The Blood Brain Barrier dysfunction in Brain tumor pathology

1. introduction

Brain tumors are rare, yet they present a major cause of cancer mortality, especially in children and young adults where they account respectively for about 30% and 20% of cancer deaths (McNeill, K. A., 2016). Studies have demonstrated that approximately 30% of brain tumors are metastatic and derive from lung cancer, breast cancer, and melanomas (Norden, A.D. *et al.*,2005), and the commonest brain tumor types in adults are Meningiomas, pituitary tumors, and malignant gliomas (McNeill, K. A., 2016).

The latter is the most common malignant primary brain tumor. It is a category of tumors deriving from glial or glial precursor cells including astrocytomas, oligodendrogliomas, ependymomas, and other cell types. Together, gliomas account for approximately 75% of malignant primary brain tumors, and the vast majority are glioblastoma (Ostrom QT, *et al.*, 2018).

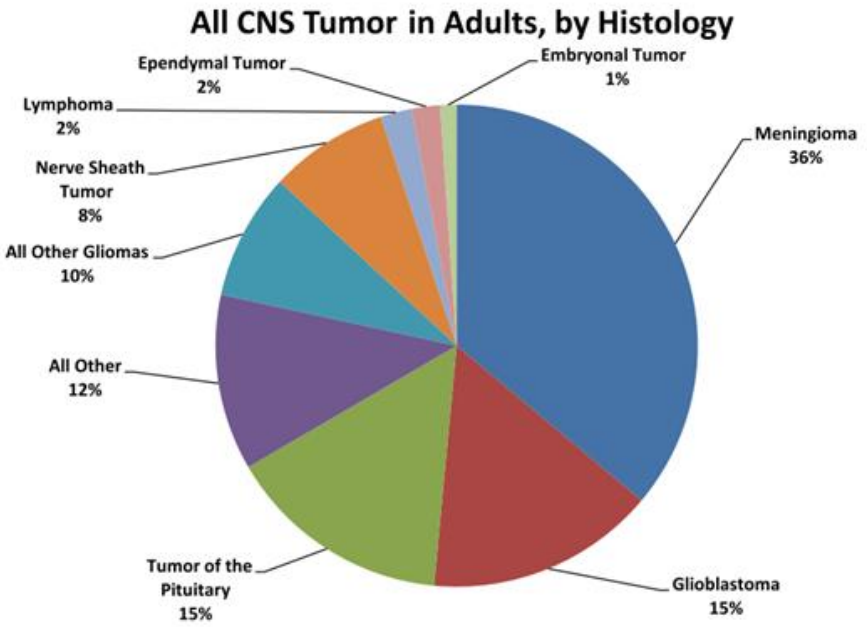

Fig 1: CNS tumors in adults (McNeill, K. A., 2016)

We wanted to further explore one prevalent brain tumor; Glioblastoma, in an effort to confirm our pipelines. Hence, we performed two case studies.

Case study1

We investigated single-nucleotide polymorphism (SNPs) involved in BBBD in Glioblastoma (GBM) and adenocarcinoma brain metastasis (BM), to validate our pipeline for single-end sequences. Brain tumors have been indeed proved to damage BBB integrity and permeability while promoting the emergence of a blood-tumor barrier (BTB) that is very heterogeneous and characterized by numerous unique features, including non-uniform permeability and the active efflux of molecules (Arvanitis, C.D. et al 2020; Groothuis, D.R et al 1991).

Some studies have identified the breakdown of inter-endothelial TJs in gliomas and metastatic adenocarcinoma in humans (Norden, A.D. et al., 2005; Long, D.M., 1970). The expression of TJ proteins, CLN-5, OCLN, and CLN-1, have been identified to be downregulated in the brain microvessels of patients with GBM even though the expression of ZO-1 (a scaffold protein that crosslinks and links the TJ strand proteins to the cell cytoskeleton), remained unaltered (Liebner S et al., 2000). The explanation behind the lack of TJs in the microvessels of brain tumors remains elusive.

The role of VEGF and the cytokines produced by the tumor cells certainly play an important role in the elevated BBB vascular permeability and the formation of cerebral edemas (De Vries et al., 1996; Saunders N.R. et al., 2000). The latter has also been linked to the substantial upregulation of AQP4 in various brain tumors, including astrocytoma and metastatic adenocarcinoma, and has been correlated with the opening of the BBB (Saadoun, S. et al., 2002). Secreted VEGF induces the damage of existing BBB architecture and the growth of altered capillaries from the existing vessels (Hardee ME et al., 2012; Plate KH et al., 2012). This resulted tumoral vascular endothelium displays an irregular expression profile of transporters and receptors to accommodate the high metabolic demands of associated tumor cells (Madden SL et al., 2004; Zhan C et al., 2012). In fact, these newly formed capillaries are structurally disrupted and are more permeable than even non-BBB peripheral capillaries in contrast with the normal brain vessels from which they originate (Hobbs SK et al., 1998).

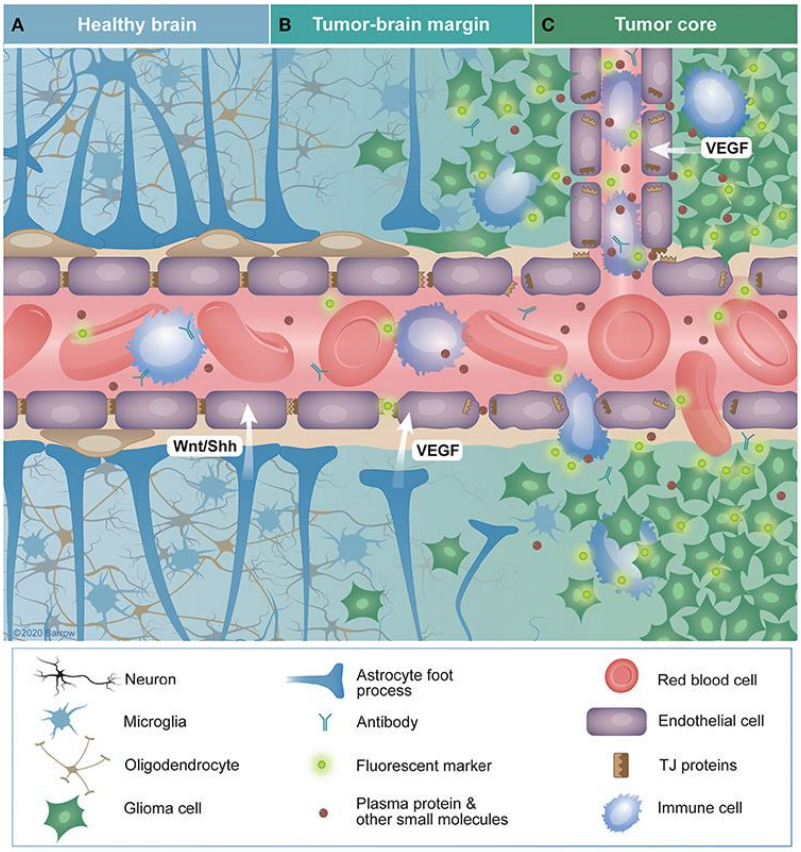

Fig 2: The blood-brain (BBB) and blood-brain tumor barriers characteristics in glioma. (Belykh E, et al., 2020)

(A) The healthy BBB selectively disrupts the spreading of blood contents across the central nervous system (CNS) endothelium. Controlled expression of tight-junction proteins and the endothelial cells provide a physical barrier to the passage of these solutes into the brain parenchyma. Sonic hedgehog and Wnt-family proteins secreted by astrocytes and pericytes are crucial for BBB preservation. **(B)** In tumor-brain margin zones, glioma cells may infiltrate into otherwise healthy parenchyma, resulting in the disruption of local neural structures, including the BBB. This infiltration damages the connections between members of the neuro vascular unit, causing the downregulation of tight-junction proteins and the degradation of the BBB through tumor-secreted vascular endothelial growth factor VEGF. The resulting vascular permeability causes the extravasation of blood contents, including solutes, fluorescent markers, antibodies, and immune cells. On the other hand, glioma cells may infiltrate without disrupting the BBB, effectively shielding themselves with an unimpaired BBB. **(C)** Inside the tumor core, BBB disruption is mostly the greatest, especially in high-grade gliomas. The increased density of highly metabolically active cells in this area stimulates hypoxia-

driven expression of VEGF that promotes angiogenesis. The new vessels are usually immature, and lack typical CNS barrier properties, resulting in characteristics such as aberrant transporter expression, increased interstitial pressure, and edema. [TJ: tight junctions; VEGF: vascular endothelial growth factor; Wnt/Shh: Wnt and sonic hedgehog family proteins.]

Gene	Location	SNP	Allele, genotype, model association with	Gene product
EGF	4q25	**rs4444903** (c.61A > G)	A allele vs G;G (OR = 1.30, 95% CI: 0.91–1.87), A;A vs. G;G (OR = 1.76, 95% CI: 0.82–3.77)	Epidermal growth factor
GSTP1	11q13	**rs1695** (c.313A > G)	A;A, A;G + G;G, (HR = 0.390 (0.196–0.775)	Glutathione S-transferase pi-1
EGFR	7p11.2	**rs730437** (c.748-49G > A) **rs1468727** (c.1631 + 781C > T)	C;C, C allele (OR = 1.32; 95% CI: 1.05–1.66) C;C, C allele (OR = 1.31, 95% CI: 1.04–1.65)	Epidermal growth factor receptor
MGMT	10q26.3	**rs1625649** (c. 485C < A)	A;A vs. C;C, C;A (HR = 2.876, HR = 5.835)	O-6-methylguanine-DNA methyltransferase
MC4R	18q21.32	**rs489693** **(rs1673474)**	A;A vs. A;C/C;C (HR = 3.26, 1.29–8.22)* (HR = 7.02, 2.44–20.2)** *association between SNP and PFS **association between SNP and OS	Melanocortin 4 receptor
PTEN	10q23.31	**rs701848** (c.*1516 =)	C;C vs. C;T + T;T (OR = 1.169, 95% CI: 1.061–1.288;53)	Phosphatase and tensin homolog
VEGFA	6p21-p12, 6p21.1	**rs699947** (c.-2578C > A) **rs1570360** (c.-1154G > A)	C;C + C;A (OR = 2.56 (1.36–4.80) C allele (OR = 1.53 (1.14–2.03) G;G (OR = 1.53 (1.03–2.29), G allele (OR = 1.39 (1.01–1.91)	Vascular endothelial growth factor A
MTOR	1p36.22	**rs143119651** (c.7496A > G, c.6248A > G, c.6815A > G)		Mechanistic target of rapamycin kinase
GFAP (*ALXDRD*)	17q21.31	**rs11558961** (c.*28C > G, c.*28C > A)	G allele (OR = 0.77, 95% CI: 0.61–0.97 C;G (OR = 0.68, 95% CI: 0.49–0.95)	Glial fibrillary acidic protein

Note. SNP = single-nucleotide polymorphism.

Table 1: Polymorphic variants in genes associated with risk of glioblastoma (Alexandr N.Chernov et al., 2021)

Case Study 2

A second case study, to validate the pipeline for the paired-end sequences, was conducted as to look into implicated CNVs in glioblastom's mechanisms.

Chr	*Start Position*	*End Position*	*P-values*
20	40202935	40206372	6.66E-16
20	9539112	9539112	2.34E-14
20	22493112	22494453	2.34E-14
20	14835190	14835190	2.85E-14
20	14831186	14831615	1.49E-13
20	40202087	40202445	1.50E-13
20	14835230	14835333	2.86E-13
20	44538839	44540560	4.44E-13
20	40207824	40209580	4.90E-13
20	37756863	37772698	1.05E-12
20	14832703	14832703	1.60E-12
20	12716527	12718370	1.96E-12
20	14897154	14897201	1.96E-12

20	43743401	43743401	9.42E-12
20	9539204	9539204	1.07E-11
20	22488619	22492962	1.07E-11
20	40212060	40220009	1.07E-11
20	14831127	14831127	2.26E-11
20	4444102	4450364	2.60E-11
8	2119465	2120782	3.01E-11
20	22495347	22505099	3.01E-11
20	33108019	33108775	6.02E-11
20	37725829	37726311	6.02E-11
20	40319262	40337470	6.02E-11
20	40862648	40867876	6.02E-11
20	9533479	9535866	6.21E-11
20	23883085	23883085	6.21E-11
20	24258855	24259342	6.21E-11

Table 2: Significally associated CNVs with glioblastoma (p-value < 1.00E-10)

(Momiao Xiong et al., 2010 ;10.1109/ICBBE.2010.5516437)

Pathway	*FDR*
Metabolism of xenobiotics by cytochrome P450	2.7E-06
Calcium signaling pathway	1.5E-06
Axon guidance	2.9E-03
Colorectal cancer	2.3E-03
Tight junction	3.4E-03
Regulation of eIF2 pathway	6.3E-03
Double Stranded RNA Induced Gene Expression	7.0E-03
Glioma	6.6E-03
Glycan structures - biosynthesis 1	6.1E-03
Jak-STAT signaling pathway	5.6E-03
Drug metabolism - cytochrome P450	6.8E-03
Keratinocyte Differentiation pathway	7.9E-03
Telomerase RNA component gene hTerc Transcriptional Regulation	9.3E-03
Skeletal muscle hypertrophy is regulated via AKT/mTOR pathway	2.3E-02
BCR Signaling pathway	4.4E-02

Table 3: Pathways that harbor CNVs and associated with glioblastoma

(Momiao Xiong et al., 2010 ;10.1109/ICBBE.2010.5516437)

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