Genome Medicine

RESEARCH HIGHLIGHT

Circulating microRNAs as biomarkers - True Blood?

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Abstract

MicroRNAs are post-transcriptional regulators that are involved in many physiological and pathophysiological conditions. A recent study compared the expression profiles of hundreds of blood-borne microRNAs across a variety of nonmalignant and malignant diseases to identify disease-specific expression patterns. The resulting microRNA expression data could be used to discriminate disease samples with a high level of accuracy, demonstrating the potential for using microRNA signatures for the blood-based diagnosis of disease.

Screening of blood-derived microRNAs for tomorrow's diagnosis

The first connection between microRNAs (miRNAs) and the pathogenesis of cancer was described by Calin *et al.* in 2002 [1]. Since then, it has become clear that miRNA expression patterns reflect the identities of both normal and malignant cells. The majority of reported miRNA expression data have been generated from solid tissues, but differentially expressed microRNAs have also been identified in body fluids, such as serum and cerebrospinal fluid [2]. This finding has led researchers to explore the diagnostic and predictive relevance of miRNAs in whole blood, the most accessible organ of the human body.

In an article recently published in *Nature Methods*, Keller and colleagues [3] addressed this issue by investigating the miRNA transcriptome (which they call the 'miRNome') of blood samples from patients with a variety of diseases, including lung cancer and myocardial infarction. A total of 454 blood samples, taken from individuals affected by one of 14 selected diseases (Box 1) or from healthy controls, were profiled using miRNA microarray technology. This led to the identification of multiple miRNAs that are deregulated in the disease state, that is, their expression was either up- or down-regulated in patient samples compared with matched controls. The dysregulated miRNA profiles could be used to discriminate between healthy controls and patient samples with an average accuracy of 88.5%, or 72.5% using only two miRNAs. Considering the lack of diagnostic markers for many diseases, the work of Keller *et al.* [3] highlights the potential for the future integration of blood-based miRNAs into routine diagnostics.

Serum, plasma or whole blood?

Despite the high discriminative potential, the predictive value of the total miRNome amounted on average to only 67.45%. Confounding variables for this experimental approach include existing co-morbidities and varying number of cells in the blood (blood counts), which might distort disease-specific miRNA profiles. The heterogeneity of blood, which is a mixture of platelets, erythrocytes, myeloid cells and lymphoid cells, might be another source of confounding factors.

Until now, most published studies focused on the detection of miRNAs from serum. However, the study by Keller and colleagues [3] demonstrate that miRNomes of blood cells can reflect the presence of diseases that are not necessarily blood-borne. It is not fully understood how the various types of blood cell change their miRNome in response to disease; neither is it understood which cells in the hematopoietic system alter to reflect individual disease. Could it be that the miRNome of monocytes is altered following myocardial infarction, a necrotic condition, whereas immune diseases induce changes in the miRNome of B lymphocytes? To answer this question, a direct comparison between the miRNomes of serum, whole blood samples and hematopoietic subpopulations, such as lymphocytes, from different diseases would be helpful. The use of serum might decrease the above mentioned confounding variables, possibly allowing the detection of a more concentrated disease-related miRNome by decreasing blood cell-related background expression of miRNAs through co-morbidities, for example. In addition, most published works focused on the comparison between serum-derived miRNomes of healthy controls with one particular disease. However, a comparison between whole blood and serum across multiple malignant and non-malignant diseases is still

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Box 1. The 14 diseases profiled by Keller *et al.* [3]

Prostate cancer
Acute myocardial infarction
Chronic obstructive pulmonary disease
Gastric tumors
Lung cancer
Melanoma
Multiple sclerosis
Ovarian cancer
Pancreatic ductal adenocarcinoma
Pancreatic tumors
Pancreatitis
Periodontitis
Sarcoidosis
Wilms' tumor

missing, leaving unanswered the question which approach has more of a predictive miRNome.

miRNAs: new tools for the pathologist?

Many studies have demonstrated that miRNA expression patterns can classify cancers and form cancer-specific networks describing the developmental lineage and differentiation state of tumors [4,5]. However, is this enough to justify the integration of miRNAs into routine diagnostics? The answer depends on the type of cancer. A major problem lies in the varying number of miRNAs necessary to distinguish and classify specific cancer entities. For example, the number of miRNAs that are predictors for different classes of acute myeloid leukemias (AML) with genetic abnormalities varies between 7 and 48 using a real-time PCR approach [6], whereas a signature involving only 4 miRNAs (miR-128a, miR-128b, miR-223 and let-7b) accurately discriminates between AMLs and acute lymphoblastic leukemias (ALLs) [7]. In addition, expression levels of a single miRNA, miR-181a, constitute a prognostic factor for overall survival in normal karyotype leukemia. If cancer is considered to be a developmental process similar to hematopoiesis [8,9], these findings might imply that miRNA expression alters hierarchically to reflect different stages within cancer development. Multiple miRNA predictors have been developed to classify differentiation stages of cancers; a useful tool, especially for cancers of unknown primary origin [10]. Yet it is still not clear whether these findings using tissue samples are mirrored by serum-based miRNA markers.

Challenges for miRNA-based diagnostics

The study of Keller *et al.* [3] strengthens the concept of blood-borne miRNAs as biomarkers for disease. Technical limitations include a lack of existing standards that would allow cross-comparisons between laboratories and patient samples from various locations. In addition, diagnostic tests for miRNAs need to be simplified and streamlined for rapid readout. This would allow a better integration into established pathological scoring systems that would eventually benefit patients. To this end, more diseases have to be screened for blood-borne miRNA expression profiles and correlated with clinical features. For now, miRNAs are only a fragment of the truth.

Abbreviations
AML, acute myeloid leukemia; miRNA, microRNA; miRNome, miRNA transcriptome.

Competing interests
The authors declare that they have no competing interests.

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