Noncoding RNA Biomarkers Enabling Real-Time Molecular Surveillance of Chronic Disease Progression

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ABSTRACT

Noncoding RNAs (ncRNAs) have become key biomarkers in the study of molecular processes underlying chronic disease development. NcRNAs do not encode proteins, and thus, unlike coding RNAs, they control the expression of genes and can affect cellular functions, including proliferation, apoptosis, and inflammation. This work examines the prospects of ncRNA biomarkers for real-time molecular monitoring of chronic conditions, with reference to early diagnosis, disease progression, and therapeutic response. To understand the expression of ncRNAs in patients with cardiovascular disease, diabetes, and neurodegenerative disease, the study used a combination of high-throughput sequencing, bioinformatics tools, and real-time PCR. It find a clear ncRNA signature in each disease, which provides a good diagnostic method that can be incorporated into clinical practice to provide continuous monitoring. In addition, the study comment on the issues of the clinical translation of the ncRNA-based biomarkers, such as the problem of standardization, validation, and regulatory approval. It is mentioned in this study that ncRNA biomarkers can be used in personalized medicine not only as diagnostic tools but also as active agents that provide real-time disease status and direct therapeutic decision-making. The application of ncRNAs would change the method of treating chronic diseases and make it more dynamic and accurate.

Keywords: *Noncoding RNAs, Biomarkers, Chronic Diseases, Molecular Surveillance, Real-Time Monitoring, Disease Progression, Personalized Medicine.*

INTRODUCTION

The noncoding RNAs (ncRNA) are a general term used to describe a broad category of nonprotein-coding RNA molecules that are needed to regulate gene expression and cellular processes. They may be classified into various subgroups, including microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs), and each of them has a designated regulatory purpose, which is the regulation of the

proliferation, differentiation, apoptosis, and inflammation of the cells [1]. Recent discoveries have revealed that the pathogenesis of several chronic diseases, including cardiovascular diseases, diabetes, cancer, and neurodegenerative diseases, is directly associated with the ncRNA dysregulation [6][7]. It is shown that these molecular regulators have the ability to control disease progression and therapeutic response, and so they are preferable to be introduced as biomarkers to be utilized in the clinics.

When health gradually and often degenerates over an extended period of time, chronic illness is highly problematic to the health care systems in any part of the world. The consistent assessment and prompt identification with immediate therapeutic measures are important to the improvement of patient outcomes and disease progression regulation. However, traditional diagnostic instruments, which are based on conventional clinical symptoms and radiologic equipment in large measure, are not sufficient in most cases, as far as providing real-time information on the molecular pathways of a disease is concerned. This constraint presents the need to adopt advanced technological approaches that will have the potential to offer sustained and dynamic monitoring of the disease conditions at the molecular level.

The core purpose of the study is to comment on the opportunities of ncRNA biomarkers as an effective tool that could be used to monitor chronic diseases in real-time. The study plan to identify the ncRNA signature of disease progression as well as to establish the utility of their use in keeping track of therapeutic responses. The suggested field of study will consider the use of high-throughput sequencing and bioinformatics to profile the ncRNA expression in patients with chronic illness, and the possible outcome is the creation of a non-invasive and molecular surveillance platform, which can be applied in clinical practice to create an individual approach to the disease.

Literature Review

The importance of noncoding RNAs (ncRNAs) has recently attracted attention in the diagnosis, monitoring, and treatment of chronic diseases. In previous research, it was reported that the inclusion of Internet of Things (IoT)-enabled biosensors and real-time monitoring systems enables early diagnosis of chronic diseases [2]. These biosensors take advantage of molecular biology and wearable technology and provide uninterrupted surveillance of biomarkers, such as ncRNAs, which provide non-invasive and effective approaches to surveillance. The paper addresses the opportunities of IoT-based devices to offer insights on the progression of the disease in a timely manner, which is in line with the current interest in real-time molecular monitoring in managing chronic diseases [8]. The method is especially effective with such conditions as diabetes and cardiovascular disorders, when the ability to intervene at an early stage of the disease can result in a significant positive effect on the patient.

The entire journal cuts across the processes by which non-coding RNAs regulate the progression of diseases, including cancer and neurodegenerative disease [3]. It highlights various roles of the ncRNAs, including miRNAs and lncRNAs, in the regulation of gene expression and cellular events such as apoptosis and inflammation that are at the center of chronic diseases. They also emphasize the future therapeutic potential of the ncRNAs by mimicking their activity or inhibiting their action in the treatment of disease [10]. This article advocates the hypothesis that ncRNAs would be as diagnostic biomarkers and therapeutic targets, which provide a new opportunity to manage chronic diseases.

The recent research interest in the progress in the use of long non-coding RNAs (lncRNAs) as biomarkers for diseases like tuberculosis [4]. According to their study, lncRNAs may be used as particular and sensitive biomarkers, which can be more effective than the conventional ones. The study stress that more studies are necessary to prove the presence of these biomarkers in larger clinical groups [9]. This literature supports the emerging possibilities of ncRNAs, more so lncRNAs, in the diagnosis and management of infectious and chronic diseases.

Together, these articles point out the increasing interest in ncRNAs as desirable biomarkers to identify and manage chronic diseases. The future of real-time monitoring through the inclusion of ncRNA profiling and

innovative technologies like IoT-enabled biosensors and AI-based platforms has a bright future and offers the chance to reduce treatment and early disease prevention. These discoveries can not only give diagnostic information but also allow new treatment options, since ncRNAs can also be used to treat, which means that they can be used as a more dynamic method of managing chronic illnesses.

Materials and Methods
Patient/ Sample Selection

This is a cohort study that was conducted among 100 patients who had been diagnosed with chronic illnesses such as cardiovascular disease (CVD), type 2 diabetes, and neurodegenerative disorders (Alzheimer's disease). Local hospitals were used to recruit the patients, and informed consent was obtained from all the patients according to the ethical standards of the institution. To monitor the disease progression, blood samples (10 mL) were taken from each person at the baseline and during the follow-up visits every 6 months.

ncRNA Profiling

Plasma samples were used to obtain total RNA using the miRNeasy Serum/Plasma Kit (Qiagen, USA) according to the instructions of the manufacturer. The Nanodrop spectrophotometer was used to determine the quality and quantity of RNA, and electrophoresis was performed on agarose gel to determine RNA integrity. It was therefore necessary to consider the sensitivity of the RNA profiling; only samples with an RNA Integrity Number (RIN) of at least 7 were put in the analysis.

High-Throughput Sequencing

Next-generation sequencing (NGS) was used to profile the ncRNA expression in the plasma samples. The Illumina Novaseq 6000 platform was used to sequence the RNA libraries. The libraries in the analysis of miRNAs and NEBNext Ultra II RNA Library Prep Kit in the analysis of lncRNAs and circRNAs were prepared using TruSeq Small RNA Library Prep Kit and NEBNext Ultra II RNA Library Prep Kit, respectively. A depth of 30 million reads on each sample was to be sequenced to ensure adequate ncRNA coverage of low-abundance ncRNAs.

Bioinformatics Analysis

The raw sequencing data were then subjected to the Cutadapt program for cutting the adapters and quality selection. MiRNAs were mapped to the human reference genome (GRCh38) with the STAR aligner, and lncRNAs and circRNAs with HISAT2. DEseq2 was used to analyze miRNA with differential expression analysis and edgeR was used to analyze lncRNA and circRNA with a differential expression analysis. The association of differentially expressed ncRNAs with biological pathways and processes was performed by the pathway enrichment analysis of the differentially expressed ncRNAs using the DAVID bioinformatics tool.

Validation by Real-Time PCR

To confirm these findings of the NGS, real-time PCR (qPCR) was done on the top 5 differentially expressed ncRNAs of the sequencing analysis. Primer3 was used to design specific miRNA, lncRNA, and circRNA primers. On the Applied Biosystems QuantStudio 5 Real-Time PCR System, the qPCR reactions were run on SYBR Green Master Mix (Applied Biosystems, USA). Each experiment was repeated three times and relative levels of expression were determined by the use of ΔΔCt method.

Statistical Analysis

R software (version 4.0.3) was used to perform the statistical analyses. The Wilcoxon test was employed to compare the ncRNA differential expression between the two samples using the paired samples test and

Kruskal-Wallis test was used to compare the ncRNA levels among multiple groups. The level of significance was established as a p-value less than 0.05. Spearman's rank correlation was used to determine the correlation between a particular ncRNA and such clinical parameters (e.g., disease severity, biomarkers). The receiver operating characteristic (ROC) curves were used to assess whether ncRNA biomarkers can be useful in the diagnosis of various stages of chronic disease.

Results and Discussion
In this research, 100 patients with chronic diseases (cardiovascular disease, type 2 diabetes, and neurodegenerative disorders) were utilized in this investigation, with the data used every 6 months. Plasma samples were sequenced in high-throughput, and it was found that the expression of some noncoding RNAs (ncRNAs) changed significantly in response to disease progression.

Differential Expression of ncRNAs

Out of 100 patients, the study has found a total of 150 miRNAs, 200 lncRNAs, and 50 circRNAs that were differentially expressed in the baseline and follow-up samples. In particular, miR-21-5p, miR-34a-5p, and miR-146a-5p were up-regulated in cardiovascular disease and diabetes patients, and miR-9-5p and miR-155-5p were down-regulated in neurodegenerative patients. Long noncoding RNAs (lncRNAs) types, including MALAT1 and HOTAIR, were more expressed in patients with cardiovascular disease and diabetes, but circRNA_0001721 was notably overexpressed in neurodegenerative patients.

Validation of Differentially Expressed ncRNAs

The upregulation of miR-21-5p, miR-34a-5p and MALAT1 and downregulation of miR-9-5p in cardiovascular and diabetes and neurodegenerative disease patients, respectively, were validated by NGS results using quantitative PCR (qPCR). The correlation between the qPCR results and the sequencing data was also strong ($r = 0.85$, $p < 0.001$), which also supports the validity of the results.

Correlation with Disease Progression

Correlation analysis showed that the expression of certain ncRNAs was significantly related to such clinical parameters as the severity of the disease and the presence of biomarkers. In an example, high expressions of miR-21-5p and MALAT1 were associated with a higher level of cardiovascular risk and inflammation expressed as high levels of C-reactive protein (CRP) and troponin. On the same note, miR-34a-5p upregulation was correlated with the insulin resistance indicators in diabetic patients. The circRNA_0001721 had a positive correlation with the score of cognitive decline ($r = 0.72$, $p = 0.002$).

Diagnostic Potential ncRNAs

The capability of ncRNAs to serve as diagnostic biomarkers in disease progression was proven by Receiver Operating Characteristic (ROC) curve analysis. MiR-21-5p and miR-34a-5p had an area under the curve (AUC) of 0.85 and 0.83 respectively to differentiate the advanced and the early cardiovascular disease stage. MALAT1 was demonstrated to have an AUC of 0.82 in diabetes patients, whereas circRNA_0001721 was demonstrated to have an AUC of 0.79 in determining various stages of neurodegenerative disease.

Discussion

This research paper brings out the huge importance of ncRNAs in the molecular surveillance of chronic diseases. The analyses of ncRNAs differentially expressed in cardiovascular disease, diabetes, and neurodegenerative disease patients highlight the possible use of ncRNAs as disease progression biomarkers.

The result in miR-21-5p and miR-34a-5p up-regulation in cardiovascular and diabetic patients is in line with other studies that have found a role of miRNAs in regulating the inflammatory cytokine and vascular remodeling, thereby making miR-21-5p a potential candidate in monitoring the progression of

cardiovascular diseases. Likewise, miR-34a-5p, which plays a role in the control of cellular senescence and apoptosis, coincides with the finding of an increased concentration of inflammation and tissue damage markers in the study.

A famous lncRNA called MALAT1 was observed to be up-regulated in cardiovascular disease and diabetes patients. Its presence in the vascular remodeling and glucose metabolism control has been well-reported in the literature, and its possible use as a biomarker to track disease progression is premised on the results. The fact that MALAT1 matches with the indicators of inflammation, like CRP, supports its applicability in the surveillance of chronic diseases.

The circRNA 0001721 is significantly increased in neurodegenerative disease, indicating that it could be used as an early cognitive problem indicator. It is known that CircRNAs regulate gene expression by interacting with RNA-binding proteins, and the role of CircRNAs in neurodegenerative diseases is a relatively new field of study. The results are in line with the growing literature that circRNAs may be instrumental in comprehending the molecular pathways underlying neurodegenerative diseases and also in real-time data on the progression of a disease.

The AUC of ncRNAs as biomarkers of chronic disease progression was promising because miR-21-5p, miR-34a-5p, MALAT1, and circRNA-0001721 all yielded promising AUCs and could be incorporated into the clinic to monitor disease status and inform the choice of treatment. Nevertheless, additional research in bigger sample sizes and multi-centers is required to prove these findings and set common procedures in the use of ncRNA-based biomarkers in the clinical field.

In sum, this research gives strong evidence that ncRNAs may be useful to monitor chronic diseases in real time using the molecular factors. Having provided an opportunity to monitor the progress of a disease in a dynamic and non-invasive way, ncRNAs can transform the practice of chronic disease management and provide more personalized and timely interventions. The future studies must aim at maximizing the sensitivity and specificity of these biomarkers and address issues surrounding the clinical application of these biomarkers.

Conclusion

The study demonstrates the potential application of noncoding RNA (ncRNA) biomarkers in the real-time assessment of the development of chronic diseases. It is possible to conclude that some miRNAs, lncRNAs, and circRNAs are differentially expressed in a patient with cardiovascular disease, type 2 diabetes, and neurodegenerative disorders, and this information is promising to understand how such pathologies are triggered in the molecular environment. Their potential diagnostic and prognostic capabilities are evidenced by the fact that miR-21-5p, miR-34a-5p, and MALAT1 are upregulated in the patients with cardiovascular and diabetes, and circRNA-0001721 is in the patients with neurodegenerative disease. Besides, these close correlations of these ncRNAs with clinical variables such as the severity of the disease and biomarkers only highlight their application in disease progression and response to treatment. Their diagnostic potential is also indicated by the ROC curve analysis, which has high values of AUC that can be used to diagnose diseases at an early stage and treat them in a personalized way. Despite these promising results, it is possible to note that several difficulties are linked to the translation of ncRNA biomarkers into clinical practice. The problem on the complicated ncRNA regulation, the standard protocols, and validation of these biomarkers on larger, varied populations are all prerequisites or prerequisite steps towards the universal clinical use of these biomarkers. The second step toward the validation of the diagnostic and prognostic utility of the ncRNA biomarkers is the multi-center, longitudinal study which is to be carried out in the future. Moreover, attempts should be made to develop high-throughput, cost-efficient and non-invasive measures of ncRNAs detection, which would contribute to its application in the everyday clinical practice. Lastly, ncRNA-based molecular surveillance has the potential to revolutionize the existing paradigm of treating chronic diseases since it enables real-time and dynamic monitoring and offers more personalized treatment plans.

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