Liquid Biopsy of Brain Tumors

Subjects: Medicine, Research & Experimental | Clinical Neurology | Pathology
Contributor: Robert Eibl

Direct biopsies obtain tissue material from the primary tumor, either via neurosurgical removal of all or most parts of a tumor or via stereotactic tissue biopsy. In contrast, a liquid biopsy uses body fluids collected distant to the brain tumor, such as venous blood from the arm or cerebrospinal fluid (CSF) via lumbar or cisternal puncture.

Keywords: liquid biopsy ; brain tumor ; circulating tumor cell-CTC ; circulating tumor (ct)DNA ; extracellular vesicle ; microRNA-miR ; biomarker ; glioblastoma ; medulloblastoma ; glioma

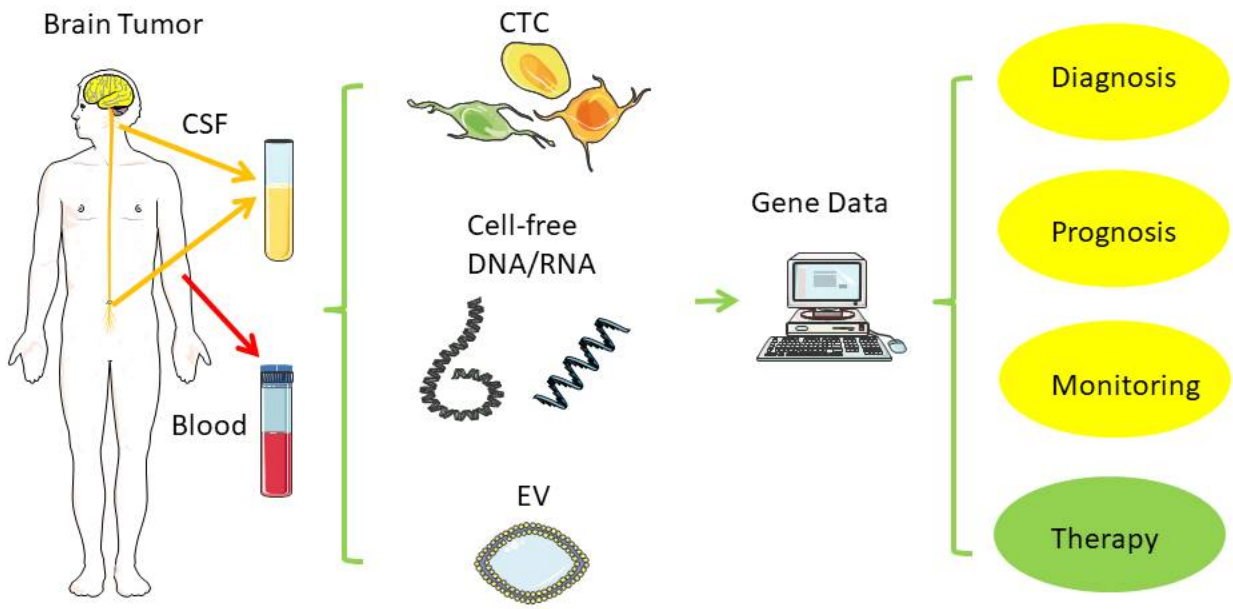

Figure 1. Liquid biopsy. Distant from the original brain tumor, samples from blood and cerebrospinal fluid (CSF) can typically serve as low-risk source of tumor-derived nucleic acids (RNA, DNA) for further analysis. Notes: CSF - cerebrospinal fluid; EV - extracellular vesicle; CTC - circulating tumor cell. Created/modified with https://smart.servier.com (accessed on 8 August 2021), licensed under Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/ (accessed on 8 August 2021)).

1. Circulating Tumor Cells—CTCs

Circulating tumor cells (CTCs) are cells derived from a tumor which enter the bloodstream and other body fluids, e.g., the CSF (**Figure 1**). Unfortunately, they are extremely rare for almost all cancers; so far, a widely accepted standard method for identifying and collecting CTCs is still lacking. Since brain tumors do not usually metastasize via the bloodstream [1][2][3][4][5][6], it was quite a surprise to find CTCs in 20–40% of glioblastoma patients [7][8][9][10][11][12]. In carcinoma, clusters of tumor cells are increasingly associated with easier metastatic spread compared to single CTCs; similar clusters can be found in glioblastomas [11], supporting the idea that glioblastoma-derived CTCs can cross the blood–brain barrier as clusters of cells, although they almost never establish metastases [13]. Applying the seed-and-soil hypothesis from Paget

from 1889, they may have the ability to spread (seed), but cannot find the right target tissue or endothelium (soil), where they can egress, survive, and grow [14]. The limited overall survival time of the patients may also prevent micrometastases from growing larger after seeding. Different approaches exist to isolate CTCs for many tumor entities. To isolate CTCs from carcinoma, an elegant method uses epithelial cell adhesion molecule (EpCAM) as a surface marker, which is exclusively expressed on epithelia and epithelial-derived neoplasms; since EpCAM is not expressed on leukocytes, it can be used to selectively accumulate CTCs. In contrast, EpCAM is not expressed on brain tumor cells and, therefore, cannot be used as a selection marker for these cancers. Currently, no adequate surface marker for brain tumor CTCs has been established. Unfortunately, other isolation methods, which use the bigger size of tumor cells, are still less efficient. However, in at least 50% of the cases, CTCs from brain tumors can in fact be isolated from the blood or CSF. These can be extremely informative for molecular diagnostics with subclassification of tumors, as well as prognosis and repeated monitoring of progression and therapy resistance. The specificity of mutations found in CTCs is very high, but low sensitivity remains a big challenge, i.e., not all liquid biopsies from brain tumor samples allow the isolation of CTCs. Not only the genetic analysis of CTCs is tempting to be applied for diagnostics, but also pharmacological and [15] tests are in progress [16][17][18][19][20][21][22][23][24][25]. It is noteworthy that brain tumor CTCs are easier to collect from CSF than from blood. Interestingly, cisternal puncture appears to give even better results than lumbar puncture, although the CSF is moving downward and is fully exchanged 3–5 times per day. Interestingly, circulating epithelial, i.e., noncancerous, cells have also been found in benign, inflammatory bowel diseases [26]; this points to the need for molecular characterization of cells obtained for CTC testing. Collection of CSF is usually performed easily, with a rare risk of damage to the brain. Major challenges for CTCs in brain tumors are the low sensitivity and the need for standardization, e.g., the amount of material and the source (blood, serum, plasma, or CSF from lumbar or cisternal puncture). If possible, cisternal puncture appears to be superior, but this procedure is not as easily performed as a lumbar puncture. For blood samples, 7.5 mL may become a standard for some, although higher amounts of blood increase the chance of finding CTCs, which is why others recommend over 30 mL. Due to these limitations, CTCs are only useful for a few patients with brain tumors. The collection of a sufficient amount of CSF may appear to be an additional challenge for children with brain tumors, such as medulloblastomas, but usually not for adults; it is also possible to use a previously implanted shunt from the ventricle to collect CSF. In carcinomas, the number of CTCs was correlated with time of survival, i.e., patients with more than a certain number died early, while others survived up to 10 times longer [27].

2. Cell-Free DNA and Circulating Tumor DNA

In 1948, two French scientists found nucleic acids in human blood [28], but only 60 years later was this finding applied to detect specific mutations found in colon cancer [29]. In cancer patients, circulating tumor DNA (ctDNA) represents only a fraction of the total cell-free DNA (cfDNA) (**Figure 1**). In a xenograft model, the principal fragment length of human glioblastoma ctDNA was typically shorter than the background rat cfDNA, 134–144 bp vs. 167 bp, respectively [30]. Such distinct differences between normal cfDNA and tumor-derived ctDNA allow selection for the shorter cfDNA to increase sensitivity.

Earlier studies showed that specific mutations well known from tissue biopsies [31][32][33][34] can also be detected in serum [35][36][37][38], in plasma [39][40][41], or in both [36]. More recent studies changed from searching for single, specific mutations to sequencing panels of tumor-related genes in plasma [42][43][44]. CSF showed a significantly higher sensitivity than serum or plasma in such multigene assays [45][46][47][48][49]. With next-generation sequencing (NGS), it was shown that CSF-derived ctDNA represented genomic alterations of brain tumors better than blood-derived ctDNA [50]. The first detection of frequent and important histone H3 mutations in CSF in children with usually unresectable midline glioma supports the clinical utility of such an approach [51], since CSF is more safely accessible than tissue biopsy from the brainstem or thalamus.

Despite a high specificity to detect tumor-associated mutations in ctDNA from blood or CSF, variable sensitivity limits the use of ctDNA for routine clinical applications [49][51]. Independent of tumor size, entity, and grading, a close location to a neighboring CSF reservoir correlated with a higher sensitivity to detect the ctDNA of medulloblastomas, ependymomas, and high-grade gliomas [49], although, surprisingly, not all tumors (ependymoma, low-grade glioma) abutting CSF space were detectable in this way. However, under certain conditions, liquid biopsy can be beneficial for some patients in order to (1) differentiate between pseudoprogression and real tumor progression, (2) monitor tumor response after surgery, chemotherapy, or radiation therapy, or (3) monitor tumor relapse before image diagnostics.

Over two decades, several mutations and detection methods from blood and CSF have evolved (more or less) chronologically (**Table 1**). The initial focus was on methylation-specific PCR of the MGMT promoter, p16, DAPK, RASSF1A, p73, PTEN promoter, p15INK4B, and p14ARF, as well as the LOH of 10q, 1p, and 19q, and sequencing specific mutations of PTEN, IDH1/2, EGFR, TP53, PIK3CA, EPHB1, telomerase reverse transcriptase (TERT), ANK,

FTH1, OR51D1, NF2, AKT1, Met, ATRX, H3F3H, HIST1H3B, BRAF, JAK2, NF1, NRAS, GNAS, ATM, 1P19Q, and CIC [35][36][37][38][39][40][41][48][49][52][53][54]. More recent studies analyzed a number of genes by sequencing panels of genes from 54–70 genes up to whole-genome analysis [40][43][47]. Some of these typical mutations known from surgical biopsies are also relevant for therapeutic decisions [55][56]. Detection limits vary, and sensitivity appears to be better from CSF. Currently, a standard to use ctDNA in brain tumors needs to be established.

Table 1. ctDNA markers tested in liquid biopsy of brain tumors.

Year	Gene	Variation	Source	Method	Tumor
2003 [35]	MGMT (promoter)	Methylation	Serum	MS-PCR	GBM
2006 [41]		Methylation	Plasma	MS-PCR	GBM, AA
2010 [37]		Methylation	Serum	MS-PCR	Astrocytic tumors (WHO III, IV), oligodendroglial tumors (WHO II, III)
2013 [38]		Methylation	Serum	MS-PCR	Glial tumors (II, III, IV), meningioma
2003 [35]	p16	Methylation	Serum	MS-PCR	GBM
2006 [41]		Methylation	Plasma	MS-PCR	GBM, AA, AOA
2003 [35]	DAPK	Methylation	Serum	MS-PCR	GBM
2003 [35]	RASSF1A	Methylation	Serum	MS-PCR	GBM
2013 [38]		Methylation	Serum	MS-PCR	Glial tumors (II, III, IV), meningioma
2006 [41]	p73	Methylation	Plasma	MS-PCR	GBM
2010 [37]	PTEN	Methylation	MS-PCR	MS-PCR	Astrocytic tumors (WHO III, IV)
2014 [36]		Mutation	Plasma, serum	Digital PCR, sequencing	Glioma II, AA, GBM
2010 [37]	10q	LOH	Serum	LOH	Astrocytic (WHO III, IV), Oligodendroglial (WHO II, III)
2010 [37]	1p	LOH	Serum	LOH	Oligodendroglial (WHO II, III)
2010 [37]	19q	LOH	Serum	LOH	Oligodendroglial (WHO II, III)
2012 [39]	IDH1	Mutation (R132H)	Plasma	digital PCR	Glioma (WHO grade II, III, IV)
2014 [36]		Mutation	Plasma, serum	Digital PCR, sequencing	Glioma II, AA, GBM
2013 [38]	p15INK4B	Methylation	Serum	MS-PCR	Glial tumors (II, III, IV), meningioma
2013 [38]	p14ARF	Methylation	Serum	MS-PCR	Glial tumors (II, III, IV), meningioma
2014 [36]	TP53	Mutation	Plasma, serum	Digital PCR, sequencing	Glioma II, AA, GBM
2014 [36]	EGFR	Mutations	Plasma, serum	Digital PCR, sequencing	Glioma II, AA, GBM
2014 [36]	PIK3CA	Mutation	Plasma, serum	Digital PCR, sequencing	Glioma II, AA, GBM

Year	Gene	Variation	Source	Method	Tumor
2015 [50]	TP53 (R114C) EPHB1 TERT PIK3CG IDH1 (R132H) ANK (K2337X) EGFR (C620S) PTEN (D162) FTH1 (R108K) OR51D1 (R135C)	Mutations	CSF, (plasma)	ddPCR, MAF	GBM
2015 [49]	Genome	Mutations	CSF	TAS/WES	AA III, PA I, ependymoma, medulloblastoma IV, GBM, LGG II, diffuse astrocytoma
2015 [48]	Gene panel (587 genes) including NF2, AKT1	Mutations	CSF, plasma, serum	ddPCR/TAS	Vestibular schwannoma, atypical meningioma
2017 [40]	Gene panels (54, 68, 70 genes) including p53, EGFR, Met	Mutations	Plasma	NGS	Brain tumors (not specified)
2018 [45]	IDH1, IDH2, TP53, TERT, ATRX, H3F3A, HIST1H3B	Mutations	CSF	sequencing	Diffuse gliomas
2018 [47]	Genome	SCNAs and fragmentation	CSF	WGS	Glioma
2018 [57]	TERT	Mutation	CSF, (plasma)	PCR, sequencing	GBM
2019 [58]	BRAF	Mutation (V600E)	CSF, plasma, serum	dPCR	PXA, ganglioglioma, PA, pilomyxoid astrocytoma
2019 [43]	Genome including TP53, JAK2, NF1, EGFR, BRAF, IDH1, NRAS, GNAS, ATM	Mutations	Plasma	NGS	Astrocytic/oligodendral tumors grades I–IV, including GBM, medulloblastoma, meningioma, and ependymoma
2019 [46]	IDH1 1P19Q CIC ATRX TP53	Mutations	CSF	NGS	LGG, GBM

Notes: MS-PCR = methylation-specific PCR; AA = anaplastic astrocytoma; AOA = anaplastic oligoastrocytoma; GBM = glioblastoma multiforme; LGG = low-grade glioma; LDA = low density array; MAF = mutant allelic frequency; PA = pilocytic astrocytoma; PXA = pleomorphic xanthoastrocytoma; qRT-PCR = quantitative reverse transcriptase polymerase chain reaction; RNA = ribonucleic acid; WHO = World Health Organization tumor grading; I, II, III, IV = tumor grade I, II, III, IV (not necessarily identical to WHO grading); NGS = next generation sequencing; TAS = targeted analysis sequencing; WES = whole exome sequencing; WGS = whole genome sequencing.

3. MicroRNA—miRNA—miR

MicroRNAs (miRNA, miR) are only 20–24 nucleotides long, i.e., very small, noncoding RNA molecules derived from just 1% of the whole genome. They are strongly involved in regulation of the stability and translation of mRNA in health and disease. Although first found in 1993 in the nematode *Caenorhabditis elegans* [59], the potential biological effects of up to 1900 miRNAs in humans are not completely understood. Many seem to play a role in tumor biology, angiogenesis and immunology and some can be considered as promising prognostic factors or as potential therapeutic targets in glioblastoma (**Table 2**) [52].

Most of the over 20 studies on miRNAs in gliomas showed variable, reasonable degrees of sensitivity and specificity, both often over 80% to 90%. The miRNAs relevant for brain tumors are often upregulated with a worse prognosis, but can also be downregulated compared to others: miR-10b, miR-15b, miR-15b-5p, miR-16-5p, miR-19b-3p, miR-20a-5b, miR-20a-5p, miR-21, miR-23a, miR-29, miR-106a-5p, miR-125, miR-128, miR-125, miR-125b, miR-128, miR-130-3p, miR-133a, miR-145-5p, miR-150, miR-181b-5p, miR-182, miR-182-5p, miR-197, miR-205, miR-208a-3p, miR-210, miR-221, miR-

222, miR-222-3p, miR-223, miR-320, miR-320e, miR-328-3p, miR-339-5p, miR-340-5p, miR-374-3p, miR-376a, miR-376b, miR-376c, miR-454, miR-485-3p, miR-486, miR-486-5p, miR-497, miR-543, miR-548b-5b, and RNU6-1 [60][61][62][63][64][65][66][67][68][69][70][71][72][73][74][75][76][77][78][79]. Upregulation of miR-21 may serve as an early diagnostic but also as prognostic [62] and monitoring marker [80], whereas panels of different miRNAs were found to be potential markers for diagnostics and tumor grade, as well as prognostics [81]. In an elegant new model, urine samples from mainly glioma patients and noncancer individuals were used to develop with artificial intelligence (AI) a diagnostic model for the detection of such tumors in urine samples [82]. A panel of 23 miRNAs was found to separate noncancerous from glioma patients. However, a common standard needs to be established and validated to diagnose tumor patients not only from noncancer individuals, but also from patients with other diseases, such as inflammations or degenerating diseases. Future studies may also include circular RNAs (circRNA) as possible markers. They are more stable than single-stranded RNA, and some of them can serve as a functionally antagonistic sponge for specific miRs and, therefore, are significantly involved in gene regulation [53].

Table 2. Examples of circulating miRNA markers in brain tumors.

Year	miR	Variation	Source	Method	Tumor
2016 [80]	miR-10-b miR-21	Up/progression	Serum	qPCR	HGG
2016 [69]	miR-205	Down/diagnostics	Serum	qPCR	Glioma
2018 [66]	Panel of 7 miRNAs	Diagnostic signature	Serum EV	NGS	GBM
2020 [64]	miR-21 miR-20e miR-223	Up/progression	Serum	ddPCR	LGG, GBM
2020 [65]	miR-17-5p miR-125b miR-221	Up/progression	Serum	qPCR	GBM
2020 [83]	miR-486	Up/diagnostic	EV from tumor microenvironment/neurosurgical aspirate fluid	NGS	GBM
2021 [84]	miR-21 miR124-3p miR-222	Up/progression	Serum EV	qPCR	HGG
2021 [82]	Panel of 23 miRNAs	Screening signature	Urine	nanowire	GBM, glioma

4. Extracellular Vesicles—EVs

Tumor and normal cells can release small, extracellular vesicles into body fluids, such as blood and CSF (**Figure 1**). In addition to proteins, these vesicles contain DNA and RNA, including miRNA, which are protected by the cellular membrane. EVs can be analyzed to reliably detect tumor-specific mutations, including amplification of wild-type EGFR [85][86][87]. CSF appears to have an advantage over serum, perhaps due to the reduced number of EVs from leukocytes compared to blood. For example, IDH1 mutation G395A was detected in CSF-derived EVs of glioma grades II, III, and IV with a sensitivity of 63% and a specificity of 100%, but not in frozen serum [54]. Using quantitative PCR changes in wildtype IDH1 levels can also be used to monitor tumor burden and treatment response when the tumor does not have an IDH-1 mutation [54]. Most glioblastomas have an amplification of the wild-type epidermal growth factor receptor gene (EGFR), which results in an increased RNA expression; this amplification can be detected (indirectly) by quantitative reverse transcriptase-PCR (qRT) of CSF-derived EV RNA [85]. Using the same method, another typical mutation in glioblastomas can be detected, EGFRvIII, which lacks several exons. This deletion mutant was also detected in EV from blood in high-grade gliomas (III and IV) [86][87] and may serve as a good biomarker. EVs from serum or from neurosurgical fluid were also used to detect miRNAs from glioblastomas [66][83].

References

1. Eibl, R.H.; P. Kleihues; P. S. Jat; O. D. Wiestler; A model for primitive neuroectodermal tumors in transgenic neural transplants harboring the SV40 large T antigen.. *The American Journal of Pathology* **1994**, *144*, 556-564, .
2. Wiestler, O.D.; Aguzzi, A.; Schneemann, M.; Eibl, R.; von Deimling, A.; Kleihues, P; Oncogene complementation in fetal brain transplants.. *Cancer Research* **1992**, *52*, 3760-3767, .
3. Radner, H.; El-Shabrawi, Y.; Eibl, R.H.; Brüstle, O.; Kenner, L.; Kleihues, P; Wiestler, O.D.; Tumor induction by ras and myc oncogenes in fetal and neonatal brain: modulating effects of developmental stage and retroviral dose. *Acta Neuropathologica* **1993**, *86*, 456-465, .
4. Wiestler, O.D.; Brüstle, O.; Eibl, R.H.; Radner, H.; von Deimling, A.; Plate, K.; Aguzzi, A.; Kleihues, P.; A new approach to the molecular basis of neoplastic transformation in the brain. *Neuropathology and Applied Neurobiology* **1992**, *18*, 443-453, .
5. Wiestler, O.D.; Brüstle, O.; Eibl, R.H.; Radner, H.; Aguzzi, A.; Kleihues, P.; Retrovirus-mediated oncogene transfer into neural transplants.. *Brain Pathology* **1992**, *2*, 47-59, .
6. Wiestler, O.D.; Brüstle, O.; Eibl, R.H.; Radner, H.; Aguzzi, A.; Kleihues, P.; Oncogene Transfer into the Brain. *Recent Results Cancer Res.* **1994**, *135*, 55-66, .
7. Sullivan, J.P.; Nahed, B.V.; Madden, M.W.; Oliveira, S.M.; Springer, S.; Bhere, D.; Chi, A.S.; Wakimoto, H.; Rothenberg, S.M.; Sequist, L.V.; et al. Brain Tumor Cells in Circulation Are Enriched for Mesenchymal Gene Expression. Cancer Discov. 2014, 4, 1299–1309.
8. Müller, C.; Holtschmidt, J.; Auer, M.; Heitzer, E.; Lamszus, K.; Schulte, A.; Matschke, J.; Langer-Freitag, S.; Gasch, C.; Stoupiec, M.; et al. Hematogenous Dissemination of Glioblastoma Multiforme. Sci. Transl. Med. 2014, 6, 247ra101.
9. Perryman, L.; Erler, J.T. Brain Cancer Spreads. Sci. Transl. Med. 2014, 6, 247fs28.
10. Macarthur, K.M.; Kao, G.D.; Chandrasekaran, S.; Alonso-Basanta, M.; Chapman, C.; Lustig, R.A.; Wileyto, E.P.; Hahn, S.M.; Dorsey, J.F. Detection of Brain Tumor Cells in the Peripheral Blood by a Telomerase Promoter-Based Assay. Cancer Res. 2014, 74, 2152–2159.
11. Krol, I.; Castro-Giner, F.; Maurer, M.; Gkountela, S.; Szczerba, B.M.; Scherrer, R.; Coleman, N.; Carreira, S.; Bachmann, F.; Anderson, S.; et al. Detection of Circulating Tumour Cell Clusters in Human Glioblastoma. Br. J. Cancer 2018, 119, 487–491.
12. Gao, F.; Cui, Y.; Jiang, H.; Sui, D.; Wang, Y.; Jiang, Z.; Zhao, J.; Lin, S. Circulating Tumor Cell Is a Common Property of Brain Glioma and Promotes the Monitoring System. Oncotarget 2016, 7, 71330–71340.
13. Eibl, R.H.; Pietsch, T.; Moll, J.; Skroch-Angel, J; Heider, K.H.; von Ammon, K.; Wiestler, O.D.; Ponta, H.; Kleihues, P.; Herrlich, P.; et al. Expression of variant CD44 epitopes in human astrocytic brain tumors. *Journal of Neuro-Oncology* **1995**, *26*, 165-170, .
14. Paget, S. The Distribution of Secondary Growths in Cancer of the Breast. Lancet 1889, 133, 571–573.
15. Gonzalez-Beltran, A.N.; Masuzzo, P.; Ampe, C.; Bakker, G.-J.; Besson, S.; Eibl, R.H.; Friedl, P.; Gunzer, M.; Kittisopikul, M.; Dévédec, S.E.L.; et al. Community standards for open cell migration data. *GigaScience* **2020**, *9*, giaa041, .
16. Eibl, R.H.; Benoit, M; Molecular resolution of cell adhesion forces. *IEE Proceedings - Nanobiotechnology* **2004**, *151*, 128-132, .
17. Eibl, R.H.; Moy, V.T.; Atomic Force Microscopy Measurements of Protein–Ligand Interactions on Living Cells. *Protein-Ligand Interactions* **2005**, *305*, 439-450, .
18. Eibl, R.H.; Comment on “A method to measure cellular adhesion utilizing a polymer micro-cantilever” [Appl. Phys. Lett. 103, 123702 (2013)]. *Applied Physics Letters* **2014**, *104*, 236103, .
19. Eibl, R.H.; Single-Molecule Studies of Integrins by AFM-Based Force Spectroscopy on Living Cells. *Scanning Probe Microscopy in Nanoscience and Nanotechnology 3* **2012**, *3*, 137-169, .
20. Pax, M.; Rieger, J.; Eibl, R.H.; Thielemann, C.; Johannsmann, D.; Measurements of fast fluctuations of viscoelastic properties with the quartz crystal microbalance. *The Analyst* **2005**, *130*, 1474-1477, .
21. Eibl, R.H.; Cell Adhesion Receptors Studied by AFM-Based Single-Molecule Force Spectroscopy. *Scanning Probe Microscopy in Nanoscience and Nanotechnology 2* **2010**, *2*, 197-215, .
22. Eibl, R.H.; Moy, V.T.; AFM-based adhesion measurement of single receptor-ligand bonds on living cells. In: Recent Res. Devel. Biophys., 3(2004), 235-246, ISBN: 81-7895-130-4, Transworld Research Network, Kerala

23. Eibl, R.H.; Direct Force Measurements of Receptor-Ligand Interactions on living cells. In: Applied Scanning Probe Methods XII - Characterization. Bhushan B, Fuchs H (Editors), Springer, pp. 1-31, (2009)

24. Eibl R.H.; First measurement of physiologic VLA-4 activation by SDF-1 at the single-molecule level on a living cell. In: Advances in Single Molecule Research for Biology and Nanoscience. Hinterdorfer P, Schuetz G, Pohl P (Editors),Trauner, Linz, p40-43, ISBN 3854991630 (2007)

25. Eibl, R.H.; Atomic force microscopy measurement of SDF-1 mediated affinity modulation of single VLA-4 - VCAM-1 bonds. In: Immunology 2004: Cytokine Network, Regulatory Cells, Signaling and Apoptosis. Monduzzi Ed. (Editor: E. Skamene), MEDIMOND S.r.l., Bologna, Italy, (1):115-120 ISBN 978-88-7587-070-6 (2004)

26. Pantel, K.; Denève, E.; Nocca, D.; Coffy, A.; Vendrell, J.-P.; Maudelonde, T.; Riethdorf, S.; Alix-Panabières, C. Circulating Epithelial Cells in Patients with Benign Colon Diseases. Clin. Chem. 2012, 58, 936–940.

27. Mazard, T.; Cayrefourcq, L.; Perriard, F.; Senellart, H.; Linot, B.; de la Fouchardière, C.; Terrebonne, E.; François, E.; Obled, S.; Guimbaud, R.; et al. Clinical Relevance of Viable Circulating Tumor Cells in Patients with Metastatic Colorectal Cancer: The COLOSPOT Prospective Study. Cancers 2021, 13, 2966.

28. Mandel, P.; Metais, P. Nuclear Acids In Human Blood Plasma. C. R. Seances Soc. Biol. Fil. 1948, 142, 241–243.

29. Diehl, F.; Schmidt, K.; Choti, M.A.; Romans, K.; Goodman, S.; Li, M.; Thornton, K.; Agrawal, N.; Sokoll, L.; Szabo, S.A.; et al. Circulating Mutant DNA to Assess Tumor Dynamics. Nat. Med. 2008, 14, 985–990.

30. Underhill, H.R.; Kitzman, J.O.; Hellwig, S.; Welker, N.C.; Daza, R.; Baker, D.N.; Gligorich, K.M.; Rostomily, R.C.; Bronner, M.P.; Shendure, J. Fragment Length of Circulating Tumor DNA. PLoS Genet. 2016, 12, e1006162.

31. Ohgaki, H; Eibl, R.H.; Wiestler, O.D.; Yasargil, M.G.; Newcom, E.W.; Kleihues, P.; p53 mutations in nonastrocytic human brain tumors.. *Cancer Research* **1991**, *51*, 6202-6205, .

32. von Deimling, A.; Eibl, R.H.; Ohgaki, H.; Louis, D.N.; von Ammon, K.; Petersen, I.; Kleihues, P.; Chung, R.Y.; Wiestler, O.D.; Seizinger, B.R.; et al. P53 Mutations Are Associated with 17p Allelic Loss in Grade II and Grade III Astrocytoma.. *Cancer Res.* **1992**, *52*, 2987-2990, .

33. Ohgaki, H.; Eibl, R.H.; Schwab, M.; Reichel, M.B.; Mariani, L.; Gehring, M.; Petersen, I.; Höll, T.; Wiestler, O.D.; Kleihues, P.; et al. Mutations of thep53 tumor suppressor gene in neoplasms of the human nervous system. *Molecular Carcinogenesis* **1993**, *8*, 74-80, .

34. Kleihues, P.; Ohgaki, H.; Eibl, R.H.; Reichel, M.B.; Mariani, L.; Gehring, M.; Petersen, I.; Höll, T.; von Deimling, A.; Wiestler, O.D.; et al. Type and Frequency of p53 Mutations in Tumors of the Nervous System and Its Coverings. *Methods in Molecular Biology* **1994**, *135*, 25-31, .

35. Balaña, C.; Ramirez, J.L.; Taron, M.; Roussos, Y.; Ariza, A.; Ballester, R.; Sarries, C.; Mendez, P.; Sanchez, J.J.; Rosell, R. O6-Methyl-Guanine-DNA Methyltransferase Methylation in Serum and Tumor DNA Predicts Response to 1,3-Bis(2-Chloroethyl)-1-Nitrosourea but Not to Temozolamide plus Cisplatin in Glioblastoma Multiforme. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 2003, 9, 1461–1468.

36. Bettegowda, C.; Sausen, M.; Leary, R.J.; Kinde, I.; Wang, Y.; Agrawal, N.; Bartlett, B.R.; Wang, H.; Luber, B.; Alani, R.M.; et al. Detection of Circulating Tumor DNA in Early- and Late-Stage Human Malignancies. Sci. Transl. Med. 2014, 6, 224ra24.

37. Lavon, I.; Refael, M.; Zelikovitch, B.; Shalom, E.; Siegal, T. Serum DNA Can Define Tumor-Specific Genetic and Epigenetic Markers in Gliomas of Various Grades. Neuro-Oncology 2010, 12, 173–180.

38. Majchrzak-Celińska, A.; Paluszczak, J.; Kleszcz, R.; Magiera, M.; Barciszewska, A.-M.; Nowak, S.; Baer-Dubowska, W. Detection of MGMT, RASSF1A, P15INK4B, and P14ARF Promoter Methylation in Circulating Tumor-Derived DNA of Central Nervous System Cancer Patients. J. Appl. Genet. 2013, 54, 335–344.

39. Boisselier, B.; Gallego Perez-Larraya, J.; Rossetto, M.; Labussiere, M.; Ciccarino, P.; Marie, Y.; Delattre, J.-Y.; Sanson, M. Detection of IDH1 Mutation in the Plasma of Patients with Glioma. Neurology 2012, 79, 1693–1698.

40. Schwaederle, M.; Chattopadhyay, R.; Kato, S.; Fanta, P.T.; Banks, K.C.; Choi, I.S.; Piccioni, D.E.; Ikeda, S.; Talasaz, A.; Lanman, R.B.; et al. Genomic Alterations in Circulating Tumor DNA from Diverse Cancer Patients Identified by Next-Generation Sequencing. Cancer Res. 2017, 77, 5419–5427.

41. Weaver, K.D.; Grossman, S.A.; Herman, J.G. Methylated Tumor-Specific DNA as a Plasma Biomarker in Patients with Glioma. Cancer Investig. 2006, 24, 35–40.

42. Bagley, S.J.; Nabavizadeh, S.A.; Mays, J.J.; Till, J.E.; Ware, J.B.; Levy, S.; Sarchiapone, W.; Hussain, J.; Prior, T.; Guiry, S.; et al. Clinical Utility of Plasma Cell-Free DNA in Adult Patients with Newly Diagnosed Glioblastoma: A Pilot Prospective Study. Clin. Cancer Res. 2020, 26, 397–407.

43. Piccioni, D.E.; Achrol, A.S.; Kiedrowski, L.A.; Banks, K.C.; Boucher, N.; Barkhoudarian, G.; Kelly, D.F.; Juarez, T.; Lanman, R.B.; Raymond, V.M.; et al. Analysis of Cell-Free Circulating Tumor DNA in 419 Patients with Glioblastoma and Other Primary Brain Tumors. CNS Oncol. 2019, 8, CNS34.

44. Zill, O.A.; Banks, K.C.; Fairclough, S.R.; Mortimer, S.A.; Vowles, J.V.; Mokhtari, R.; Gandara, D.R.; Mack, P.C.; Odegaard, J.I.; Nagy, R.J.; et al. The Landscape of Actionable Genomic Alterations in Cell-Free Circulating Tumor DNA from 21,807 Advanced Cancer Patients. Clin. Cancer Res. 2018, 24, 3528–3538.

45. Martínez-Ricarte, F.; Mayor, R.; Martínez-Sáez, E.; Rubio-Pérez, C.; Pineda, E.; Cordero, E.; Cicuéndez, M.; Poca, M.A.; López-Bigas, N.; y Cajal, S.R.; et al. Molecular Diagnosis of Diffuse Gliomas through Sequencing of Cell-Free Circulating Tumor DNA from Cerebrospinal Fluid. Clin. Cancer Res. 2018, 24, 2812–2819.

46. Miller, A.M.; Shah, R.H.; Pentsova, E.I.; Pourmaleki, M.; Briggs, S.; Distefano, N.; Zheng, Y.; Skakodub, A.; Mehta, S.A.; Campos, C.; et al. Tracking Tumour Evolution in Glioma through Liquid Biopsies of Cerebrospinal Fluid. Nature 2019, 565, 654–658.

47. Mouliere, F.; Mair, R.; Chandrananda, D.; Marass, F.; Smith, C.G.; Su, J.; Morris, J.; Watts, C.; Brindle, K.M.; Rosenfeld, N. Detection of Cell-free DNA Fragmentation and Copy Number Alterations in Cerebrospinal Fluid from Glioma Patients. EMBO Mol. Med. 2018, 10, e9323.

48. Pan, W.; Gu, W.; Nagpal, S.; Gephart, M.H.; Quake, S.R. Brain Tumor Mutations Detected in Cerebral Spinal Fluid. Clin. Chem. 2015, 61, 514–522.

49. Wang, Y.; Springer, S.; Zhang, M.; McMahon, K.W.; Kinde, I.; Dobbyn, L.; Ptak, J.; Brem, H.; Chaichana, K.; Gallia, G.L.; et al. Detection of Tumor-Derived DNA in Cerebrospinal Fluid of Patients with Primary Tumors of the Brain and Spinal Cord. Proc. Natl. Acad. Sci. USA 2015, 112, 9704–9709.

50. De Mattos-Arruda, L.; Mayor, R.; Ng, C.K.Y.; Weigelt, B.; Martínez-Ricarte, F.; Torrejon, D.; Oliveira, M.; Arias, A.; Raventos, C.; Tang, J.; et al. Cerebrospinal Fluid-Derived Circulating Tumour DNA Better Represents the Genomic Alterations of Brain Tumours than Plasma. Nat. Commun. 2015, 6, 8839.

51. Huang, T.Y.; Piunti, A.; Lulla, R.R.; Qi, J.; Horbinski, C.M.; Tomita, T.; James, C.D.; Shilatifard, A.; Saratsis, A.M. Detection of Histone H3 Mutations in Cerebrospinal Fluid-Derived Tumor DNA from Children with Diffuse Midline Glioma. Acta Neuropathol. Commun. 2017, 5, 28.

52. Floyd, D.; Purow, B. Micro-Masters of Glioblastoma Biology and Therapy: Increasingly Recognized Roles for MicroRNAs. Neuro-Oncology 2014, 16, 622–627.

53. Ding, Y.; Lu, C.; Zhang, W.; Wang, Y.; Li, Y.; Zhu, Y.; Lv, S.; Zhang, J. The Emerging Role of Circular RNAs in Cardiovascular Diseases. J. Physiol. Biochem. 2021, 77, 343–353.

54. Chen, W.W.; Balaj, L.; Liau, L.M.; Samuels, M.L.; Kotsopoulos, S.K.; Maguire, C.A.; Loguidice, L.; Soto, H.; Garrett, M.; Zhu, L.D.; et al. BEAMing and Droplet Digital PCR Analysis of Mutant IDH1 MRNA in Glioma Patient Serum and Cerebrospinal Fluid Extracellular Vesicles. Mol. Ther. Nucleic Acids 2013, 2, e109.

55. Preuss, I.; Eberhagen, I.; Haas, S.; Eibl, R.H.; Kaufmann, M.; von Minckwitz, G.; Kaina, B. O6-Methylguanine-DNA Methyltransferase Activity in Breast and Brain Tumors. Int. J. Cancer 1995, 61, 321–326.

56. Preuss, I.; Haas, S.; Eichhorn, U.; Eberhagen, I.; Kaufmann, M.; Beck, T.; Eibl, R.H.; Dall, P.; Bauknecht, T.; Hengstler, J.; et al. Activity of the DNA Repair Protein O6-Methylguanine-DNA Methyltransferase in Human Tumor and Corresponding Normal Tissue. Cancer Detect. Prev. 1996, 20, 130–136.

57. Juratli, T.A.; Stasik, S.; Zolal, A.; Schuster, C.; Richter, S.; Daubner, D.; Juratli, M.A.; Thowe, R.; Hennig, S.; Makina, M.; et al. TERT Promoter Mutation Detection in Cell-Free Tumor-Derived DNA in Patients with IDH Wild-Type Glioblastomas: A Pilot Prospective Study. Clin. Cancer Res. 2018, 24, 5282–5291.

58. García-Romero, N.; Carrión-Navarro, J.; Areal-Hidalgo, P.; Ortiz de Mendivil, A.; Asensi-Puig, A.; Madurga, R.; Núñez-Torres, R.; González-Neira, A.; Belda-Iniesta, C.; González-Rumayor, V.; et al. BRAF V600E Detection in Liquid Biopsies from Pediatric Central Nervous System Tumors. Cancers 2019, 12, 66.

59. Lee, R.C.; Feinbaum, R.L.; Ambros, V. The C. Elegans Heterochronic Gene Lin-4 Encodes Small RNAs with Antisense Complementarity to Lin-14. Cell 1993, 75, 843–854.

60. Jones, J.; Nguyen, H.; Drummond, K.; Morokoff, A. Circulating Biomarkers for Glioma: A Review. Neurosurgery 2021, 88, E221–E230.

61. Roth, P.; Wischhusen, J.; Happold, C.; Chandran, P.A.; Hofer, S.; Eisele, G.; Weller, M.; Keller, A. A Specific MiRNA Signature in the Peripheral Blood of Glioblastoma Patients. J. Neurochem. 2011, 118, 449–457.

62. Wang, Q.; Li, P.; Li, A.; Jiang, W.; Wang, H.; Wang, J.; Xie, K. Plasma Specific MiRNAs as Predictive Biomarkers for Diagnosis and Prognosis of Glioma. J. Exp. Clin. Cancer Res. 2012, 31, 97.

63. Yang, C.; Wang, C.; Chen, X.; Chen, S.; Zhang, Y.; Zhi, F.; Wang, J.; Li, L.; Zhou, X.; Li, N.; et al. Identification of Seven Serum MicroRNAs from a Genome-Wide Serum MicroRNA Expression Profile as Potential Noninvasive Biomarkers for Malignant Astrocytomas. Int. J. Cancer 2013, 132, 116–127.

64. Morokoff, A.; Jones, J.; Nguyen, H.; Ma, C.; Lasocki, A.; Gaillard, F.; Bennett, I.; Luwor, R.; Stylli, S.; Paradiso, L.; et al. Serum MicroRNA Is a Biomarker for Post-Operative Monitoring in Glioma. J. Neurooncol. 2020, 149, 391–400.

65. Swellam, M.; Bakr, N.M.; El Magdoub, H.M.; Hamza, M.S.; Ezz El Arab, L.R. Emerging Role of MiRNAs as Liquid Biopsy Markers for Prediction of Glioblastoma Multiforme Prognosis. J. Mol. Neurosci. 2021, 71, 836–844.

66. Ebrahimkhani, S.; Vafaee, F.; Hallal, S.; Wei, H.; Lee, M.Y.T.; Young, P.E.; Satgunaseelan, L.; Beadnall, H.; Barnett, M.H.; Shivalingam, B.; et al. Deep Sequencing of Circulating Exosomal MicroRNA Allows Non-Invasive Glioblastoma Diagnosis. NPJ Precis. Oncol. 2018, 2, 28.

67. Zhao, H.; Shen, J.; Hodges, T.R.; Song, R.; Fuller, G.N.; Heimberger, A.B. Serum MicroRNA Profiling in Patients with Glioblastoma: A Survival Analysis. Mol. Cancer 2017, 16, 59.

68. Huang, Q.; Wang, C.; Hou, Z.; Wang, G.; Lv, J.; Wang, H.; Yang, J.; Zhang, Z.; Zhang, H. Serum MicroRNA-376 Family as Diagnostic and Prognostic Markers in Human Gliomas. Cancer Biomark. Sect. Dis. Markers 2017, 19, 137–144.

69. Yue, X.; Lan, F.; Hu, M.; Pan, Q.; Wang, Q.; Wang, J. Downregulation of Serum MicroRNA-205 as a Potential Diagnostic and Prognostic Biomarker for Human Glioma. J. Neurosurg. 2016, 124, 122–128.

70. Xiao, Y.; Zhang, L.; Song, Z.; Guo, C.; Zhu, J.; Li, Z.; Zhu, S. Potential Diagnostic and Prognostic Value of Plasma Circulating MicroRNA-182 in Human Glioma. Med. Sci. Monit. 2016, 22, 855–862.

71. Lai, N.-S.; Wu, D.-G.; Fang, X.-G.; Lin, Y.-C.; Chen, S.-S.; Li, Z.-B.; Xu, S.-S. Serum MicroRNA-210 as a Potential Noninvasive Biomarker for the Diagnosis and Prognosis of Glioma. Br. J. Cancer 2015, 112, 1241–1246.

72. Zhi, F.; Shao, N.; Wang, R.; Deng, D.; Xue, L.; Wang, Q.; Zhang, Y.; Shi, Y.; Xia, X.; Wang, S.; et al. Identification of 9 Serum MicroRNAs as Potential Noninvasive Biomarkers of Human Astrocytoma. Neuro-Oncology 2015, 17, 383–391.

73. Sun, J.; Liao, K.; Wu, X.; Huang, J.; Zhang, S.; Lu, X. Serum MicroRNA-128 as a Biomarker for Diagnosis of Glioma. Int. J. Clin. Exp. Med. 2015, 8, 456–463.

74. Manterola, L.; Guruceaga, E.; Gállego Pérez-Larraya, J.; González-Huarriz, M.; Jauregui, P.; Tejada, S.; Diez-Valle, R.; Segura, V.; Samprón, N.; Barrena, C.; et al. A Small Noncoding RNA Signature Found in Exosomes of GBM Patient Serum as a Diagnostic Tool. Neuro-Oncol. 2014, 16, 520–527.

75. Wu, J.; Li, L.; Jiang, C. Identification and Evaluation of Serum MicroRNA-29 Family for Glioma Screening. Mol. Neurobiol. 2015, 52, 1540–1546.

76. Ivo D'Urso, P.; Fernando D'Urso, O.; Damiano Gianfreda, C.; Mezzolla, V.; Storelli, C.; Marsigliante, S. MiR-15b and MiR-21 as Circulating Biomarkers for Diagnosis of Glioma. Curr. Genom. 2015, 16, 304–311.

77. Zhang, R.; Pang, B.; Xin, T.; Guo, H.; Xing, Y.; Xu, S.; Feng, B.; Liu, B.; Pang, Q. Plasma MiR-221/222 Family as Novel Descriptive and Prognostic Biomarkers for Glioma. Mol. Neurobiol. 2016, 53, 1452–1460.

78. Wei, X.; Chen, D.; Lv, T.; Li, G.; Qu, S. Serum MicroRNA-125b as a Potential Biomarker for Glioma Diagnosis. Mol. Neurobiol. 2016, 53, 163–170.

79. Shao, N.; Wang, L.; Xue, L.; Wang, R.; Lan, Q. Plasma MiR-454-3p as a Potential Prognostic Indicator in Human Glioma. Neurol. Sci. 2014, 36, 309–313.

80. Siegal, T.; Charbit, H.; Paldor, I.; Zelikovitch, B.; Canello, T.; Benis, A.; Wong, M.L.; Morokoff, A.P.; Kaye, A.H.; Lavon, I. Dynamics of Circulating Hypoxia-Mediated MiRNAs and Tumor Response in Patients with High-Grade Glioma Treated with Bevacizumab. J. Neurosurg. 2016, 125, 1008–1015.

81. Ali, H.; Harting, R.; de Vries, R.; Ali, M.; Wurdinger, T.; Best, M.G. Blood-Based Biomarkers for Glioma in the Context of Gliomagenesis: A Systematic Review. Front. Oncol. 2021, 11, 2134.

82. Kitano, Y.; Aoki, K.; Ohka, F.; Yamazaki, S.; Motomura, K.; Tanahashi, K.; Hirano, M.; Naganawa, T.; Iida, M.; Shiraki, Y.; et al. Urinary MicroRNA-Based Diagnostic Model for Central Nervous System Tumors Using Nanowire Scaffolds. ACS Appl. Mater. Interfaces 2021, 13, 17316–17329.

83. Hallal, S.; Ebrahim Khani, S.; Wei, H.; Lee, M.Y.T.; Sim, H.-W.; Sy, J.; Shivalingam, B.; Buckland, M.E.; Alexander-Kaufman, K.L. Deep Sequencing of Small RNAs from Neurosurgical Extracellular Vesicles Substantiates MiR-486-3p as a Circulating Biomarker That Distinguishes Glioblastoma from Lower-Grade Astrocytoma Patients. Int. J. Mol. Sci. 2020, 21, 4954.

84. Olioso, D.; Caccese, M.; Santangelo, A.; Lippi, G.; Zagonel, V.; Cabrini, G.; Lombardi, G.; Dechecchi, M.C. Serum Exosomal MicroRNA-21, 222 and 124-3p as Noninvasive Predictive Biomarkers in Newly Diagnosed High-Grade Gliomas: A Prospective Study. Cancers 2021, 13, 3006.

85. Figueroa, J.M.; Skog, J.; Akers, J.; Li, H.; Komotar, R.; Jensen, R.; Ringel, F.; Yang, I.; Kalkanis, S.; Thompson, R.; et al. Detection of Wild-Type EGFR Amplification and EGFRvIII Mutation in CSF-Derived Extracellular Vesicles of Glioblastoma Patients. Neuro-Oncology 2017, 19, 1494–1502.
86. Manda, S.V.; Kataria, Y.; Tatireddy, B.R.; Ramakrishnan, B.; Ratnam, B.G.; Lath, R.; Ranjan, A.; Ray, A. Exosomes as a Biomarker Platform for Detecting Epidermal Growth Factor Receptor–Positive High-Grade Gliomas. J. Neurosurg. 2018, 128, 1091–1101.
87. Skog, J.; Wurdinger, T.; van Rijn, S.; Meijer, D.; Gainche, L.; Sena-Esteves, M.; Curry, W.T.; Carter, R.S.; Krichevsky, A.M.; Breakefield, X.O. Glioblastoma Microvesicles Transport RNA and Protein That Promote Tumor Growth and Provide Diagnostic Biomarkers. Nat. Cell Biol. 2008, 10, 1470–1476.