



NON-INVASIVE DIAGNOSIS OF GLIOBLASTOMA USING MICRO-RNA AND TUMOR-EDUCATED PLATELETS: A REVIEW OF EMERGING BIOMARKERS

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ABSTRACT

Glioblastoma (GB) represent a dynamic and prevalent form of cancer in the central nervous system. Its diagnosis typically relies on physical examination and imaging techniques, often complemented or confirmed by a surgical biopsy. Given the highly invasive nature of these procedures, there is an urgent need to develop non-invasive diagnostic and therapies for GB patients. In this context, liquid biopsy has emerged as a promising alternative, offering tumor-derived information from minimally invasive samples. Platelets, which can absorb or internalize tumor-derived molecules like microRNAs (miRNAs) during tumor progression, undergo changes in their own RNA expression profiles. These miRNAs not only regulate platelet reactivity but, when influenced by tumor signals, also serve as indicators of tumor presence. Such changes in platelet profiles develop tumor-educated platelets (TEPs). TEPs are implicated in cancer progression and metastasis according to their interplay with tumor cells. This review focuses on the potential of TEPs and miRNAs as tumor biomarkers in GB, providing new insights for the development of strategies aimed at cancer prevention and therapy.

Keywords: glioblastoma, tumor-educated platelets, liquid biopsy, tumor biomarkers.

Introduction

Glioblastoma (GB) represent an active malignant tumor of the central nervous system (CNS), emerged from neuroepithelial glial cells. It is expressed by highly invasive behavior, and marked genetic heterogeneity, contributing to its poor prognosis and frequent therapies failure. To date, no reliable biomarkers have been identified in blood or cerebrospinal fluid (CSF) for the monitoring, or prognostic evaluation of GB (1).

Liquid biopsies showed to be a non-invasive method for early detection of tumors, and monitoring (2). It is known that blood contain tumor-associated biomarkers like mononuclear

cell fraction, encompassing leucocytes, plasma and serum, which contain cell-free deoxyribonucleic acid (DNA), ribonucleic acid (RNA), plasma proteins, metabolites, and tumor-educated platelets (TEPs) (2-4).

TEPs are blood platelets that act as systemic biomarkers in the presence of tumors (5), and thereby are exposed to tumour-mediated platelet education, sequestering extracellular vesicles-derived RNAs (6, 7) and proteins (8). In the last years, few studies have been shown the TEPs roles in blood-based cancer diagnosis via RNA-sequencing based-methodology (9-12). Platelets are anucleate cell fragments that lack a nucleus, derived from megakaryocytes, their precursor

cell present in the bone marrow (13). Platelets contain also small RNAs including microRNAs (miRNAs) (14).

MiRNAs are small, evolutionary conserved, non-coding RNA, that assisted in the post-transcriptionally regulation of gene expression (15) like cell proliferation, differentiation, angiogenesis and apoptosis (16). Dysregulation of miRNAs is implicated in the progression of GB, besides other tumors (17). Some of circulating miRNAs showed to have an important role in GB pathogenesis, becoming a suitable aspirant for a noninvasive diagnosis of GB, like miR-21, miR-15b, miR-23a, miR-128, miR-342-3p, miR-150, miR-197, miR-497, and miR-185 (18-22).

The aim of the present review is to ensure updated information about the role of miRNA and TEPs in GB diagnosis, with an emphasis on liquid biopsy, formulating procedures to prevent and treat cancer.

Tumor-educated Platelets and microRNA as a Potential Biomarkers

To date, GB is mainly diagnosed based on clinical presentation, magnetic resonance imaging, and tissue biopsy. However, these approaches have important limitations, as they are highly invasive and associated with potential side effects. Since the introduction of the 2016 World Health Organization classification, routine diagnostic evaluation of GB has required molecular genetic profiling to support prognostic stratification. Among the most clinically relevant biomarkers are the mutational status of IDH1/2 and methylation of the MGMT promoter (23). To overcome the limitations associated with tissue sampling, blood-based liquid biopsies have been proposed as an alternative (23, 24). These approaches offer several advantages, including the potential for earlier cancer detection, reduced costs through personalized molecular-targeted therapies, and enhanced support for clinical decision-making in oncology. Nevertheless, currently available blood-derived biomarkers showed limited sensitivity for cancer detection, particularly in patients with localized disease (25).

Moreover, brain tumors remain especially

challenging, using blood-based methods. To date, commonly investigated bio-sources such as exosomes, and circulating tumor cells (CTCs) have shown limited applicability in GB, hindering their use in early diagnosis (3, 26). This limitation may be addressed by TEPs, which have been reported to enable effective blood-based cancer detection (10, 22).

Study published by Best and contributors (9) showed that molecular investigation of blood platelets offers valuable diagnostics information for patients which present six different tumor type, inclusive GB, suggesting that platelets may be employed as all in one biosource to detect the molecular part of tumors. Another study of Sol and contributors using thromboSeq platelet RNA sequencing pipeline and the digital SWARM algorithm sustained that TEPs can become a minimally invasive biosource from blood-based diagnostics of GB patients (27).

Tumors can influence both signaling pathways and protein synthesis in platelets. Traditionally, platelets were considered biologically limited, leading to the supposition that their content lacked transcriptional and translational activity. Interestingly, some studies have showed that platelets retain the ability to synthesize proteins, playing a crucial role in signaling-dependent activation processes (28). These processes may result in what is described as “platelet education,” characterized by alterations in RNA and proteomic profiles. Through this transformation, normal platelets can acquire oncogenic properties, becoming so-called TEPs. The concept of TEPs has gained increasing attention as advances in technology continue to reveal the complex interplay between megakaryocytes, platelets, and cancer (29).

Several miRNAs have been identified in vitro and some in serum, plasma, and circulating exosomes of GB patients (30). This observation indicates that, as in other malignancies, miRNAs may serve as promising noninvasive biomarkers for the early detection of GB. Notably, miRNAs whether enclosed within exosomes or present in a free form showed high stability and can be readily detected across a variety of biofluids like CSF. Beyond their diagnostic potential, a number of miRNAs have recently been shown to provide valuable insight into patients' responses to GB

therapy. Therefore, the application of miRNAs in the evolution of the next RNA-based therapeutic approaches for GB holds considerable promise (31).

Liquid Biopsy from Glioblastoma

However, liquid biopsy showed to be a valuable approach in the identification of cancer biomarkers. Its growing relevance is largely due to its minimally invasive nature in sample collection (32). Although blood is the most commonly used sample in GB, CSF is also used as biomarker detection. Among the most extensively studied biomarkers are circulating free nucleic acids, as well as circulating tumor cells, extracellular vesicles, volatile organic compounds, and TEPs. For clinical applicability, these molecules must be tumor-specific and present in detectable concentrations (33).

Although the blood-brain barrier is known to be compromised in GB, it still represents a significant obstacle that must be overcome to achieve therapeutically effective concentrations of molecules, while minimizing off-target effects in other organs (34). Liquid biopsy focuses on the detection and quantification of tumor-derived components, including cell-free nucleic acids, which can encapsulate DNA, RNA, and other biomolecules (i.e., CTCs, proteins, metabolites, and TEPs) (35). These components can disseminate to distant sites and can be detected in various body fluids (36).

Such tumor-derived elements can serve as valuable cancer biomarkers, often providing information comparable to, or even more comprehensive than obtained through tissue biopsy. They may help identify the tumor's origin and support the monitoring of disease progression and treatment response. Due to its ability to analyze tumor-specific components in real time, liquid biopsy is increasingly being applied across multiple cancer types, offering important insights into diagnosis, prognosis, and ongoing tumor dynamics (37).

Conclusions

Both miRNAs and TEPs show considerable potential as sources of biomarkers, although

their combined use has not yet been thoroughly investigated. With continued collaborative efforts, noninvasive methods such as liquid biopsy may become a key element of personalized neuro-oncology.

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