Role of Personalized Medicine in the Identification and Characterization of Parkinson's Disease in Asymptomatic Subjects

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Medical physicians and scientists alike have traditionally viewed and interpreted diseases at the `visual' clinical level. With the advent of genomics and proteomics technologies, personalized medicine offers the promise and potential of uncovering the largely `unseen' details of disease causality, onset and progression [1]. We hypothesized that changes in the expression profile of biological indices (biomarkers) in the plasma and in circulating Peripheral Blood Mononuclear Cells (PBMC) may provide an ideal and clinically assessable “window” into the brain, reflecting molecular alterations associated with the onset and progression of Parkinson's disease (PD). The objective of our subsequent study was to test whether the differential expression of micro (mi) RNAs in PBMCs could be used to distinguish true PD from non-PD cases at baseline, prior to any definitive clinical diagnosis.

We explored whether specific changes in microRNA (miRNA) [2] from circulating PBMCs might reflect central nervous system neuropathology, such as in PD. We analyzed miRNA expression profiles in PBMC specimens that were collected at the baseline visit (recruitment) from 23 randomly chosen subjects with suspected PD, but whose mild clinical phenotypes precluded definitive PD diagnoses. Follow-up assessments conducted one year later, when patients had developed sufficiently robust clinical symptoms for unambiguous disease diagnosis, confirmed that thirteen of the patients met the clinical criteria for true PD (ten were deemed not to have PD). The objective of this study was then to test whether differential expression of miRNAs in PBMCs could be used to distinguish true-PD from non-PD cases at baseline, prior to definitive clinical diagnosis.

Results from our high throughput miRNA expression profile analyses of baseline PBMC specimens using the Agilent miRNA platform led to the identification of three candidate miRNA biomarker species (hsa-mir-29C, hsa-mir-424 and hsa-mir-30e5p) that were expressed at significantly higher levels in baseline PBMC specimens from true-PD compared to non-PD cases. In follow-up analyses, we used independent quantitative real-time Polymerase Chain Reaction (qPCR) techniques to examine the differential regulation of the three candidate miRNAs in the PBMCs of true-PD versus non-PD cases. Consistent with our high-throughput miRNA expression profile analyses, results from our qPCR studies confirmed that hsa-mir-29c, hsa-mir-424 and hsa-mir-30e5p expression levels were significantly higher (by more than two- to four-fold) in baseline PBMC specimens of true-PD compared to non-PD cases (data not shown). Thus, our studies demonstrated for the first time that the regulation of specific peripheral molecular indices (e.g., miRNA biomarkers in...
PBMCs) is associated with the onset and/or progression of PD. Using receiver operating characteristics (ROC) analyses, we assessed the predictive accuracy of using miRNA biomarker contents in PBMCs to correctly identify true PD cases among patients with suspected PD. We found that the combination of the hsa-mir-29c, hsa-mir-30e-5p, and hsa-mir-424 miRNA biomarker species (the three miRNA model) provided 95% accuracy in correctly diagnosing “true” PD. Moreover, this model resulted in 92% sensitivity, missing 8% of true PD cases (false negatives), and 100% specificity, not incorrectly identifying any of the non-PD cases as true PD (false positives) (Table 1).

Based on the efficacy of miRNA biomarker contents in PBMCs in distinguishing true-PD from non-PD cases among our study cohort, we continued to explore potential changes in the regulation of the three miRNA biomarkers in the brains of PD cases. In recent ongoing feasibility studies, we found higher contents of the three miRNA biomarkers in substantia nigra specimens of PD compared to normal cases (data not shown). Our observation suggests that the content of hsa-mir-29c, hsa-mir-30e-5p, and hsa-mir-424 miRNA biomarker species in clinically accessible PBMCs provides a “window to the brain”, reflecting molecular changes in the PD brain. More importantly, our new feasibility evidence suggests that hsa-mir-29c, hsa-mir-30e-5p, and hsa-mir-424 miRNA might have potential roles in PD onset and/or progression. Based on this, we used the Diana-mirPath Software (http://diana.cslab.ece.ntua.gr/?sec=home) to assess the potential roles of the identified PD biomarker miRNAs in biological processes relevant to PD. We conducted the Diana-mirPath analysis using 2 of the miRNA biomarkers (hsa-mir-29C and hsa-mir-424) that are part of the Diana-mirPath database; hsa-mir-30e5p is not part of the database and was therefore excluded from this analysis. Interestingly, we found that potential target genes that might be regulated by hsa-mir-29C and hsa-mir-424 are significantly (p<5.1E-06) enriched in a cellular pathway relevant to PD pathophysiology, particularly in relation to cellular dopamine metabolism/release. Our observations suggest that these biomarkers might have direct pathological relevance to PD onset/progression.

Collectively, our findings suggest that changes in miRNA biomarker contents in PBMCs reflect biological features relevant to PD and provide a means to help identify true-PD from non-PD cases at early stages of the disease, prior to definitive clinical diagnoses. Future studies will continue searching for potential relationships between miRNA biomarker contents in PBMCs and indices of dopamine uptake in the brain. Outcomes will test the potential value of PBMC miRNA biomarker contents as an index of brain dopaminergic function in PD. The development of biomarkers in personalized medicine will provide impetus for future investigations aimed at the early detection of disease, possibly at asymptomatic stages of PD and other age-related neurodegenerative disorders, and will help to devise future preventative therapeutic interventions.

Acknowledgments

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References

Table 1

The 3 miRNA model provides a sensitive and specific criterion for distinguishing true PD from non-PD among patients with suspected PD.

<table>
<thead>
<tr>
<th>miRNA species</th>
<th>Specificity</th>
<th>Sensitivity</th>
<th>Accuracy</th>
<th>area under the curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-29c</td>
<td>80%</td>
<td>92%</td>
<td>86.9%</td>
<td>0.892</td>
</tr>
<tr>
<td>miR-30-5p</td>
<td>90%</td>
<td>62%</td>
<td>73.9%</td>
<td>0.762</td>
</tr>
<tr>
<td>miR-424</td>
<td>100%</td>
<td>92%</td>
<td>94.7%</td>
<td>0.927</td>
</tr>
<tr>
<td>miR-29c + miR-30-5p</td>
<td>100%</td>
<td>77%</td>
<td>87.0%</td>
<td>0.931</td>
</tr>
<tr>
<td>miR-29c + miR-424</td>
<td>100%</td>
<td>83%</td>
<td>90.0%</td>
<td>0.969</td>
</tr>
<tr>
<td>miR-30-5p + miR-424</td>
<td>100%</td>
<td>83%</td>
<td>90.0%</td>
<td>0.969</td>
</tr>
<tr>
<td>miR-29c + miR-30-5p + miR-424</td>
<td>100%</td>
<td>92%</td>
<td>95.0%</td>
<td>0.979</td>
</tr>
</tbody>
</table>

The concentrations of individual miRNA assessed by quantitative RT-PCR (data not shown) were used in the ROC analysis. The table presents results from ROC analysis using individual miRNA species or using a combination of 2 or all 3 miRNA species (the 3 miRNA model).