Blood platelets as an RNA biomarker platform for neuro-oncological diseases

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Abstract
Blood-based liquid biopsies are an upcoming approach for earlier cancer detection, diagnostics, prognostics, therapy-response prediction, and therapy monitoring, including in patients with tumors of the central nervous system. Among these, liquid biopsies are plasma-derived markers such as cell-free DNA, RNA and proteins, extracellular vesicles, circulating glioma cells, immune cells, and blood platelets. Blood platelets are involved in the local and systemic response to the presence of cancer, thereby sequestering and splicing RNAs, which may be clinically useful as blood-based biomarkers. In this review, we discuss the available literature regarding the role of blood platelets in gliomas and provide suggestions for future research efforts.

Key words
blood | brain tumor | glioma | glioblastoma | liquid biopsies | neuro-oncology | RNA | tumor-educated platelets

Liquid Biopsies in Glioma

Liquid biopsies are a minimally invasive way of obtaining molecular information associated with the presence of a tumor.1-3 Such biomarkers can provide diagnostic information, as well as prognostic value, information on therapy-response prediction, and may guide therapy monitoring. Several blood-based biosources, such as plasma, serum, plasma-derived extracellular vesicles, and circulating tumor cells, with different biomolecules such as DNA, RNA, proteins, and metabolites, are currently being evaluated for their use for many types of cancer,4,5 including in patients with glioma.6,7 Despite these efforts, brain tumors remain notorious for their difficult detectability in blood.5,8,9 For example, many plasma-derived protein biomarkers, including GFAP, YKL-40, interleukines, and angiogenic factors, and miRNA biomarkers, including miR-21, have been proposed, of which most show contrary results in the published studies.10 Also, when analyzing multiple point mutations and genomic rearrangements in plasma cell-free DNA, in less than 10% of the patients, a trace of the presence of glioblastoma was identified.11 For IDH1-mutations in plasma DNA, in only 60% of the IDH1-mutant glioma patients, this biomarker was correctly identified.12 Interestingly, identification and filtering of cell-free DNA fragments drastically improved detection accuracies in patients with glioblastoma,13 and methylated nucleic acids are also promising as glioma biomarkers.14,15 Finally, circulating glioma cells were detected in 20-73% of patients with glioblastoma depending on the detection method applied.16-18 Alternatively, it has been proposed to employ cerebrospinal fluid as a biosource for molecular biomarkers in both pediatric and adult brain tumors.19,20 Thus far, none of the identified circulating biomarkers is ready for implementation in a clinical setting, and novel biomarkers with high accuracy levels are desired, as individual biomarkers and/or as combined biomarker panels.10

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Over the past decades, there has been a growing interest in the role of blood platelets in immune and inflammatory activities in health and disease, including in cancer progression. In the 19th century, the first observations of the role of platelet in cancer correlated a reduced metastatic capability to decreasing platelet counts. This led to the hypothesis that platelets are primarily involved in the process of cancer metastasis. More recently, platelets have been found to be able to protect circulating tumor cells from immune cells, allowing tumor cells to safely migrate to metastatic sites in mice with melanoma, fibrosarcoma, or lymphoma. Platelets can form a cell-fibrin-platelet aggregate, surrounding the circulating tumor cells. Platelets provide protection while transferring MHC class I proteins to the circulating tumor cells, thereby shielding the circulating tumor cells from natural killer cells. Additionally, platelets can supply the tumor with pro-angiogenic factors like VEGF, PDGF, and bFGF, of which the VEGF levels were significantly elevated in platelets of patients with glioblastoma (Figure 1). These pro-angiogenic growth factors are released during platelet activation, creating a microenvironment that can promote tumor growth, as observed in in vivo mouse models of metastatic melanoma and lung carcinoma.

Data from in vitro experiments showed that cytokines released from activated platelets in mice injected with human glioblastoma cells increased angiogenic activity, cell proliferation, and cell migration. Alternatively, the same study showed that nude mice injected with human glioblastoma cells that were depleted of platelets did not demonstrate significantly reduced tumor growth as opposed to control mice, indicating that the effect may be at least partially driven by immune cells. Another study revealed that circulating platelets reduce tumor cell apoptosis and anoikis when detached from the primary tumor through YAP1 activation. Finally and surprisingly, patients with an IDH1 mutant glioma are at risk for the development of venous thrombo-embolism, whereas those with IDH1 wild-type are not. This effect is attributed to the potent anti-thrombotic effect of IDH1, potentially via reduced tissue factor protein levels and D-2-hydroxyglutarate activity, indicating that such tumor-intrinsic factors act on a systemic level.

This also positions platelets as potential targets for cancer therapy. There is a long debate ongoing regarding the potential beneficial and protective benefit of the platelet cyclo-oxygenase inhibitor aspirin, which has been shown to reduce mortality rates in colorectal cancer. In vitro experiments to study the potential benefits of aspirin as an additive in glioblastoma therapy with temozolomide showed that aspirin has an inhibitory effect on VEGF-promoted angiogenesis triggered by temozolomide therapy, indicating a synergistic effect. Also in vitro and in vivo experiments on glioblastoma cell lines have shown a similar sensitization of temozolomide treatment with temozolomide/aspirin-coloaded microspheres.

Till date and to the best of our knowledge, no prospective clinical trials investigated this hypothesis. In all, multiple lines of evidence indicate that blood platelets contribute to the progression of gliomas.
Blood Platelets as a Tool for Blood-Based Cancer Diagnostics

Platelets are an attractive diagnostics biosource as they have already been shown to be useful in predicting the presence of cancer. An increased platelet count is associated with increased mortality rates in among others, lung, renal, and gastric cancers. Platelets are anucleated cells and thus cannot transcribe novel RNA molecules. However, platelets inherit many types of RNAs from the megakaryocyte, including mRNA, IncRNAs, miRNAs, tRNAs, and circRNAs. Throughout their life-time of approximately 7–10 days, platelets dynamically regulate their RNA content and are able to translate their RNAs into proteins upon external cues. In 2011, it was uncovered that platelets sequester glioblastoma-derived EGFRvIII RNA transcripts, likely through transfer mechanisms involving extracellular vesicles. A similar observation was made for the EML4-ALK rearrangement in patients with non-small-cell lung cancer, and the PCA3 transcript in prostate cancer. These tumor-derived mutants can be easily detected by highly-sensitive PCR or deep sequencing methodologies. The mRNA content of blood platelets in patients with cancer also was investigated using shallow RNA-sequencing, termed thromboSeq, with which hundreds of mRNA transcripts were detected with different levels in patients with lower-grade glioma, glioblastoma, and brain metastases. These findings prompted a follow-up study in which blood of glioblastoma patients was longitudinally sampled following tumor surgery and concomitant chemoradiotherapy treatment. It was demonstrated that the glioblastoma signal decreased in the blood platelets following tumor resection, and that this decreased signal was also associated with tumor pseudo-progression as opposed to true tumor progression. Of note, this platelet-based diagnostics methodology may also be applied to patients with other brain disorders, such as multiple sclerosis. The exact mechanisms of these platelet alterations are currently unknown and remain a focus of follow-up research. Platelets are able to sequester circulating RNA molecules by interacting with the surrounding environment. In addition, there are considered to be different platelet subpopulations and potentially differential education of the bone marrow-resident and lung-resident megakaryocytes, as well as specific platelet-immune cell and platelet tumor-cell interactions and alternative splicing events, resulting in differential platelet activation and platelet mRNA splicing. Such biological mechanisms may be further elucidated by, for example, single platelet RNA-characterization (e.g., using CyTOF methodologies), platelet sorting based on known markers for subpopulations and subsequent characterization (e.g., using P-selectin for enrichment of younger platelets), analysis of megakaryocyte transcriptomes from both lungs and bone marrow together with the circulating pool of platelets in glioblastoma mouse models, and analysis of clusters of platelets with circulating immune cells, and potential culturing of such aggregates.

Conclusion and Future Perspectives

Liquid biopsies have been proposed as a potential non-invasive procedure to detect and monitor gliomas. Analyzing blood platelet RNA isolated from glioma patients may become a fast and easily accessible tool, yielding a plethora of potential RNA biomarkers, not excluding miRNAs, IncRNAs, tRNAs, and circRNAs. Blood-based liquid biopsies in the field of neuro-oncology will especially be of relevance for therapy monitoring purposes, complementing the current use of clinical examination and imaging modalities, for example, for the (earlier) detection of glioblastoma pseudoprogression and detection of tumor progression in patients with a lower-grade glioma. However, thus far none of the identified liquid biomarkers for patients with glioma is ready for clinical implementation. Here, we propose the following next steps: 1) the biological mechanisms of the measured biomarkers should be elucidated, and whether these are primary tumor-intrinsic or secondary response events; 2) biomarker discovery should be performed on well-defined and annotated clinical cohorts, preferentially in an unbiased manner; 3) large-scale clinical trials should be conducted for biomarker evaluation and validation, preferably in a multi-center, prospective, and blinded manner; 4) pre-clinical variables of validated biomarkers should be extensively studied and all employed tests should be well-standardized; 5) biomarker discovery studies should be focused on evaluation of multiple biosources and biomolecules in parallel, yielding biomarker panels; and 6) blood-based biomarkers should complement clinical and radiological parameters, e.g., by including such parameters in a clinical evaluation score. Also, platelets should be part of every blood biobank of patients with glioma in order to facilitate validation of upcoming to-be-discovered biomarkers. Potentially, additional knowledge on the dynamics of blood markers may also elucidate novel pathophysiological mechanisms involved in the progression of glioma.
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