Glioma "Liquid Biopsy": A New Frontier in Neurosurgery

Glioblastoma multiforme (GBM) is considered one of the most devastating cancers with poor prognosis and limited progression-free survival. GBM recurrence is usually detected by serial follow-up imaging that remains insensitive to microtumor growth; hence, delaying the detection of recurrence. For this reason, the need for biomarkers that serve for tumor recurrence detection and tumor activity monitoring is crucial for improving the prognosis of GBM. Ebrahimkhani et al[1] evaluated the role of exosome sequencing in the detection of GBM recurrence using patient serum. Exosomes are small vesicles released by cells into the bloodstream and contain a variety of molecules, enzymes, and genetic material including miRNA. They can be traced back to their cells of origin by analyzing the miRNAs they carry. The authors studied the sera of glioma patients and healthy controls and aimed to detect the miRNA signature specific to glioma patients.

Exosomes from the sera of 12 patients with GBM IDH wild-type and 12 healthy controls were studied, and their corresponding selected miRNAs were analyzed using univariate and multivariate analyses. Age and gender of both groups matched. The same analyses were repeated on 10 patients with grade II-III IDH-mutant gliomas and 10 matched healthy controls. Additional sera from 23 subjects (4 GBM patients, 9 multiple sclerosis patients, 1 ganglioglioma patient, and 9 healthy controls) were collected and served to test/validate the GBM miRNA signatures extrapolated in the experimental analyses (Figure). In the first step of the experiment, collected sera were treated with RNAase to eliminate free circulating nonexosomal miRNAs. Exosomes were then isolated using size exclusion chromatography; exosome

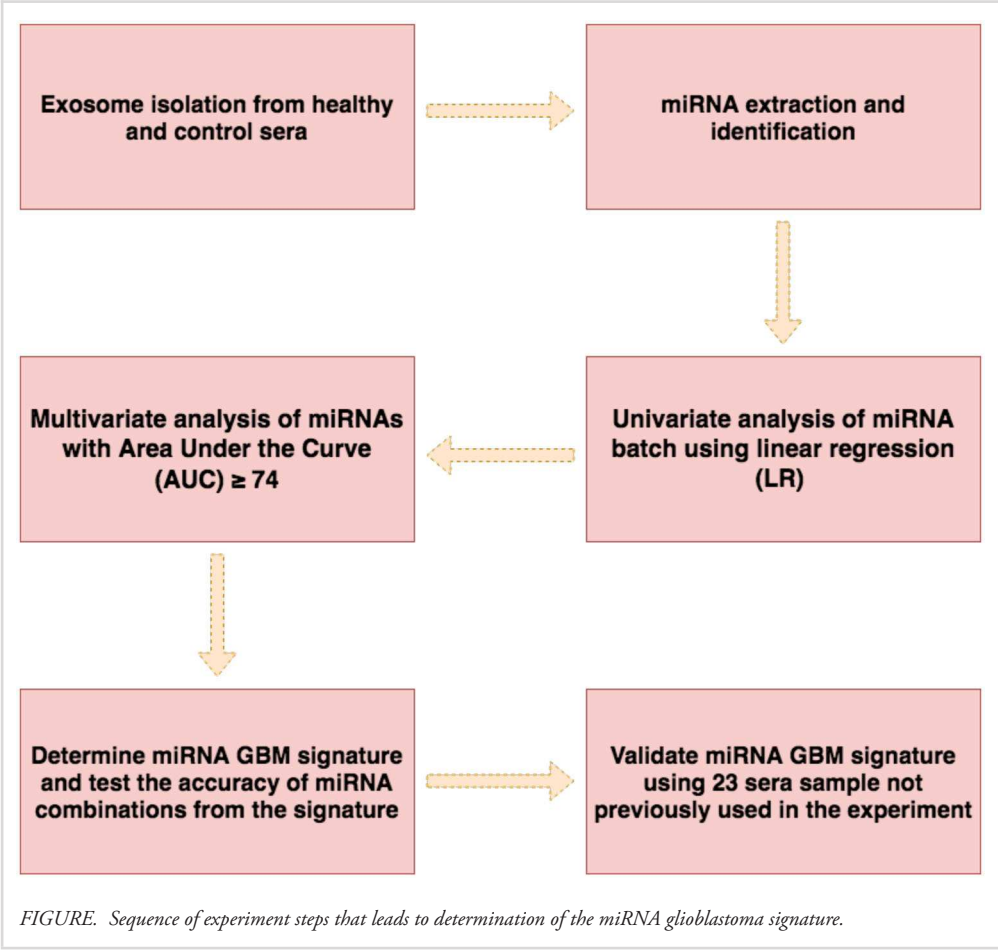


FIGURE. Sequence of experiment steps that leads to determination of the miRNA glioblastoma signature.

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heterogeneity was characterized using TEM (transmission electron microscopy) and nanoparticle tracking analysis. Mass spectrometry (MS) proteomic analysis was performed on exosomal preparations, which identified exosome marker proteins. Upon unbiased high-throughput next generation sequencing, 1939 different miRNAs in total were detected and analyzed. Prior to their analyses, the authors researched genetics libraries and matched the collected miRNAs to their target mRNAs using Ingenuity Pathway Analysis software. They found that 44 of these miRNAs are associated with cancer and neurological diseases genetic regulation—half of them being involved in GBM and glioma signaling pathways. The latter findings depict the promising role of miRNA in GBM detection.

The initial analysis done by Ebrahimkhani et al[1] consisted of univariate analysis of the miRNAs collected from the GBM samples (n = 12) and their controls (n = 12) using logistic regression models. They identified 26 miRNAs expressed by GBM cells that differentiate GBM cells from other cells with an area under receiver operator characteristic curve (AUROC) of at least .74. Among the 26 identified miRNAs, random forest multivariate modeling was used to select the miRNA signature of GBM cells. In the random forest model miRNA data is randomly tabulated and organized into decision trees. The trees get updated by the model to match the final diagnoses. Final decision trees were tested on nonselected miRNA combinations (30%) and yielded a predictive power of 91.7%. Seven of these 26 miRNAs (miR-182-5p, miR-328-3p, miR-339-5p, miR-340-5p, miR- 485-3p, miR-486-5p, and miR-543) were found in more than 75% of the decision trees and were selected as the most stable differentiators of GBM. AUROC measures of all the combinations of the 7 differentiators of GBM showed that 6 combinations (size of combinations: 2, 2, 3, 3, 4, 5) that had AUROC measures of 1, hence distinguishing GBM patients from healthy controls with 100% accuracy. The GBM miRNA signature was then tested on the 23 collected sera that did not contribute to the analyses. Testing correctly identified preoperative and recurrent GBM samples. The signature was also able to correctly differentiate and classify healthy subjects and nonglioma subjects (including multiple sclerosis patients). Linear regression and mutant glioma random forest analyses were repeated on grade II-III IDH and corresponding healthy controls sera; the models identified 23 additional miRNAs that can accurately distinguish these 2 subsets. 13 miRNAs were found to be expressed differentially between GBM IDH wild type exosomes and grade II-III IDH mutant glioma exosomes with an estimated predictive power of 77.4%.

The study by Ebrahimkhani et al[1] is a stepping stone toward the development of "liquid biopsy" in GBM. The GBM miRNA signature identified in this study includes miRNAs that are known to be implicated in GBM pathways[2] and should be considered as a biomarker for disease monitoring. Future directions in research on the use of exosomes in affecting clinical management decisions should include comparative studies with larger clinical samples to understand the feasibility and reliability of this technique.

Disclosures

The authors have no personal, financial, or institutional interest in any of the drugs, materials, or devices described in this article.

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