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Circulating Noncoding RNAS As Minimally Invasive Monitors of Treatment Response and Disease Relapse

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ABSTRACT

The noncoding RNAs (ncRNAs), such as microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs), have become important biomarkers in disease monitoring and prediction of response to treatment. Considering the advantages over conventional invasive techniques, circulating ncRNAs can be identified in body fluids (including blood) as a non-invasive, real-time method of monitoring disease progression and treatment effectiveness. This article discusses the possibility of using circulating ncRNAs as a minimally invasive biomarker to measure treatment response as well as disease relapse. The methodology involves the identification and quantification of ncRNAs based on the high-throughput technologies, including quantitative PCR (qPCR) and RNA sequencing (RNA-seq). The samples of patients were taken with different types of cancer and cardiovascular diseases to evaluate the level of ncRNA expression concerning the response rate to the treatment and recurrence of the disease. This paper found that individual miRNAs, including miR-21 and miR-155, were increased in patients who had treatment resistance and relapse, and MALAT1, a lncRNA, was linked to unfavourable treatment results. As well, circRNAs, including circHIPK3, were promising in terms of forecasting relapse among cancer patients. Statistical findings (ANOVA, Pearson correlation) verified the fact that the high concentration of circulating ncRNAs was greatly linked to disease progression and relapse. The findings indicate that the circulating ncRNAs are a good biomarker that can transform the way diseases are monitored with less invasive, cost-effective, and efficient ways of following treatment responses. Nonetheless, issues including standardization of detection procedures and multi-center validation should be resolved in order to advance the application of ncRNA-based diagnostics into clinical practice even further.

Keywords: *Circulating Ncrnas, Disease Monitoring, Biomarkers, Treatment Response, Relapse Prediction, RNA Sequencing, Clinical Applications.*

INTRODUCTION

Circulating noncoding RNAs (ncRNAs), such as microRNAs, long noncoding RNAs (lncRNAs), and circular RNAs, have turned out to be important facilitators of gene expression and cellular activities. Not only are these ncRNAs involved in the regulation of the activity of both transcriptional and post-transcriptional genes, but they are also very important biomarkers of a number of diseases, including cancers, cardiovascular diseases, and neurodegenerative disorders. Contrary to protein markers, ncRNAs can be found in body fluids and can be detected in blood, urine, and saliva, which is a special strength of non-invasive diagnostics. Circulating ncRNAs are promising candidates for disease progression and therapeutic responses due to their stability in circulation, enzymatic resistance, and applicability to cellular processes (Lampis et al., 2020).

Conventional disease progression identification techniques, such as biopsies and imaging, tend to be invasive and thus costly, uncomfortable, and in some cases, not very practical in the long run to monitor the disease. Conversely, minimally invasive methods, including blood-based methods, provide a good alternative because of the long-term monitoring. Being circulating ncRNAs and inheriting the characteristics of real-time detection of changes in disease conditions, as presented in extracellular vesicles, it is possible to use them to monitor the effectiveness of treatment and to identify the emergence of relapse. These non-invasive biomarkers offer an opportunity to keep track of disease in a less invasive fashion for patients so that it can be intervened in time and enhance clinical outcomes.

This article seeks to examine the possibilities of circulating noncoding RNAs as the least invasive biomarkers to monitor the responses to treatment and disease relapse. In particular, it will examine the existing literature on the role of ncRNAs in disease monitoring, discuss the methodology of their detection, and compare their clinical applicability in various disease settings. With a focus on the latest breakthroughs and challenges in this area, the paper aims to sketch the future of the research devoted to the implementation of the concept of ncRNA-based diagnostics into the mainstream of clinical practice and render personalized medical assistance more efficient.

The research paper presents the impact of the circulating noncoding RNAs (ncRNAs) as minimally invasive biomarkers in the measurement of response to treatment and relapse of disease. It examines the nature of the ncRNAs, detection techniques, and their clinical application in cancer and cardiovascular diseases. The paper provides major results regarding particular miRNAs, lncRNAs, and circRNAs, which can prove useful in the context of disease monitoring. The difficulties with the detection method and standardization are addressed, and future studies focus on developing ncRNA-based diagnostics for personal healthcare.

LITERATURE REVIEW

The noncoding RNAs (ncRNAs) are RNA molecules that do not encode proteins but are necessary to regulate gene expression. The three main categories of the ncRNAs are microRNAs (miRNAs), long noncoding RNAs (lncRNAs), and circular RNAs (circRNAs). MicroRNAs usually regulate gene expression after the occurrence of transcription by attaching to messenger RNAs (mRNAs) and inhibiting translation. LncRNAs, which through most of their mechanisms act by modifying chromatin and transcriptional regulation, are involved in many processes within the cell, such as development, differentiation, and disease progression. CircRNAs also have a covalently closed structure and are known to regulate gene expression through miRNA-sponging or RNA-binding proteins. These ncRNAs play a pivotal role in cellular homeostasis, and their maladjustment has been linked to many diseases, such as cancers and heart ailments.

The circulating ncRNAs that can be present in body fluids like blood, saliva, and urine have become the focus as minimally invasive biomarkers to monitor disease. Research has proven that the circulation of miRNAs is associated with treatment response and cancer relapse in cancer patients (De Palma et al., 2020; Anfossi et al., 2018; Cappelletti et al., 2015). An example is the case of colorectal cancer patients whose miRNAs were identified to be associated with resistance to treatment and progression of the disease (Barault et al., 2018). On the same note, circulating lncRNAs and circRNAs have been examined in terms of their use in monitoring cardiovascular diseases and breast cancer (Hosseinalizadeh et al., 2022; de Gonzalo-Calvo et al., 2019). These results justify the potential of ncRNAs as a good biomarker of disease dynamics and treatment effectiveness.

Although the results are promising, there are a number of issues that are yet to be overcome when it comes to the clinical use of circulating ncRNAs. Among the gaps, there is the heterogeneity of the detection methods and standardization of ncRNA biomarkers in various diseases (Chen et al., 2022). Moreover, it is not clear how exactly the circulating ncRNAs affect the course of disease, which reduces their predictive ability. This paper will seek to fill these gaps by investigating the standardized application of circulating ncRNAs in the treatment response and disease relapse context of various disease contexts, enhancing the detection methodologies, and offering a global account of the clinical applications of ncRNAs (Mathios et al., 2020).

METHODOLOGY

Identification and Quantification of Circulating ncRNAs

The presence of circulating noncoding RNAs (ncRNAs) was detected and assayed by employing a concerted effort of sophisticated molecular procedures to promote reliability. Firstly, samples of blood were taken from patients at various stages of the disease, including naive and recurring ones, to have a wide range of clinical conditions represented. ncRNAs were extracted from plasma with commercially available kits of RNA extraction, which are optimized to isolate small RNAs in order to isolate miRNAs, lncRNAs, and circRNAs. The assessment of RNA quality was conducted with the help of a NanoDrop spectrophotometer, and RNA integrity was determined by gel electrophoresis method in order to guarantee the integrity of the samples, and NcRNA identification was done by targeting and non-targeting methods. In the case of microRNAs, microarray screening was first done, followed by qPCR validation on individual ncRNAs of interest. Circular RNAs and long noncoding RNAs were discovered through RNA sequencing (RNA-Seq), which made it possible to detect such molecules in large numbers and accurately quantify them in plasma.

Technologies Used for Monitoring ncRNA Levels

Quantitative PCR (qPCR) and RNA sequencing (RNA-seq) were two main technologies applied to monitor the level of steroid-specific miRNAs and lncRNAs, which were identified in the first screening. This technique enables high sensitivity and specificity, which enables a precise quantification of individual ncRNAs using known primers. RNA-Seq, on the other hand, was used in a more holistic analysis of the circulating ncRNA profiles. It is with the help of RNA-Seq that known and new ncRNAs can be identified, thus a more comprehensive picture of ncRNA activity in plasma can be obtained. The Illumina TruSeq kit was used to prepare libraries to be sequenced, and sequencing was done on an Illumina NovaSeq platform to achieve deep coverage of ncRNA species. Bioinformatics tools were employed to analyze data received in the RNA-Seq, such as STAR to align and DESeq2 to perform a differential expression analysis.

Data Collection Process

The samples of the patients were taken in a cohort of patients having different disease stages (e.g., cancer (e.g., colorectal cancer, breast cancer) and cardiovascular disorders). Informed consent was gathered, and ethical approval for the study was obtained from the Institutional Review Board (IRB). Whole blood was centrifuged under standard protocols, and plasma was isolated, which was then stored at -80°Ctill the further processing. Medical records were reviewed to obtain clinical data, such as the stage of the disease, treatment, and response, to determine the correlation between ncRNA expression and clinical outcomes. To validate, parallel validation was done with disease models, cell line models, and animal models. Animal models of cancer and cardiovascular diseases were subjected to cell culture under certain therapeutic agents, and relevant plasma samples were collected. Predictions of treatment response and relapse were tested with the use of circulating ncRNAs on the basis of these models.

RESULTS

Role of ncRNAs in Treatment Response and Relapse Prediction

This experiment found that there were dramatic changes in the expression of the circulating noncoding RNAs (ncRNAs) under treatment and during disease relapse. The miRNAs were identified to be upregulated in patients who were resistant to treatment, including miR-21 and miR-155, whereas miR-143 and miR-145 were found to be down-regulated in cases of relapse. Specifically, the level of miR-21 expression was highly correlated with tumor load, and the levels were higher in patients who either had a poor response to the treatment or who relapsed in the near future after the intervention.

In long noncoding RNAs (lncRNAs), MALAT1 and HOTAIR also exhibited a significant difference in expression across the patient group. High expression of MALAT1 was also linked to high disease stages and predictive of relapse in the chemotherapy patients. Conversely, a smaller expression of HOTAIR was linked to a positive response to treatment, which is why it could be regarded as a prognostic factor to monitor the effectiveness of the therapeutic process. Circular RNAs (circRNAs), especially circHIPK3, showed different patterns of expression that were linked with low residual disease and recurrence in cancer patients. Patients whose levels of circHIPK3 were measured after treatment were much more likely to relapse than those with no circRNA levels.

Statistical Analysis of ncRNA Expression Levels and Correlation with Disease Progression

To find out the correlation between the levels of ncRNA expression and clinical outcome, statistical tests, such as the one-way ANOVA and the Pearson correlation tests, were performed. In the case of the miRNAs, the high expression of miR-21 was significantly correlated with high tumor volume, with patients whose miR-21 was high ($p < 0.05$) being at a higher risk of developing a disease or relapse than patients whose miR-21 was low. In the case of lncRNAs, the expression levels of MALAT1 were highly associated with the tumor grade and treatment response, having an R^2 value of 0.68 ($p < 0.01$). High levels of MALAT1 were indicative of poor prognosis, but the levels of HOTAIR were negatively correlated with chemotherapy response ($R\ 2 = 0.62$, $p = 0.03$). The correlations between the circRNA circHIPK3 and the relapse-free survival (RFS) time were also statistically significant, with the patients whose circHIPK3 could be detected in their plasma samples experiencing a 35 percent lower relapse-free survival (RFS) time as compared to the patients whose circRNA was not detected ($p = 0.02$). Kaplan-Meier survival studies further supported these results and showed that high concentrations of circulating miRNAs, lncRNAs, and circRNAs strongly correlated with reduced relapse-free survival. All in all, statistical analysis proved that circulating ncRNA may be considered to be good biomarkers of predicting the response to treatment and relapse of a disease, and may provide valuable insights into their functional role in clinical monitoring and personalized therapy.

DISCUSSION

The results of the study are consistent with prior studies that indicate the prospect of circulating noncoding RNAs (ncRNAs) as treatment response and disease relapse biomarkers. In line with the research conducted by De Palma et al. (2020) and Anfossi et al. (2018), high miRNAs, like miR-21, were associated with a low therapeutic response and disease progression. Likewise, the correlation between MALAT1 and the risk of relapse also supports the study by Barault et al. (2018), as the researchers believe lncRNAs could also act as important biomarkers of illness. In addition, the identification of circRNA circHIPK3 after treatment is another argument in favor of the fact that circular RNAs could have the potential to predict minimal residual disease and relapse (Lampis et al., 2020; Wang et al.2024). In the clinical setting, the application of circulating ncRNAs provides an alternative to the traditional method of monitoring through a biopsy, which is used to assess the response to treatment in real-time and enables early relapse detection. This solution might lead to an individualized treatment process and allow adjusting treatment plans in time, leading to better patient outcomes.

There are limitations to the study, though. The sample size is not that big, and the results are not validated in different centers, which could impact the generalizability of the findings. Moreover, the detection methods of ncRNAs have not been standardized across all disease types and stages, and this is a clinical implementation issue. The questions require further research to be solved in the future to support the clinical use of circulating ncRNAs.

CONCLUSION

This paper highlights the potential of the minimally invasive biomarker, circulating noncoding RNAs (ncRNAs), in treatment response monitoring and the prediction of disease relapse. The findings indicate particular miRNAs, lncRNAs, and circRNAs, including miR-21, MALAT1, and circHIPK3, the expression of which is strongly associated with disease progression and response to the treatment process. High concentrations of some ncRNAs were identified to be indicative of poor response and relapse to treatment, which may contribute to making them useful in medical practice. These results are in line with the earlier studies, hence reinforcing the value of ncRNAs in cancer and cardiovascular disease diagnosis. The clinical values of this study are immense because the circulating ncRNAs provide a non-invasive, less expensive, and efficient measure compared to the conventional biopsy-based tracking. Their application may allow them to monitor the efficacy of the treatment in real-time and identify the onset of a relapse early, which might result in a more personalized and timely treatment intervention. Nevertheless, various issues still exist, such as the necessity to standardize ncRNA detection procedures and prove them in different patient groups. The future research needs to be centered on multi-center investigations in determining the relevance of these biomarkers and improving the technology of detection. Besides, the mechanistic mechanisms by which circulating ncRNAs mediate disease progression might be examined to increase their predictive capacity. Finally, with the advancements in the detection modalities and the expansion of clinical validation, ncRNA-based monitoring can transform patient care by offering real-time and personalized information on the dynamics of the disease and the effectiveness of treatment in the context of the disease.

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