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Commentary

Written in Blood: Kissing Disease miRNAs Could Predict Outcome of Patients With Chronic Lymphocytic Leukaemia

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Chronic lymphocytic leukaemia (CLL) is the most common adult leukaemia in the Western world and is characterized by the accumulation of CD5+/CD19+/CD23+ mature B cells in the peripheral blood, bone marrow and lymphoid tissue of patients. Despite major improvements in therapy over the past decades, CLL remains essentially an incurable disease. The clinical course of CLL is highly variable with life expectancies running from months to decades. The presence of recurrent chromosomal aberrations along with the mutation status of *IGHV* genes in the tumour cells of CLL patients are the current gold standards of clinical prognostication. However, these tests are laborious to implement and require techniques not readily available in all diagnostic laboratories. In this issue of *EBioMedicine*, Ferrajoli and colleagues propose that plasma and tumour cell-derived microRNAs (miRNAs) encoded by Espstein–Barr virus (EBV), the causative agent of infectious mononucleosis (also known as *kissing disease*), could be useful diagnostic and prognostic biomarkers for this disease (Ferrajoli et al., 2015). Plasma biomarkers are an appealing prospect for clinical practice as the ability to directly measure molecules in the blood of CLL patients (or other types of cancer) without needing to purify tumour cells would almost certainly lead lower costs and shorten turnaround times for patients.

The presence of nucleic acids (DNA) in the blood was first demonstrated over 60 years ago (Mandel and Metais, 1948), although their potential as a non-invasive biomarker of cancer was not recognised until some years later. It was however the 2008 discovery of microRNAs (miRNAs) in the blood of cancer patients (Lawrie et al., 2008; Mitchell et al., 2008) that has put the field in the spotlight and there are now more than 4500 publications on the subject (http://www.ncbi.nlm.nih.gov/pubmed). MiRNAs are particularly attractive candidates as non-invasive biomarkers as they are able to sub-classify cancers to a greater degree of accuracy than traditional gene expression analysis (Lu et al., 2005), and are protected from the high levels of RNase activity present in blood (Mitchell et al., 2008). Furthermore, unlike protein biomarkers which can persist in the blood for many weeks, the short half-life of circulating RNA makes it eminently suitable as a tool to monitor the evolution of a cancer in real-time allowing treatment decisions to be taken much more efficiently. Despite its promise, the study of circulating miRNA is very much in its infancy reflected in the many non-overlapping and even contradictory studies have been published. On many occasions this disparity is due to technical factors particularly variability in the choice of starting material (*i.e.* exosomes, sera or plasma), purification techniques, detection platforms and/or statistical methodologies. Perhaps the most serious barrier to translation of these findings into the clinic is the low number of patients typically used for these studies. To their credit the study by Ferrajoli et al. not only studied a high number of CLL patient samples (n = 516) with separate test and validation cohorts, but also used three different platform technologies to detect miRNAs. Moreover, they measured expression variability of candidate reference genes in the samples. This often overlooked consideration is crucial for the reliability of miRNA detection in blood as expression levels of the most commonly used reference miRNAs can vary significantly amongst samples depending upon the pathology studied (Friedman et al., 2012). In the present study they found that levels of the EBV-miRNA BHRF1-1 were significantly higher in plasma samples from CLL patients than healthy individuals, and that patients with higher levels also had high B2M and higher Rai stage, both indicators of more advanced disease.

Perhaps the most surprising finding of this publication is that EBV miRNAs were detectable in individuals without an apparent history of EBV infection at all (*i.e.* EBNA-1 IgG seronegative). Plasma samples from fifteen CLL patients were identified as being seronegative (also negative for active EBV infection), yet all of them contained detectable levels of BHRF1-1 and BART4 miRNAs. The authors suggest that this anomaly is a reflection of the lack of sensitivity of conventional detection methods. If this really is the case then further research is needed to address why some infected individuals do not seroconvert. This could have important implications for the development of vaccines against EBV infection that has been linked to a range of different malignancies that account for ~200,000 new cancers every year.

Although EBV infection is intimately associated with many different forms of haematological malignancy, the link with CLL remains controversial not least of all because EBV infection is only rarely detected in CLL tumour cells, and cells are generally considered refractory to infection *in vitro* (Teramoto et al., 2000). However the publication by Ferrajoli et al. appears to dispute these results as it provides convincing evidence for the presence of EBV miRNAs in tumour cells of CLL patients detected by small RNA sequencing, qRT-PCR and *in situ* hybridisation. The authors suggest that the tumour cells are infected but at a level that it is not detected by conventional methodology. However, an

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alternative hypothesis is that the tumour cells are not infected at all (consistent with lack of LMP-1 or EBER expression seen in this study), but rather that they acquire EBV miRNAs *via* exosomal transfer. It has been demonstrated that functionally competent EBV-miRNAs can be exported from infected B-cells as exosomes and taken up by dendritic cells (Pegtel et al., 2010). Conversely, malignant B-cells have been shown to import (non-viral) miRNAs packaged within exosomes (Mittelbrunn et al., 2011). Therefore it seems plausible that non-infected CLL tumour cells might obtain EBV-miRNAs in a similar fashion, perhaps from infected non-malignant B-cells or epithelial cell donors (Temple et al., 2014). Irrespective of the source, these miRNAs are proposed to play a pathogenic role in CLL, as the authors of the present study demonstrate that over-expression of BHRF1-1 in tumour cells can inhibit TP53 expression, and that this is consistent with the observation that CLL patients with high BHRF1-1 expression have shorter OS.

Although the study by Ferrajoli et al. proposes novel biomarkers for CLL, the clinical utility of these findings is questionable as 30–50% of patients never require therapy at all, and therefore, outside of a clinical trial context, this information is unlikely to result in changes to patient management. Nevertheless its publication represents an important breakthrough in the rapidly emerging field of circulating miRnomics.

Conflict of Interest Disclosure

The authors declare no conflicts of interest.

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