Commentary

Progress of research on microRNAs with diagnostic value in asbestos exposure: A call for method standardization

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Summary

Malignant mesothelioma (MM) is an insidious, lethal asbestos-related cancer that is poorly responsive to current treatments. Specific and sensitive biomarkers providing early MM diagnosis in exposed subjects, who are at high-risk of developing it, are sorely needed. MicroRNAs (miRNAs) are endogenous, non-coding small RNAs with a well-established diagnostic role in cancer and pollution exposure. In a recent systematic review and qualitative meta-analysis followed by a functional investigation, we examined all the available data on the miRNA biomarkers involved in asbestos exposure and MM pathways. This invited commentary aims to provide an insightful critique into the state of the art of the research into clinically relevant miRNA biomarkers, highlighting the strengths and weaknesses of current research efforts in this field. It also reviews the suggestions advanced to improve biomarker development productivity and the translation of research results into clinical practice, stressing that multicenter multidisciplinary studies adopting standardized methods and protocol sharing are the key to move from the workbench to the clinic.

Keywords: Asbestos, mesothelioma, microRNA, mesomiRs, molecular pathological epidemiology

1. Introduction

Asbestos and asbestos-like fibers are naturally occurring crystalline silicates whose exceptional physicochemical properties have led to their extensive use in innumerable industrial applications worldwide (1,2). Occupational or environmental exposure to asbestos is associated to the development of asbestos-related diseases (ARDs) through accumulation of asbestos fibers and bodies in the lungs (2).

ARDS are characterized by a slow onset and an insidious course. They induce a range of non-malignant inflammatory diseases (asbestosis) due to formation of plaques in the pleura and to permanent fibrosis, which promote carcinogenesis (3). Malignant ARDs include bronchogenic carcinoma and mesothelioma of the pleura (80-90%), peritoneum (10-15%), and other mesothelial surfaces (< 5%) (4). MM is rare in the general population, but common in exposed cohorts. It is a lethal cancer characterized by considerable latency (≥ 30-60 years) (5), poor prognosis and quality of life, and unresponsiveness to currently available treatments. Symptoms are non-specific and the differential diagnosis (by pleural biopsy) is complex, invasive, and often late (6). The success and applicability of current multimodal therapeutic protocols depend on tumor stage, patient performance status and co-morbidities (7). Patients with advanced, unresectable, and poorly differentiated disease as well as co-morbidities have a worse prognosis (7). Increased treatment effectiveness through patient-tailored care and management depend on monitoring exposed subjects and early disease detection. The discovery and validation of MM-specific, non-invasive biomarkers, a goal that has been pursued for more than 20 years (8), would enable disease detection at the asymptomatic stage. Recent studies have found that microRNAs (miRNAs) play an

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important role in MM biology and have the potential to be employed both as biomarkers and as therapeutic targets (9). They are short, non-coding RNAs with a key role as post-transcriptional regulators in physiological and pathological processes; they interact with target mRNAs in a sequence-specific manner, and are differentially expressed in several diseases including cancer onset and progression. High-quality miRNAs are tissue-specific and easy to extract from tissue, cells, and body fluids (10,11); circulating cell-free miRNAs are highly stable because they are bound to specific carriers, such as microvesicles, Argonaute proteins, and high-density lipoproteins (12-14). These evidence have led to suggest a role for them as clinical molecular markers. Given the invasive nature of MM diagnosis and differential diagnosis from other cancers or benign proliferations, a variety of technological approaches and study designs have been applied over the past 10 years to test the value of miRNAs as non-invasive MM biomarkers. However, although a myriad putative miRNAs having diagnostic/prognostic relevance have been identified, the translation of research findings to clinical practice has met with limited success.

2. Diagnostic value of miRNAs in asbestos exposure: state of the art

In 2015, our group undertook a systematic review to collect and analyze the best evidence on the question (15). The miRNAs reported to have diagnostic potential since the earliest studies were comprehensively reviewed in an effort to find an evidence-based consensus on their biomarker potential in asbestos-exposed subjects and MM patients. Secondary data analysis has huge possibilities to identify high-quality evidence in these datasets and to provide guidance when the literature is inconsistent and studies disagree. The results of our work, the first systematic review and qualitative/quantitative meta-analysis on the issue, suggested that miRNAs may indeed play a key role in the diagnosis of asbestos exposure and ARDs and through it also improve prognosis and survival. A systematic search of the major biomedical databases for miRNA expression signatures related to asbestos exposure and MM provided a number of promising candidates, which were subjected to functional and bioinformatic analysis to assess their biomarker potential (15). The evidence-based picture thus obtained highlighted some major strengths and weaknesses of miRNA research in the field.

2.1. Strengths of MM-miRNA research

The literature search found a number of promising miRNAs, designated as "mesomiRs" (MM-associated miRNAs), with early diagnostic potential (15). In particular, two signatures, one found in blood and another in tissue, are expressed differently in asbestos-exposed subjects vs. MM patients:

i) the circulating miRNAs mir-126-3p, mir-103a-3p, and mir-625-3p were seen to provide a particularly promising multi-marker panel in combination with mesothelin and/or fibulin-3 for early, non-invasive diagnosis and screening of high-risk, exposed subjects (15); circulating miRNAs have ideal biomarker value, because they are non-invasive, stable, not expensive to test, and vary little in the general population.

ii) the tissue miRNAs that have been described most consistently (miR-16-5p, miR-126-3p, miR-143-3p, miR-145-5p, miR-192-5p, miR-193a-3p, miR-200b-3p, miR-203a-3p, and miR-652-3p) were pooled into a meta-signature that was found to have diagnostic value (15).

Application of these two miRNA panels, which are endowed with high sensitivity and specificity, has the potential to supply a more accurate assessment of the likelihood of MM development by asbestos-exposed subjects compared with other biomarkers; it may even allow to assess patients, the rate of cancer progression, and prognosis based on relative miRNA expression (15). The performance of the two biomarker panels should also be assessed in terms of surveillance of high-risk patients and early MM detection, so that adjuvant - systemic or targeted - therapies can be instituted at an earlier time point. This work should be supplemented by validation studies carried out in the population at risk, using a sensitive detection method and large cohorts of patients and controls. These studies would enable miRNA research to be translated into clinical practice. The evaluation of biomarker panels, rather than single molecules, provides the conceptual framework for defining the status of biological systems in health and disease and falls into the sphere of molecular pathological epidemiology (MPE). This novel discipline, which has been defined as "epidemiology of molecular pathology and heterogeneity of disease" (16), straddles traditional pathology and epidemiology and assesses how particular exposures influence disease risk through the search for and evaluation of molecular pathological markers also in relation to exogenous (e.g. exposure) and endogenous factors (17,18). Under the umbrella of the "the unique disease principle" (19), MPE is intertwined with precision medicine (16,20) and is the research branch capable of identifying potential biomarkers for the new frontier of personalized medicine (21).

Analysis of the meta-dataset suggested to us that there could be a correlation between deregulated circulating and tissue miRNAs and the pathogenic process triggered by asbestos exposure (15). These miRNA pools should be further evaluated not only as diagnostic instruments, but also as possible therapeutic targets by assessing their molecular role. Another key task, beside the evaluation of their up- or down-regulation, is the validation of their targets and regulators, which would clarify how miRNAs
2. Weaknesses of MM-miRNA research

An interesting consequence of the evaluation of the works collected in the meta-analysis was the insight we gained into the problems that hamper the translation of research findings into clinical applications. In particular, the lack of study design and method standardization seriously hampers the reproducibility of results obtained in different laboratories, magnifying inconsistencies. Major obstacles were identified in the pre-analysis, intra-analysis, and post-analysis stages. Pre-analytical factors include:

i) patient selection bias, represented by high interindividual variability in exposure levels and genetics (MM subtypes, MM stage, benign proliferation, rate of cancer progression) and by the method adopted to assess asbestos exposure.

ii) sample availability, especially in rare diseases like MM.

iii) lack of standardization in sample collection, handling, and storage (MM samples included fresh/frozen biopsy specimens; formalin-fixed paraffin-embedded (FFPE) tissue; macro-dissected tissue; laser-captured micro-dissected tissue; tissue collected after treatment; plasma, serum, and blood cell fraction, and cell lines).

iv) control sample inconsistency: FFPE biopsies of healthy pleura tissue, patient-matched non-neoplastic pleura, lung, pericardium, healthy lung from asbestos-exposed subjects, specimens from a range of cancers, non-neoplastic proliferations, plasma/serum from healthy or exposed subjects, blood cell fraction of healthy/exposed subjects, immortalized cell lines, and normal human mesothelial cell cultures. In addition, some studies comparing MM isotypes did not envisage a control group of normal samples (15).

Analytical factors include:

i) different performance of platforms and variability within and across the analytical methods applied for the discovery and quantification of novel biomarkers. MiRNA quantification approaches were also widely different, including real-time quantitative PCR, qRT-PCR array, microarray, in situ hybridization-based assays, and variants thereof.

ii) limited sample size, statistically underpowered datasets impairing robustness of evidence.

iii) lack of appropriate reference standards and quality control.

Obstacles in the post-analysis phase include different qRT-PCR normalization methods and statistical approaches, poor study design, particularly in the validation phase (which is often missing or is conducted in the same patient cohort used for screening), and finally the failure to report important preanalytical issues related to specimen provenance and biomarker assay protocols (15).

We hope that this overview of the strengths of the approaches that have been applied to discover promising miRNAs and of the limitations that hinder the progress of biomarker research toward clinical validation of results may induce a greater focus of research efforts.

3. A call to action to turn the biomarker potential of miRNAs into a clinical reality

We share the view that all investigations should begin with a clearly defined, appropriately designed study that eventually confers clinical benefits on patients (22). As regards MM, the poor prognosis and quality of life of patients and the lack of an effective cure urge strong and effective action. Finding biomarkers capable of non-invasive diagnosis or of early disease prediction in high-risk subjects would have enormous implications (15). However, the search for biomarkers is a complex process whose steps include verification, validation, demonstration of analytical validity, evaluation of clinical value and, ultimately, assessment of clinical effectiveness (23). The major limitations hampering the translation of basic biomarker research into useful clinical assays are reported in Figure 1. Our conclusions are in line with those reached by the National Biomarker Development Alliance (NBDA) after a two-year review and consultation process. This unique trans-sector alliance - which is dedicated to solve the problems hindering biomarker research and discovery to accelerate their clinical application - has identified a number of difficulties affecting the whole biomarker research pipeline, from discovery to clinical validation and regulatory approval (24,25). According to the NBDA, key shortcomings of biomarker research translation also include the lack of common standards for data reporting and exchange, for database design and interoperability, for longitudinal integration of discovery and clinical development data, and for the integration of molecular profiling data into electronic medical records. A further weakness is the present reliance on isolated facilities endowed with high technical specialization, whereas coherent systems-based approaches that reflect the "multidimensional technical, clinical and regulatory complexities required to validate a new generation of multiplex molecular diagnostics" would ultimately provide better results (24).

These considerations provide a further call to action, to plan well-designed studies for the rapid validation of miRNAs with biomarker potential, alone or combined with mesothelin or fibrin-3, and to test their clinical value in high-risk individuals. In parallel, further basic research work should be aimed to investigate the molecular pathways that are regulated by aberrantly expressed miRNAs. Experimental methods, patient
populations, sample type, and specimen handling and storage protocols should carefully be defined and standardized because they can make the difference between success and failure. Shared quality control guidelines for pre- and post-analytical steps and their documentation would also considerably enhance the definition and refinement of robust miRNA biomarkers. However, such studies, especially those involving rare diseases, take several years, require large samples, and are generally not feasible by single laboratories, both in terms of specimen availability and of resources.

Joint research programs are all the more critical when investigating rare diseases, since they expand sample size and increase statistical power. Success in biomarker research and discovery "demands integration of multidisciplinary expertise and trans-sector collaboration between academia, clinical medicine regulators, industry, payers and patients" (24). Future efforts should thus be directed at developing and coordinating transnational research efforts where researchers, clinicians, public health experts, funders, and politicians join forces. This will also help i) prevent duplication of efforts and waste of money and time by ensuring efficient use of resources; ii) maximize the reliability of the data obtained; iii) improve early diagnosis, monitoring, and prognosis; and iv) hone treatment strategies. Ideally, highly specialized biomarker research teams investigating miRNAs, their targets and regulators, and related functions could be convened under the NBDA umbrella to lay down the standards, best practices, and guidelines required to set up a systems-based approach.

4. Conclusion

The peculiarities of ARDs, especially the latency of their onset and their distribution, involve that a large number of asbestos-exposed individuals worldwide are still to become MM patients. It is therefore essential to accelerate the search for novel, effective tools and strategies to prevent, diagnose, detect early, and cure these diseases. A multicenter, multidisciplinary and, critically, closely regulated, integrated and standardized systems-based approach would be the method ensuring the fastest return.

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References

4. Delgermaa V, Takahashi K, Park E-K, Le GV, Hara T,

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