Circulating microRNAs: promising biomarkers in aplastic anemia

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MicroRNAs (miRNAs) are short non-coding RNAs that play key regulatory roles in gene expression through complementary binding to the 3'-untranslated regions (3'-UTRs) of target mRNAs, leading to subsequent translational repression.[1] Since miRNAs were first identified in 1993, there has been continuous growing interest in better understanding the roles of these molecules in the regulation of both normal cell function as well as numerous disease processes.[2] Furthermore, miRNAs released into the circulation after cell death, or in extracellular vesicles, have been identified in a number of different diseases.[3,4] These circulating miRNAs can be measured in the blood, and represent promising new biomarkers for both the diagnosis of disease and the assessment of treatment responses.

Acquired aplastic anemia represents a significant clinical problem and it is of unclear etiology in the majority of cases. There has been extensive evidence for T cell-mediated bone marrow destruction, leading to a characteristic clinical presentation with hypocellular bone marrow and pancytopenia on blood work.[5] In line with this, upfront immunosuppressive therapy (IST) consisting of horse antithymocyte globulin (ATG) and cyclosporine (CsA) has significant activity and is the standard of care. Bone marrow transplantation represents an additional approach and has specific indications for some groups of aplastic anemia patients, however, even with a HLA-matched sibling donor (MSD), transplant related mortality, including the risk of graft-*versus*-host disease, exists.[6] Further, in individuals lacking a MSD, unrelated donor transplantation carries an even greater risk of graft-*versus*-host disease; alternatively, haploidentical donor transplantation remains experimental for this condition.[7]

While upfront treatment with IST is the standard of care for aplastic anemia, predicting responses to immunosuppression is difficult. Response rates for IST are estimated at about 70%, with refractory aplastic anemia patients requiring additional rounds of IST or consideration for bone marrow transplantation.[8,9] As such, biomarkers to monitor responses to IST throughout treatment have the potential to change clinical decision making and improve outcomes in aplastic anemia. Although several biomarkers to monitor responses in aplastic anemia have been proposed, these biomarkers are largely non-specific (e.g., age, blood counts), and molecular biomarkers represent a more sophisticated approach for follow-up in these patients.[10]

In the current issue of the journal, Hosokawa and colleagues build upon their previous research to establish circulating miRNAs as potential biomarkers in aplastic anemia.[11] The authors used an unbiased PCR-based panel to identify miRNAs differentially regulated in patients with severe aplastic anemia as compared to patients with myelodysplastic syndrome or healthy volunteer controls. Of note, none of these patients had received IST prior to sample collection. After identifying 19 dysregulated miRNAs in a discovery set of 179 miRNAs, the authors further validated their findings in 108 patients, and identified three miRNAs dysregulated with at least a 1.5-fold change. Interestingly, the two miRNAs upregulated in the aplastic anemia group (miR-150-5p and miR-146b-5p) have previously described roles in T cell development and regulation of innate immunity, while the role of the one miRNA downregulated in the aplastic anemia group (miR-1) may play a part in autoimmunity.[12-14]

Perhaps the most interesting finding of Hosokawa and colleagues is the identification of miR-150-5p as a marker for treatment responses to immunosuppression in aplastic anemia. The authors analyzed 40 aplastic anemia patients before and after 6 months of IST, and identified statistically significant decreases in miR-150-5p and miR-146b-5p, and a statistically significant increase in miR-1. These findings mirror the authors' other findings comparing the levels of these miRNAs in aplastic anemia patients and healthy controls. When the authors specifically compared the effect of IST on these miRNAs in responders and non-responders, miR-150-5p demonstrated a significant decrease only in responders. Surprisingly, miR-1 demonstrated a significant increase after IST regardless of whether or not the patients responded to the treatment. These findings suggest that some miRNAs differentially expressed in aplastic anemia can be used to monitor treatment response, while others cannot.

The results of the work of Hosokawa and colleagues are interesting and potentially important. Prospective studies will be required to further validate whether miR-150-5p monitoring can identify responders from non-responders to IST. Future efforts should also focus on determining the earliest time point when meaningful changes can be observed. Clinically, early identification of potential IST non-responders could conceivably trigger an earlier consideration for bone marrow transplantation. Conversely, identifying potential IST responders prior to hematologic recovery may serve a purpose in selecting so-called "late responders", for whom hematologic recovery can take up to 6 months to observe. Beyond having important clinical implications, this report also advances our understanding of the mechanisms of immune-mediated failure. The role of miRNAs in the pathophysiology of aplastic anemia and other bone marrow failure syndromes has been generally unclear. By providing evidence that miRNAs can be used to distinguish aplastic anemia from healthy patient controls, the work of the authors suggests the involvement of miR-150-5p in the immune-mediated failure. However, further research into the relevant targets of this, and other miRNAs, is needed. The authors provide some insight into this through pathway analysis, which identifies potential immune-related targets of these miRNAs. These targets will need to be validated in future studies using molecular biology techniques classically used to study miRNA biology. In summary, Hosokawa and colleagues suggest a promising new approach to monitor response to immunosuppression in aplastic anemia, an autoimmune regulated disease that lacks

useful biomarkers. Future studies will need to uncover the underlying mechanisms driving the observed changes in circulating relevant miRNAs in the disease, and how immunosuppression modulates such levels.

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Risk stratification in myelofibrosis: the quest for simplification

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Risk-stratification systems in hematologic malignancies can serve a myriad of clinical and research purposes. They facilitate rational bedside discussion regarding the likely trajectory of a disease, provide an objective screen to ensure clinical trial enrollment reproducibility, and help guide decision-making with regard to risky interventions.

The ideal prognostic model would be that derived from the experience of patients very similar to those who are seen in your clinic; thus, generalizable. It would utilize data that you have at hand, or at least can easily and accurately obtain, and it would reliably predict the future clinical course of your patient's health condition, providing greater precision when discussing sometimes highly heterogeneous diseases.

Myeloproliferative neoplasms (MPNs) are a group of malignant conditions known for such heterogeneity. For essential thrombocythemia and polycythemia vera, two of the lower-risk subtypes of MPNs, risk-stratification models have always been remarkably simple – perhaps due to the limited number of therapeutic interventions employed. A thorough patient history, complete blood count, and, in the case of essential thrombocythemia, knowledge of the *JAK2V617F* mutation status, allow the physician to sort patients into standard and high-risk categories, and assign therapy accordingly.

However, in primary myelofibrosis (PMF), a disease where survival can range from months to over a decade, there has been continuous re-evaluation of the prognostic models used. Initially, those utilized in myelodysplastic syndrome, such as the International Prognostic Scoring System (IPSS), were opted for. In the last few years, two PMF-specific models have become the standard of care: dynamic IPSS (DIPSS), and DIPSS-plus. Each of these works with relatively easy to obtain inputs including age, blood count, symptoms, peripheral blood blast percentage, transfusion history, and karyotype. Typically, clinicians use the system that best fits the situation at hand – for example, if one were discussing transplantation with a younger than average patient, one might calculate the DIPSS score since the retrospective results published by Nicolaus Kröger *et al.*, comparing transplant to non-transplant outcomes, were stratified using that same score.[1] For a patient under consideration for Ruxolitinib therapy, one might use the IPSS score since it was the model chosen for eligibility in the pivotal registration studies for this agent.[2,3]

Since 2005, when a mutation in the *JAKV617F* gene was first identified as a seminal pathologic event in polycythemia vera, an increasing number of somatic mutations have been described in association with PMF. In general, *JAK2*, *CALR* and *MPL* are considered driver mutations, though there are elegant studies examining how acquisition order dictates phenotypic destiny.[4] Additional somatic mutations found in the disease include *LNK*, *CBL*, *TET2*, *ASXL1*, *IDH1/2*, *IKZF1*, *EZH2*, *DNMT3A*, *TP53*, *SF3B1*, *SRSF2*, and *U2AF1*, a list that is likely not exhaustive. While we await additional research on the mechanistic consequences of these aberrations, retrospective studies are already looking into the prognostic importance of mutations, or groups of mutations, in patients. How these molecular mutations should be integrated into pre-existing scores, such as the DIPSS, remains a significant conundrum