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Assessment of Vascularity in Glioblastoma and Its Implications on Patient Outcomes

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Abstract

Background—There is little data on why glioblastomas (GBM) hemorrhage and how it may affect patient outcomes. The aim of this study was to investigate the mechanisms of hemorrhage in glioblastoma by examining molecular and genetic features by immunohistochemistry (IHC) and mRNA expression profiles in association with imaging and clinical outcomes.

Methods—An observational retrospective cohort analysis was performed on 43 FFPE GBM tissue samples. MR images were assessed for the presence of hemorrhage and extent of resection. Specimens were examined for CD34 and CD105 expression using IHC. Tumor mRNA expression profiles were analyzed for 92 genes related to angiogenesis and vascularity.

Results—Forty-three specimens were analyzed, and 20 showed signs of hemorrhage, 23 did not. The average OS for patients with GBM with hemorrhage was 19.12 months (95% CI 10.39 – 27.84), versus 13.85 months (95% CI 8.85 – 18.85) in those without hemorrhage ($p > 0.05$). Tumors that hemorrhaged had higher IHC staining for CD34 and CD105. mRNA expression analysis revealed tumor hemorrhage was associated with increased expression of HIF1 α and MDK, and decreased expression of F3.

Conclusions—Hemorrhage in GBM was not associated with worsened OS. Increased expression of angiogenic factors and increased CD34 and CD105 IHC staining in tumors with hemorrhage suggests that increased hypoxia-induced angiogenesis and vessel density may play a role in glioblastoma hemorrhage. Characterizing tumors that are prone to hemorrhage and mechanisms behind the development of these hemorrhages may provide insights that can lead to the development of targeted, individualized therapies for glioblastoma.

Keywords

glioblastoma; hemorrhage; gene analysis; HIF1 α ; vascularity

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Introduction

The most common primary malignant brain cancer in adults is glioblastoma (GBM) as it is diagnosed in 3.2 individuals per 100,000 in the United States each year [1]. GBM also has a poor prognosis with an average overall survival of 12–15 months and less than 10% of patients surviving 3 years [2]. The diagnosis of GBM can only be confirmed with tissue sample, procured during resection or biopsy, but the diagnosis can be highly suspected from MRI. MRI can be used to evaluate different aspects of the tumor such as location, size, contrast enhancement, infiltrative nature, perfusion, spectroscopic profile, presence of hemorrhage and vascularity. Glioblastomas are often highly vascular with 5–19.3% of patients presenting with hemorrhage [3–6]. Overall, gliomas have been found to hemorrhage in up to 42–53% of patients and many of these are glioblastoma [3, 5].

While it has been established that patients with GBM may have an increased risk of intracranial hemorrhage, the mechanisms and effects of these hemorrhages are still being elucidated [4, 7–12]. Various reports have described tumor hemorrhage and a number of the case reports of GBM and other CNS tumors that hemorrhaged have suggested possible etiologies of hemorrhage – hypertension, anticoagulation, trauma, surgical intervention, infiltration of blood vessels by malignant cells, degenerative changes such as widened intracellular junctions of fenestrated capillaries, inadequate blood supply leading to necrosis, degree of vascularity, and occlusion or thrombosis leading to hemorrhage [1, 3, 4, 6–34]. None of these have provided an adequate explanation for the event or if there is any clinical significance beyond potential acute effects from the hemorrhage.

The molecular mechanisms behind the increased risk of hemorrhage could be the result of altered gene expression driving increased angiogenesis. VEGF, angiogenin, angiopoietin, and many other genes are involved in the recruitment and development of new blood vessels to increase oxygen and nutrient delivery to the growing tumors [35–39]. The rapid development of new blood vessels in the chaotic environment of tumor growth can lead to immature and abnormal vessels [36, 40]. This increased vessel density and inferior vessel quality could directly contribute to increased intracranial hemorrhage. Many genes have been found to be associated with hemorrhage in tumors. For example, in CNS lymphomas that hemorrhage there is higher immunoreactivity to VEGF, and increased VEGF expression is associated with a poor prognosis [11, 38]. VEGF and matrix metalloproteinases (MMPs) have been associated with hemorrhage in pituitary adenomas with the presumptive mechanism being that rapid growth and overexpression of VEGF and MMPs leads to the breakdown of vessels around the tumor [41]. One factor upstream of VEGF that has been shown to play a role in cancer is hypoxia-inducible factor-1-alpha (HIF1 α). HIF1 α regulates several genes involved with angiogenesis along with tumor invasion, metastasis, chemotherapy resistance, metabolism, and stem cell maintenance [38]. While significant research has been completed to elucidate the role of these genes and associated pathways, there are still significant gaps in our understanding of how these genes and others affect the propensity of CNS tumors, particularly GBM, to hemorrhage and how that affects patient outcomes.

Angiogenesis is a complex process involving the remodeling of the extracellular matrix (ECM) and migration and proliferation of endothelial cells [42, 43]. This process is a hallmark of cancer and has been implicated as a prognostic factor in many types of cancer [44]. Increased microvessel density (MVD) has been found to be an independent prognostic factor in breast cancer [42, 45–47]. Another study investigated the mechanism of increased vascularity in GBM by examining adhesion molecules through immunohistochemistry (IHC) and demonstrated that there were increased levels of adhesion molecules in GBM that were thought to represent incomplete vascular structures [48]. Numerous additional studies have identified many other genes that are implicated as contributors to angiogenesis and ECM remodeling [36–39, 49].

Our goal for this study was to explore why hemorrhage occurs in GBM and examine how it affects patient outcomes. We aimed to identify differences in the genomic profiles and vascularity of glioblastomas that hemorrhage by analyzing IHC findings and mRNA expression profiles and correlating these to imaging findings and survival. Our hypothesis was that patients with GBM that hemorrhaged would demonstrate elevated angiogenic and vascularity related gene expression and increased vascularity on IHC and subsequently would have poorer outcomes.

Materials and Methods

Patient Inclusion and Tissue Sample Acquisition

Following IRB approval, forty-three patients with tissue specimens collected from the initial resection of newly diagnosed untreated GBM were selected for inclusion in this study. All patients were treated at the University of Nebraska Medical Center from 2007–2013 and had adequate tissue and data available. The 43 specimens, in addition to four normal brain specimens obtained from epilepsy surgeries used as controls, were analyzed. The tissue samples were formalin-fixed and paraffin-embedded (FFPE) at the time of surgery for long-term storage.

Clinical Review

A review of each patient's medical records was done to assess the patient demographics, adjuvant therapy received and patient outcomes. Patient outcomes were measured by overall survival time as defined by the length of time between date of surgery and date of death, and progression free survival was defined as the time from the date of surgery to the date of recurrence or progression shown on MRI. Overall survival was compared between patients who underwent gross total resection compared to subtotal resection. Overall survival and progression free survival were compared between patients that hemorrhaged and those that did not based on imaging findings (described below). A large majority of the patients received adjuvant chemoradiation, therefore, no further analysis was performed to evaluate the effect of adjuvant therapy on patient outcomes.

MRI Analysis

Pre-operative MR images were assessed for tumor enhancement, perfusion, and the presence of hemorrhage or significant blood products. CT scans were assessed for hemorrhage and

enhancement. These findings separated the cohort into the two groups, those that hemorrhaged and those that did not. Perfusion and enhancement were compared between these two groups. The location of each tumor was determined for lobe and side. Post-operative imaging was used to assess the extent of resection achieved for each patient and was classified as gross total resection (GTR) or subtotal resection (STR) defined by the presence of remaining contrast enhancement or FLAIR signal.

Assessing Vascular Density

Microvessel density (MVD) was assessed by IHC analysis with antibodies targeting the endothelial markers CD105 and CD34 on the 43 GBM and 4 normal brain specimens based on Weidner's previously established methods [50]. The most vascular areas in a tumor were first located at low magnification, and microvessels were counted on an image created by cellSens software using an Olympus DP72 camera attached to an Olympus BX45 microscope at 200x field. Any single or cluster of endothelial cells that was clearly separated from adjacent microvessels was considered as one countable microvessel. The average counts from the two most vascular areas were recorded for each tumor as a measure of the degree of angiogenesis. MVD was compared between the group that hemorrhaged and the group that did not hemorrhage.

Quantification of mRNA Expression

The FFPE specimens from 43 GBM and 4 normal brain controls were analyzed for mRNA expression using the Affymetrix QuantiGene Plex 2.0 Assay (Santa Clara, CA). Ninety-two genes related to angiogenesis and vascularity were chosen and custom plexes were created to run each sample in duplicate (Supplemental Table 1). The specimens were examined by a senior neuropathologist (RDM) to ensure adequate amount and consistency of quality of tissue to perform the experiments. FFPE specimen blocks were cut and placed on microscope slides. A representative slide was stained with hematoxylin and eosin (H&E) for histology to specifically identify tumor tissue. Unstained FFPE tissue slides were deparaffinized and the tumor region, identified from H&E, was macrodissected. The specimens were then homogenized and mRNA was isolated and subsequently assessed using the QuantiGene Custom QuantiGene Plex 2.0 panels. The panels utilize specific different colored beads that use complimentary sequences that hybridize to and capture specific mRNA sequences to evaluate the gene expression of the 92 identified genes related to angiogenesis and vascularity along with six housekeeping genes. Specific to our assay was the use of two separate panels consisting of 46 different reporter genes and the same six housekeeping genes (52-magnetic bead multiplex) for cross-panel normalization and gene expression comparison of the multiplexed wells. The captured mRNA was amplified with branched DNA molecules that were labeled with a biotin probe. The amplified mRNA signal was then measured based on luminescence following the addition of streptavidin phycoerythrin with a Luminex 200 instrument that classifies each bead while simultaneously measuring the amount of luminescence signal for each specific mRNA. Tumor or brain lysate concentrations were optimized by running a serial dilution of representative samples and assessing gene expression using the custom QuantiGene Plex 2.0 panels to ensure target signals were in the linear range. Samples were run in duplicate. The technical replicates were averaged. Individual mRNA readings were excluded if they were below the level of

detection (mean of the blank background wells + 3 * standard deviation of the blank wells) for that specific gene as defined by the Affymetrix QuantiGene 2.0 Reagent protocol. If more than 10 samples had mRNA levels below the level of detection for a given gene, the gene in its entirety was excluded from further analysis. The remaining readings for each target mRNA were background subtracted (mean blank well reading). The mRNA expression for each sample was then normalized to its mean global mRNA expression (housekeeping gene expression was not used for normalization as these genes demonstrated variable expression based on the length of time since initial surgical resection and FFPE processing) and analyzed by comparing the normalized expression between the groups that did and did not hemorrhage with the normal brain controls.

Statistical Analysis

Kaplan-Meier survival analyses were used to evaluate the differences in overall patient survival between patients based on extent of resection and hemorrhage status. Genes were compared statistically and graphically using the mean expression levels and standard deviations between GBM with hemorrhage and those without. Genes appearing to have differences underwent further analysis. The significance of differential gene expression of HIF1A, MDK, and F3 and IHC staining for CD105 and CD34 in tumors that hemorrhaged compared to those that did not was assessed by t-test. Significance of laterality in hemorrhage was assessed based on a Chi Squared Fisher's two-tailed exact t-test analysis.

Results

Based upon a preliminary data assessment for quality, four of the forty-three GBM samples were excluded from further analysis due to low global mRNA expression (> 20 of the remaining probes for a given sample were below the level of detection). Therefore, we evaluated a cohort of 39 GBM for analysis. On review of our cohort's records, there were 19 females and 20 males. During resection, 21 patients achieved gross total resection, 16 patients achieved subtotal resection, one underwent an excisional biopsy only and 1 had an unknown resection status. Following surgery, 29 patients had adjuvant chemoradiation, 1 received only radiation, 3 received chemotherapy alone, 4 received no additional treatment, and 2 received unknown treatment (Table 1).

Based on pre-operative imaging, 17 tumors showed signs of hemorrhage (visible hemorrhage or blood products) and were designated as hemorrhagic glioblastomas. In contrast, 22 patients did not have any visible hemorrhage or blood products on MRI imaging (Table 1). All but one tumor enhanced pre-operatively. MRI perfusion was elevated in 21 of the 25 patients examined (data not available in 14/39 patients).

Kaplan-Meier survival analysis demonstrated that patients achieving gross total resection (median survival = 17.65 months) of their tumor had a longer overall survival than those who had sub-total resection (median survival = 7.715) ($p = 0.0104$) (Fig. 1A). Kaplan-Meier survival analysis was performed to assess the effect of tumor hemorrhage on survival. This analysis revealed there was no significant difference in overall survival ($p = 0.3842$) or progression free survival ($p = 0.9149$) between the patients that hemorrhaged and those that did not (Fig. 1B & C).

Twenty-four of the 39 (61.54%) GBM were incidentally found to be on the right side. Of the GBM on the right side, 14 (58.31%) hemorrhaged. Of those on the left side, 3 (20%) hemorrhaged. Hemorrhage was significantly associated with right sided location ($p=0.0244$). The right temporal lobe had 15 GBM, 11 (73.34%) of which hemorrhaged. While there were significant associations between laterality and hemorrhage, there was no association with laterality and survival ($p=0.359$).

Analysis with IHC to assess for microvessel density showed a positive relationship between CD105 staining and hemorrhage and a positive relationship approaching significance with CD34 staining and hemorrhages (Fig. 2A & B).

The thirty-nine GBM and 4 normal samples were analyzed for genetic relationships related to hemorrhage. HIF1 α and MDK mRNA expression were increased in GBM compared to normal brain controls and was increased even further in tumors that hemorrhaged (Fig. 3A & B). F3 mRNA expression was decreased in tumors that hemorrhaged to an extent that approaches statistical significance (Fig. 3C). Additionally, ANPEP, THBS1, and COL18A1 mRNA expression demonstrated a positive association with hemorrhage particularly in tumors with lower levels of HIF1 α expression (Fig. 4A–C). ITGAV mRNA expression was inversely related to hemorrhage risk (Fig. 4D).

Discussion

The demographics of our cohort appeared to be fairly equally distributed between the group that had evidence of hemorrhage and those that did not. Age, gender, and adjuvant treatment did not appear to play a significant role in GBM hemorrhage, overall survival, or progression free survival.

Patients with gross total resection were found to have longer overall survival than sub-total resection ($p=0.0104$). This finding is in agreement with current literature [51]. There was no difference in overall survival or progression free survival between the GBM that hemorrhaged and those that did not. This does not support our initial hypothesis and is counterintuitive. One explanation for this could be that other factors, such as patient functional status, size or location of the tumor, or response to treatment, are more significant to overall and progression free survival than concurrent hemorrhage [51, 52].

The increased perfusion and enhancement among the majority of GBM in our cohort illustrates that neither of these features were associated with hemorrhage suggesting they likely represent characteristics common to GBM rather than being specific to hemorrhage. A recent study has demonstrated that increases in hemorrhage, volume and T1/FLAIR-envelope ratio (which is the relative size of the T1 abnormality in relation to the size in the T2/FLAIR images) considered together have been shown to be associated with a poorer prognosis in GBM [53]. Our results show that individually, hemorrhage did not adversely affect outcomes. Indeed, it may be that hemorrhage, in combination with other features may have a greater impact.

An incidental finding was investigated concerning the laterality of the GBM. During our analysis, it was noted that a majority of our cohort had a tumor on the right side (61.54%).

Of those, 58.31% hemorrhaged in comparison to the left side where only 20% hemorrhaged. GBM on the right side was more likely to have hemorrhaged than those on the left ($p=0.0244$). The most densely populated region was in the right temporal region with 15 GBM (38.46%) and 73.34% of those hemorrhaged. GBM laterality was not associated with survival in our cohort. The research concerning CNS tumor laterality is sparse. One study demonstrated that tumors on the right side have a worse quality of life [54]. A possible explanation of these findings is that gliomas on the right side are often less symptomatic and might be able to advance before becoming overtly symptomatic and clinically recognized. A tumor with a larger distribution of infiltration may not be as amenable to gross total resection. Another study approaching statistical significance demonstrated that low-grade gliomas and meningiomas occur more often on the left side and high-grade gliomas and acoustic neuromas occurred more often on the right side [55]. While our aim was not to determine the importance of laterality on outcomes, we found that more tumors were located on the right side and more of them had hemorrhage.

Our IHC findings suggest that increased angiogenesis was associated with hemorrhage. We found that increased CD105 and CD34 staining were associated with hemorrhage. CD105 is a marker for endoglin, a vascular endothelium glycoprotein that is often found on primitive hematopoietic cells and associated with angiogenesis and tumor growth [56]. CD34 stains for a glycoprotein (Hematopoietic progenitor cell antigen CD34) which plays a role in early hematopoiesis and is thought to act as an adhesion molecule and contribute to stem cell attachment to the extracellular matrix [57]. Both of these are commonly used as markers of vascular density and angiogenesis [56, 57].

A number of genes were found to be associated with hemorrhage with some showing both positive and negative correlations. Increased expression of HIF1 α was significantly associated with tumor hemorrhage in patients with GBM ($p=0.0073$). HIF1 α encodes for the alpha subunit of the transcription factor hypoxia-inducible factor-1, which functions as a regulator of systemic and cellular homeostatic response to hypoxia [58]. HIF1 α leads to the activation of genes whose products aim to overcome hypoxia by increasing angiogenesis and inducing apoptosis [58]. HIF1 α has been found to play an important role in embryonic vascularization and tumor angiogenesis [59]. Our finding that increased HIF1 α expression was associated with hemorrhage suggests that hypoxia induced angiogenesis may contribute to hemorrhage.

Another gene that was found to have increased expression in tumors that hemorrhaged was Midkine (Neurite Growth-Promoting Factor 2) or MDK. MDK codes for a secreted growth factor that responds to retinoic acid and binds to heparin, which promotes cell growth, migration, and angiogenesis, especially in tumors [58]. MDK has been targeted for therapeutics in a variety of disorders including non-small cell lung cancer [60]. Our finding that increased MDK expression was associated with hemorrhage suggests that high levels of cell growth and angiogenesis could contribute to tumor hemorrhage. The increased expression of both HIF1 α and MDK in tumors that hemorrhaged is consistent as both genes contribute to similar cellular processes and ultimately suggest that increased angiogenesis in the context of high cell turnover results in an increased risk of hemorrhage. A possible mechanism for hemorrhage is that hypoxia and subsequent necrosis leads to increased

expression of HIF1 α and MDK, which in turn primarily promote angiogenesis. Rapid angiogenesis can lead to the development of immature and abnormal vessels that are more prone to hemorrhage.

Increased expression of a few additional genes were found to be associated with GBM that hemorrhage, particularly in tumors that did not have increased HIF1 α expression (ANPEP, THBS1, COL18A1). These were less conserved across patient tumors and likely represent unique mechanisms contributing to tumor hemorrhage. ANPEP codes for aminopeptidase N, a protein that is found on cell plasma membranes and digests peptides [58]. It contains a sequence very similar to that of zinc-binding metalloproteinases [58]. ANPEP is involved with the metabolism of regulatory peptides for various cells including synaptic membranes of the CNS [58]. ANPEP is a marker of pericytes, mural cells of CNS blood microvessels [61]. Our findings that increased expression of ANPEP was associated with hemorrhage could be explained by an increased density of pericytes associated with increased intratumoral vascularity is prone to hemorrhage.

Increased THBS1, which encodes for a member of the thrombospondin family, was found to be associated with hemorrhages particularly in the presence of low HIF1 α levels. Thrombospondin is an adhesive glycoprotein important for cell-to-cell and cell-to-matrix connections and has been found to contribute to platelet aggregation, angiogenesis and tumorigenesis [58]. When considering the role of THBS1 in angiogenesis and tumorigenesis, these findings support our previous conclusions that increased angiogenesis may lead to hemorrhage, but suggests more than one mechanism likely contributes to an increased risk of hemorrhage.

COL18A1 encodes the alpha chain of type XVIII collagen. Endostatin, an antiangiogenic protein, is produced from cleaving the c-terminus of COL18A1. Endostatin has a role in inhibiting angiogenesis and tumorigenesis. Perhaps our finding of COL18A1 being related to hemorrhage in the presence of low HIF1 α suggests that in some patients, elevated levels of COL18A1 indicate that a mechanism is activated to prevent the proteolytic processing of COL18A1 thereby decreasing the antiangiogenic signal and allowing for increased angiogenesis.

Coagulation factor III (F3) expression was decreased in GBM that hemorrhage. F3 encodes for tissue factor (also known as thromboplastin and coagulation factor III), which is a cell surface glycoprotein that contributes to the initiation of the blood coagulation cascade [58]. Based on its known function, it is reasonable to expect that F3 expression would be protective against hemorrhage based on its role in initiating coagulation. The reverse scenario is also consistent, in that decreased F3 would limit coagulation and would increase GBM susceptibility to hemorrhage.

Another gene that was found to have decreased expression related to hemorrhage was ITGAV (Integrin, Alpha V), which encodes for a product that belongs to the integrin alpha chain family used for cell surface adhesion and signaling. Integrin has been found to possibly regulate angiogenesis and cancer progression [58]. We found ITGAV expression was inversely related to hemorrhage. This appears to offset the increasing risk of

hemorrhage with increased HIF1 α expression. A possible explanation for these findings is decreased cell to cell adhesion which could contribute to hemorrhage.

This study achieved the goal set out to discover gene expression profiles of GBM related to hemorrhage and how hemorrhage affects patient outcomes. The genes we identified as being related to hemorrhage are primarily associated with angiogenesis. Not only did the tumors that hemorrhage have increased gene signaling in pathways associated with angiogenesis, but they also demonstrated a response of an increase in vascular density as demonstrated by our IHC findings suggesting these pro-angiogenic pathways are contributing to changes in the tumor microenvironment that could be contributing to an increased risk of hemorrhage.

This study has a few limitations. First is the relatively small sample size of 39. Future studies should be completed on larger cohorts, if possible. Second, the tissue samples, although preserved in standard fashion, had varied ages. By carefully analyzing the tissue for quality and composition, we were assured reliable results, however, the degradation of proteins over time could have affected the results. Analysis of fresh frozen samples instead of FFPE may potentially affect results. Furthermore, this was a retrospective study. If a prospective study were completed, samples would still need to be stored for some time before analysis, however. Additionally, there are additional genes, CD markers for vascularity, and MRI findings that could be evaluated when looking at angiogenesis and hemorrhage in GBM. Future investigation into the significance of the laterality of GBM and how that relates to symptoms, diagnosis, progression, hemorrhage, and prognosis could be done. In fact, others have also started looking at the relationship of genomics and imaging of glioblastoma [62, 63].

Our study demonstrates that there are GBM patient populations that have increased vessel density of presumed poorer quality and specific gene expression profiles that are related to hemorrhage. Even though we found that hemorrhage in GBM does not affect overall or progression free survival, characterizing the tumors that are prone to hemorrhage and the mechanisms behind the development of these hemorrhages may provide insight that can be leveraged for the development of targeted, individualized therapies for GBM. One example of an effective therapeutic in GBM is bevacizumab, an anti-VEGF antibody that works to inhibit angiogenesis. Recent studies found that salvage treatment with bevacizumab increased progression free survival and objective response [64], and initial bevacizumab treatment increased progression free survival as well as overall survival in GBM [65]. These findings taken with our study suggest that there may be at least a subset of patients that could benefit from upfront targeting of angiogenic genes including those we identified as contributors to hemorrhage, such as HIF1 α , MDK and F3. Certainly, MDK inhibitors are being investigated as potential therapeutics for non-small cell lung cancer [60]. This may lead to improved patient outcomes as these pathways may not only be associated with an increased risk of hemorrhage, but are likely also associated with pathways that GBM are dependent on for cell survival and proliferation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A) Overall survival of patients that achieved gross total resection compared to those with subtotal resection (excluding the excisional biopsy and unknown resection status). B) Overall survival and C) progression free survival comparing glioblastomas that hemorrhaged to those that did not. PFS data not available in all patients.

Figure 2.
The relationship of A) CD105- and B) CD34-staining in GBM with hemorrhage (N=17), without hemorrhage (N=22), and controls (N=4).

Figure 3. mRNA expression of A) HIF1 α , B) MDK, and C) F3 in glioblastomas with hemorrhage (N=17), without hemorrhage (N=22), and normal brain controls (N=4). There is increased expression of HIF1 α and MDK and decreased F3 in in tumors that hemorrhaged.

Figure 4. The relationships between HIF1 α with A) ANPEP, B) THBS1, C) COL18A1, and D) ITGAV mRNA expression comparing glioblastoma with hemorrhage, without hemorrhage, and normal brain controls. ANPEP, THBS1, and COL18A1 mRNA expression demonstrated a positive association with hemorrhage particularly in tumors with lower levels of HIF1 α expression. ITGAV mRNA expression was inversely related to hemorrhage risk.

Table 1

Patient demographics, overall survival (OS), extent of resection, and adjuvant treatment stratified by hemorrhage status. (GTR=gross total resection; STR=subtotal resection)

	GBM with hemorrhage (n = 17)	GBM with no hemorrhage (n = 22)
Mean Age in Years (range)	68.22 (30–71)	60.68 (35–84)
Gender		
Female	9	10
Male	8	12
Mean OS in Months (range)	19.12 (2.52–71.19)	13.85 (0.3–37.16)
Extent of Resection		
GTR	8	13
STR	9	7
Excisional Biopsy	0	1
Unknown	0	1
Adjuvant Treatment		
chemoradiation	16	13
radiation only	1	0
chemotherapy only	0	3
none	0	4
unknown	0	2