

Identifying patients with pharmaco-resistant epilepsy using a plasma-based test

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Abstract

The identification of biomarkers in patients with pharmaco-resistant epilepsy could improve diagnosis and decrease time to efficient surgical treatment. Potential candidates to be used as biomarkers are circulating microRNAs which recently have been associated with different diseases. In this context, we worked with three microRNAs (hsa-miR-23a, hsa-miR-31, hsa-miR-134) and applied the technique of quantitative PCR assays in plasma samples. Our results indicated that hsa-miR-31 is down-regulated in patients with refractory epilepsy, making it a promising biomarker for refractory epilepsy.

Key words: Epilepsy, microRNAs, biomarkers

Introduction

Refractory epilepsy occurs in ~30% of all patients with epilepsy and major causes of pharmaco-resistant are Focal Cortical Dysplasia (FCD) and Mesial Temporal Lobe Epilepsy (MTLE). In these patients, surgical procedures can be performed in order to achieve seizure control. Nevertheless, surgery indication may be delayed due to a long clinical investigation. Therefore, the identification of biomarkers of pharmaco-resistance could potentially improve diagnosis and decrease the time to surgical treatment. Potential candidates to be used as biomarkers are circulating microRNAs (miRNAs); these are small noncoding RNA which have been recently associated with different diseases. In this context, the objectives of this study were i) to determine if changes in expression of three candidate miRNAs, previously associated with mechanisms underlying MTLE and FCDs: hsa-miR-23a, hsa-miR-31 and hsa-miR-134 are present in plasma of patients with refractory seizure and ii) to verify if plasma levels of these miRNAs are associated with response to anti-epileptic drugs (AEDs).

Results and Discussion

We determined plasma levels of these three miRNAs by quantitative PCR assays in plasma samples. MiRNAs were extracted from 40 patients with refractory epilepsy, 30 epilepsy patients who are responsive to AED treatment and 80 controls without epilepsy. Endogenous controls used in our experiment were hsa-miR-191 and hsa-miR-451.

We employed Wilcoxon test to analyze differential miRNA expression among groups and used Bonferroni test to correct for multiple comparisons. In addition, we used the Receiver Operating Characteristic (ROC) curve to evaluate power of correlation between miRNA expression and the phenotype analyzed.

To date, our results show that hsa-miR-31 is significantly down-regulated in patients with FCD and refractory epilepsy ($p=0.021$), as well as in patients with MTLE and refractory seizures ($p=0.035$), when compared to controls. In addition, hsa-miR-31 plasma levels could be used to distinguish patients with and without epilepsy, with an area under the curve (AUC) of 0.785. A second phase validation study is under way.

Conclusions

We found a significant difference in hsa-miR-31 expression between patients with refractory epilepsy and controls. Although our results are not yet finalized, we have an indication that these could have a significant impact in the treatment of patients with refractory seizures, leading to an early indication of epilepsy surgery and a better chance for patients to become seizure free.

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