CIRCULATING RNA IN BLOOD PLASMA AS DIAGNOSTIC TOOL FOR CLINICAL ONCOLOGY

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One of the key challenges facing today's oncology is the discovery of early predictors of malignant neoplasms in patients' biological samples. Liquid biopsy is a noninvasive diagnostic technique based on the detection and isolation of tumor cells, tumor-derived nucleic acid and exosomes circulating in the blood plasma of cancer patients. There is a plethora of research studies of circulating tumor DNA in patients with MN. The active proliferation of tumor cells occurs in the backdrop of altered gene expression. The presence of tissue-specific transcripts in the circulating RNA fraction suggests that levels of circulating RNA reflect the development of the primary tumor. We think that cell-free RNA circulating in the blood plasma is a promising molecular biomarker for early cancer detection.

Keywords: circulating nucleic acids, blood plasma, circulating tumor cells, circulating RNA, miRNA, biomarkers, oncology

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АНАЛИЗ ВНЕКЛЕТОЧНОЙ ФРАКЦИИ РНК ПЛАЗМЫ КАК ИНСТРУМЕНТ ДИАГНОСТИКИ В ОНКОЛОГИИ

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Одна из ключевых задач современной онкодиагностики — поиск ранних предикторов злокачественных новообразований (ЗНО) при анализе наиболее доступных видов биоматериала. Жидкостная биопсия представляет собой одну из неинвазивных методик и включает в себя обнаружение и выделение циркулирующих опухолевых клеток, циркулирующих опухолевых нуклеиновых кислот и экзосом из плазмы крови у пациентов со злокачественными заболеваниями. Множество работ посвящено исследованию внеклеточной фракции ДНК при ЗНО. Вместе с тем активную пролиферацию трансформированных клеток при развитии опухолей сопровождают значительные изменения экспрессии определенных генов. Обнаружение тканеспецифичных транскриптов в составе внеклеточной РНК плазмы крови (внРНК) позволяет предположить, что представленность циркулирующих в плазме РНК связана с развитием патологического процесса непосредственно в первичном очаге. На наш взгляд, внРНК плазмы крови представляют практическую ценность в качестве молекулярно-генетических маркеров ранней диагностики в онкологии.

Ключевые слова: циркулирующие нуклеиновые кислоты, плазма крови, циркулирующие опухолевые клетки, внРНК, микроРНК, биомаркеры, онкология

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Diagnostic tests known as liquid biopsies hold promise for the future of cancer screening. They are capable of detecting tumor-derived biomarkers in the blood serum of patients with malignant neoplasms (MN), including circulating tumor cells, circulating tumor DNA or RNA, and exosomes. Liquid biopsy samples can be analyzed using a few different types of analysis, such as quantification of individual analytes, including proteins, identification of nucleic acid sequences of the analyte, profiling DNA methylation, etc. [1]. The analysis of cell-free nucleic acids circulating in the blood plasma allows assessing the genetic heterogeneity of the tumor in response to anti-cancer therapy [2, 3].

It is known that apoptotic and necrotic cells release DNA or RNA fragments and exosomes (membrane-bound encapsulated subcellular structures containing proteins and nucleic acids derived from tumor cells) into the bloodstream [4]. From the early stages of carcinogenesis through the advanced stages of metastatic spread, tumor cells accumulate specific mutations and epigenetic modifications; these changes can be spotted by the analysis of cell-free nucleic acids.

Analysis of circulating DNA

The analysis of circulating DNA has been used in clinical oncology for over 20 years to aid the diagnosis and monitoring of the following cancers: lung [5, 6], head and neck [7], esophageal [8], breast [9], hepatic [10], colon [11], pancreatic [12], renal [13], and others. As a rule, the tests look for the presence of mutations in oncogenes, tumor suppressor genes and microsatellites [6, 9, 13]. Similarly, DNA methylation analysis has some diagnostic and prognostic value and can be employed for monitoring tumor growth [8, 14]. Quantitative aberrations of circulating DNA have been also reported in other pathologies besides MN, including preeclampsia [15], fetal chromosomal aneuploidy [16], and pernicious vomiting of pregnancy [17].

Analysis of circulating mRNA

The active proliferation of tumor cells and tumor evolution are accompanied by the pronounced changes in the abundance of various transcripts, some of which, like mRNA, can be quantified by RT-PCR [18]. RT-PCR was successfully used to measure the levels of circulating mRNA transcripts of housekeeping genes in the blood samples of healthy individuals and cancer patients [19]. Circulating RNA was also studied in patients with melanoma [20–22], follicular lymphoma [23], breast [22, 24–28], colon [23, 29], hepatic [30], esophageal [21], nasopharyngeal [31], thyroid [22], prostate [40, 41], lung [32] and other cancers. However, research into cell-free RNA is not limited to malignancies: its levels were investigated in patients with trauma [33, 34], diabetic myopathy [35], and pregnancy (fetal mRNA) [36].

A study demonstrated a statistically significant difference in hTERT mRNA levels between patients with early stages of *breast cancer* (BC) and healthy individuals. The presence of hTERT mRNA in the blood plasma of BC patients was affected by the surgical removal of the tumor [25]. However, it is unlikely that hTERT is a BC-specific marker because its concentrations also change in patients with melanoma and thyroid cancer [22]. The levels of hMAM mRNA expression in the blood plasma were correlated with unfavorable prognosis and poor survival in BC patients [26]. In another study, patients with BC were shown to have elevated Bmi-1 mRNA as compared to healthy donors [27]. According to a recent report, LincRNA-ROR (long intergenic non-protein coding RNA regulator of reprogramming) might be a potential biomarker of BC; considering that its plasma levels decline in the postoperative vs. preoperative period, this marker can be exploited to monitor a BC patient's condition [28].

It is reported that serum MMP-9 is elevated in the late stages of *ovarian cancer* and correlates with poor prognosis, which suggests the potential prognostic value of this biomarker [37]. The presence of circulating HMGA2 ctRNA may also be a promising tool for the diagnosis and monitoring of ovarian cancer [38].

Patients with advanced *prostate cancer* were shown to have higher levels of circulating cBMP6 mRNA than those with the localized lesion. At the same time, H3K27me3 is characterized by inverse distribution, and its levels are significantly lower in patients with metastatic prostate cancer than in those with early stages of the disease. Thus, post-treatment levels of circulating cBMP6 and H3K27 mRNAs are discriminators between metastatic and localized prostate cancer [39]. Levels of hTERT mRNA in the blood plasma might be another biomarker for distinguishing between localized and locally advanced prostate cancer [40].

Analysis of exosome composition

Ever more attention has been paid to the research into the extracellular vesicles (exosomes and microvesicles) secreted by the tumor that are thought to promote invasion and metastatic spread [41, 42].

Extracellular vesicles are specialized membrane organoids secreted by most cell types; they contain various molecules, including RNA, lipids, proteins, and metabolites [43, 44]. At present, extracellular vesicles are being increasingly recognized as mediators of cell-to-cell communication, transporting mRNA from cancer to normal cells across the extracellular matrix [45, 46].

Microvesicles contain microRNA, different types of long RNA, including mRNA, circular RNA and long non-coding RNA [47, 48]. RNA profiles of extracellular vesicles isolated from healthy individuals and patients with hepatocellular carcinoma are significantly different [48].

Analysis of circulating microRNA

MicroRNA comprises a group of non-coding regulatory RNA consisting of approximately 22 nucleotides and playing an essential role in the regulation of gene expression [49]. Relatively high stability makes microRNA a more advantageous biomarker than mRNA. MicroRNA is found both inside and outside exosomes [50, 51] and is highly stable due to its association with argonaute proteins [52] or lipoprotein complexes, like high density lipoproteins [53].

There were attempts to analyze circulating microRNA in patients with lymphoma [54] and in the plasma/serum samples of patients with prostate cancer [55]. Plasma levels of miR-26a can be indicative of ovarian epithelial cancer [56]. Patients with BC have significantly elevated concentrations of 4 different microRNAs (miR-148b, miR-376c, miR-409-3p, miR-801) [57]. Increased levels of miR-16, miR-21, and miR-451 and low miR-145 concentrations were observed in the plasma of patients with BC [58]. Used in combination, miR-145 and miR-451 were shown to be the best biomarkers of BC, helping to discriminate between BC patients and healthy individuals or patients with other cancers.

Challenges and limitations

Although the analysis of circulating RNA has impressive potential for the application in different fields of medicine, it is not free from drawbacks. Errors occurring during target amplification can affect the results of RNA quantification, especially when dealing with small numbers of analytes [59]. Some discrepancies might be due to the different efficacy of the applied reverse-transcriptase amplification techniques observed for different microRNA and mRNA sequences in different molecular environments. Therefore, PCR-free strategies for detecting circulating RNA seem to be most attractive [60, 61].

Today, most diagnostic approaches based on the analysis of circulating RNA have relatively low specificity and sensitivity [62]. Their improvement requires further large-scale prospective cohort studies.

CONCLUSIONS

The analysis of circulating RNA in cancer patients has a high diagnostic and prognostic value. The informative value of liquid biopsy can be considerably improved by separately analyzing the exosomal and cell-free circulating RNAs, including microRNA. Standardization of sample collection, circulating RNA extraction and the analysis of the obtained results will help to reduce the number of false-negative and false-positive results. Further large-scale prospective cohort studies are needed to select the most sensitive and specific circulating RNA panels.

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