterations? *miR-155* is likely to modulate multiple mRNA targets in CLL cells, and SHIP1 controls signalling via other cell surface receptors independent of the BCR. These modulatory interactions could also influence disease behaviour.
- How is the function of SHIP1 influenced by phosphorylation? SHIP1 phosphorylation is important for controlling the membrane localisation of SHIP1 and, therefore, its access to substrate, but was not addressed in the current study.
- To what extent is down-modulation of SHIP1 achieved by *miR-155*-mediated *SHIP1* RNA degradation versus translational inhibition? If translational control is important, to what extent does analysis of *SHIP1* RNA alone accurately reflect *miR-155* inhibitory effects on SHIP1 protein?
- How is variation in average *miR-155* expression (as measured by PCR or gene expression array) linked to intraclonal heterogeneity? Is *mir-155* differentially expressed between CLL subsets, or do apparent differences actually reflect differences in tumour cell recirculation dynamics? Do samples with high average *miR-155* have a greater proportion of recently released cells with high *miR-155*, rather than increased *miR-155 per se*? Is *miR-155* expression different between U-CLL and M-CLL is analysis if restricted to $CXCR4^{dim}CD5^{bright}$ cells?

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