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Assessment of Micro-vessel Density in Brain Gliomaby CD105 Expression

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ABSTRACT

Background & Objective: Micro-vascular proliferation is an important histological feature of brain glioma with more vascular proliferation is present in higher grades of glioma. CD 105 is expressed in new actively proliferating and immature endothelial cells in tumor environment and appears to be capable to distinguish between malignant neo-vasculature and normal vessels.

Methods: This study was designed to evaluate the Micro-Vessel Density(MVD) in different grades of brain glioma based on CD 105 expression byImmunohistochemistry method to determine whether it can be a helpful marker for rumor grading or not.

Paraffin blocks of formalin fixed samples of brain astrocyticglioma were retrieved and IHC was performed using anti-CD105 monoclonal mouse antibody.

Results: Total number of 48cases of low and high grade astrocyticgliomas were evaluated.We noted that there was a positive correlation between MVD evaluated by CD105 and tumor grade, meaning that expression was significantly greater in tumors with higher grade (P=0.019).

Conclusion: We concluded that MVD quantified by CD 105 has positive correlation with tumor grade. Also we think that expression of CD 105 specially in low-grade glioma can serve as a basis for selective treatment option in combination with current standard care.

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Introduction

Gliomas are the most common primary brain tumors in adults (1,2). Although molecular genetic features are included in recent World Health Organization (WHO) classification updates, its grade determinations are still based on histologic criteria (3).

Despite advances in treatment modalities, the prognosis of gliomas, especially higher grades, remains poor (1,4).

The median survival is about six to eight years for low-grade (I, II) tumors, but decreases to two to five years for grade III and less than two years in grade IV (glioblastoma multiforme) (1,4).

Only less than 3% of patients with glioblastoma multiforme survive more than five years (4).

Microvascular proliferation is an important histological feature of brain glioma (5) with more vascular proliferation present in higher grades of glioma. Glioblastoma is actually amongst the most vascularized tumors (6).

Histologic evaluation of tumor angiogenesis based on micro-vessel density (MVD) is an independent prognostic factor in patients diagnosed with glioma (5).

Some endothelial markers such as *CD31, CD34,* or *Factor VIII* are implicated, but they do not differentiate between mature vessels and microvasculature stimulated for tumor angiogenesis (5, 7, 8).

CD105 was originally characterized more than two decades ago (2) and is a 180 kDa integral membrane

glycoprotein, which is an accessing receptor for the transforming growth factor beta (2, 6). It is specially expressed in new actively proliferating and immature endothelial cells in tumor environment (2,5).

CD105 expression is implicated in diagnosis and prognosis assessment and as a treatment option in variable tumors including breast (2, 9), squamous cell carcinoma(2,10), pancreatic ductal carcinoma(2,11), non-small cell lung cancer and prostate cancer (6), and appears capable of distinguishing between malignant neo-vasculature and normal vessels (6).

Moreover, antibody based therapeutic strategies are considered as complementary treatment options in different neoplasms leading to notifying novel potential antigens (2).

Anti-angiogenic based target therapies with controversial results are undertaken in several clinical trials (6,12, 13)

In this regard, it is suggested that *CD105* antibody based treatment can be effective in preventing angiogenesis and inhibiting the formation of capillary-like structures with high specificity toward tumor tissue and less probable side effects (6).

On the other hand, recently, some studies denoted that the *CD105* positive vascular structures play a clinical role in biology of gliomas with influence on tumor prognosis (2), but it is not clear whether *CD105* can be used as complementary criteria for grading glioma (2).

The current study aimed at evaluating the MVD in different grades of glioma based on *CD105* expression by immunohistochemistry (IHC) method to determine whether it can be a helpful marker for rumor grading or not.

Also, expression of *CD105* in low-grade gliomas indicates a potential complementary therapeutic option in lower grade tumors in order to prevent tumor recurrence.

Materials and Methods

Paraffin blocks of formalin-fixed samples of brain glioma from 2013 to 2014 were retrieved from the archive of Pathology Department, Shariati Hospital, Tehran, Iran. The slides were regraded according to WHO criteria (14); grade I, n=8; grade II, n=16; grade III, n=8; grade IV, n=16.The questionable cases were excluded.

IHC was performed using anti-CD105 monoclonal mouse antibody (4G11, Leica) and anti-CD31 mouse monoclonal antibody (JC/70A, Biogenex) according to the manufacturers` recommendations (*CD31* study was performed to confirm localization of neo-vasculature endothelium).

The stained slides were evaluated for MVD based on CD105 staining (4, 15).

Briefly, the four most vascularized areas (hot spots) were selected at low power followed by counting each positive endothelial cell or cluster of endothelial cells (± Lumina) at high magnification. The mean number of vessels in four areas was considered as density per high power field (HPF).

After data collection, the analysis of data was conducted with SPSS version 19. P-value <0.05 was considered significant.

Results

Total number of 8, 16, 8, and 16 cases of grades I, II, III, and IV astrocytoma were evaluated, respectively.

Grades I and II tumors were categorized as low and grades III and IV as high.

The frequency of different grades regarding gender is summarized in Table 1.

Table 1. The Frequency of Different Grades Regarding Gender

Grade / Demographic Data	Gender		Total
	Female	Male	
LOW	13	11	24
High	4	20	24

The mean age of patients with lower grade tumors was 26.7 years (ranged 10 to 35) in comparison with 46.6 years (ranged 22 to 71) in cases with high grades.

Regarding the vessel density, a positive correlation between MVD and tumor grades were observed, which meant that MVD was significantly greater in tumors with higher grades (P=0.019). The data are summarized in Table 2.

Moreover, *CD31* and *CD105* revealed significant and positive correlation regarding the number of stained vessels (P=0.012) (Figure 1).

Table 2. Correlation Between MVD Evaluated by CD105 Expression and Tumor Grade

Grade	I	II	III	IV
Mean MVD expression by CD105	8.37	8.31	19.87	25.25

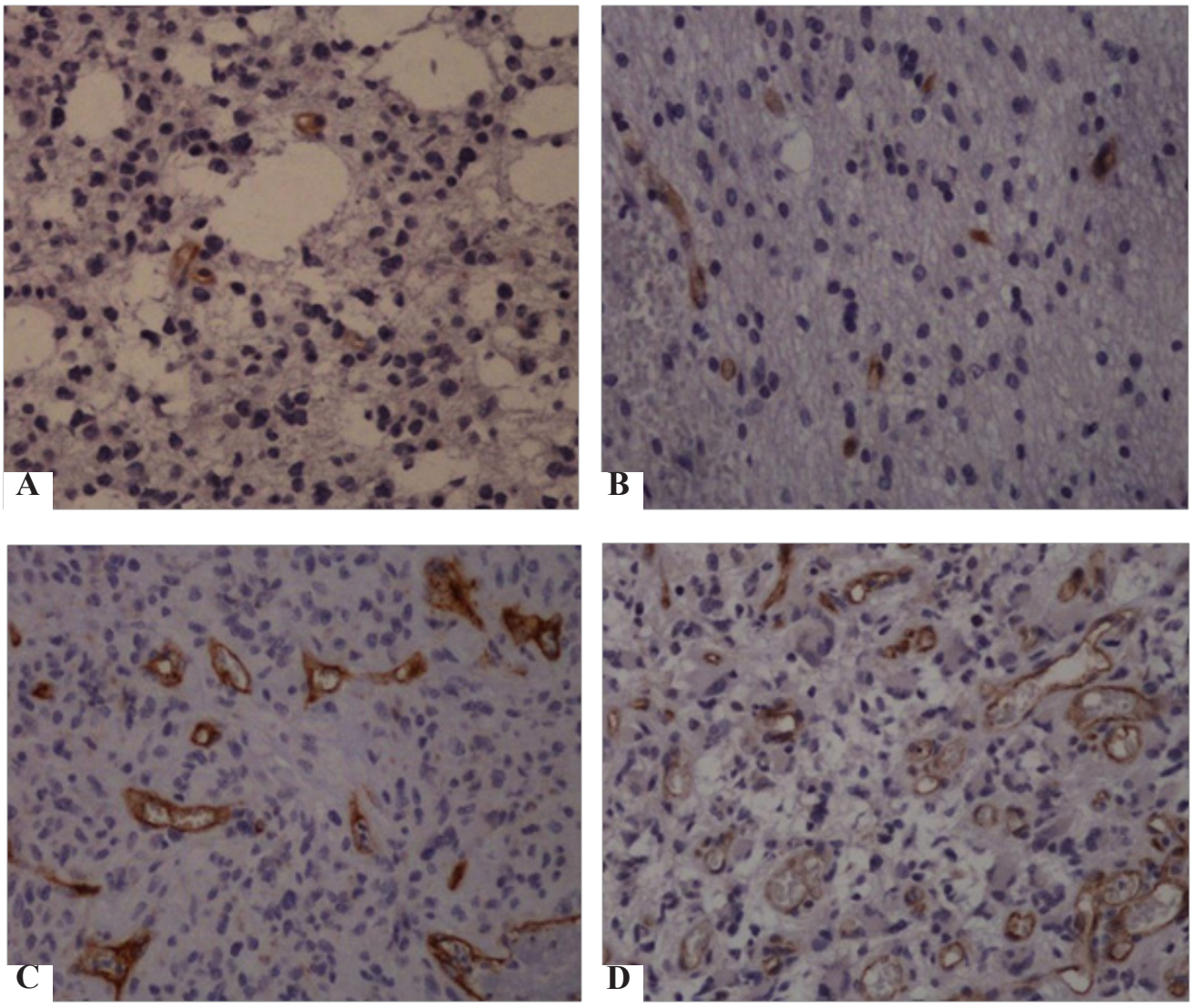

Figure 1. Expression of *CD105* in vessels of gliomas with different grades (A) I; (B) II; (C) III; (D) IV

Discussion

High-grade gliomas are highly vascular tumors (16). Angiogenesis is crucial to supply adequate nutrient and oxygenation for tumor growth and eliminate cellular waste product (16). Additionally, in combination with cellular proliferation and focal necrosis, microvascular proliferation is a key histologic feature in glioma grading (17).

It is suggested that intratumoral MVD quantified by IHC for endothelial cell markers correlate with prognosis, which meant that increase in MVD is associated with higher tumor grade (17) and shorter survival (18). Although the process of angiogenesis is subjected to many investigations, the role and function of endothelial cells still remain unclear (17). However, it is evident that tumor-associated vessels show altered shape and integrity (17). They lose blood brain barrier (BBB) properties with the increase in permeability (5), leading to fluid leakage into brain parenchyma and edema (17). Although tumor-associated vascularization was first introduced in 1940 (19-21), Dr. Judah Folkman in the early 1970s showed that targeting this neovascularization could be a vital strategy for cancer treatment (20, 22, 23).

Among solid tumors, glioblastoma shows the most angiogenic feature with marked degree of vascular proliferation and endothelial cell hyperplasia (16), suggesting a potential novel therapeutic approach to target angiogenesis (16).

There are conflicting results whether or not the degree of angiogenesis measured by MVD is of prognostic value in astrocytic tumors (8). While most studies showed positive relationship between MVD and tumor survival, some others reported opposite findings (24). This partly could be due to variable antibodies used against different pan endothelial cell markers (18,8), differences in counting method or staining procedures (18).

Efforts to standardize the method may be helpful; however, first of all, the optimal marker is an essential requirement (18).

The *CD105* is an integral membrane glycoprotein of transforming growth factor-β receptor complex (25).

As a vascular marker, *CD105* is preferentially expressed in immature vessels and some studies proposed it as a marker for tumor-associated angiogenesis (5, 16, 8); while it is barely detectable in normal tissue (20).

The utility of this marker is investigated in various neoplasms such as glioblastoma, non-small cell lung cancer, and prostate cancer (6).

It is reported that *CD105* MVD is increased with increase in tumor grade (5).

Jia et al., (5) reported that *CD105* MVD was significantly lower in low-grade glioma in comparison with that of high-grade tumors, but there was no difference between grade III and IV tumors (5).

In the current study, the mean MVD in grades I to IV gliomas were 8.37, 8.31, 19.87 and 25.25, respectively, and it was noted that the density of vessels was significantly higher in high-grade tumors (P=0.019).

The current study results were in concordant with those of Yao et al. (8). They evaluated 50 astrocytic tumors. The mean MVD in low-grade, as well as grade III and IV tumors were 24.8, 42.7, and 51.9, respectively. They found significant increase in MVD by *CD105* with increase in tumor grade. Moreover, patients with either glioblastoma or low-grade gliomas with higher MVD had significantly shorter mean survival time (8).

In another study, smith et al., (16) evaluated 150 grade III and IV pediatric glial tumors for MVD by *CD105* and *CD31*. The mean MVD per core of tissue micro-array (TMA) was 8 and 6.4 for grade IV and III astrocytic tumors, respectively (P=0.44).

They scored the density in a semi-quantitative fashion with less than 3/core TMA, 3-8, and >8 vessel/TMA. All patients that survived for more than eight years had scores <3 vessel/core. Also, they found that *CD105* expression had a significant association with poor prognosis on multivariate analysis (P <0.001) (16).

In a meta-analysis, Kong et al., (2) concluded that *CD105* overexpression in glioma tissue was strongly linked to high grade of tumors.

In the current study, *CD31* and *CD105* had positive correlation (P =0.012). However, some studies (8, 24) concluded that CD105 was a better marker than *CD31* to evaluate angiogenesis and prediction of prognosis in astrocytic tumors (8,24).

Thus, measuring MVD by this marker is superior to traditional pan-endothelial markers such as *CD1, CD34*, or *Factor VIII* (18).

Radiation is the most effective treatment for glioblastoma in combination with surgery, but the efficacy is limited due to radiation resistance (26); thus, some other therapeutic options are investigated.

Vascular endothelial growth factor (*VEGF*), for example, is another potent growth factor mediating tumor angiogenesis and overexpression of *VEGFR* is associated with poor prognosis in glioblastoma (27). Bevacizumab is a recombinant humanized monoclonal antibody that binds to VEGF and suppresses *VEGF* signaling, thereby downregulating angiogenesis (27,28). Although it was initially observed that bevacizumab was associated with high radiographic response rate and prolonged progression-free survival, no overall survival benefit of bevacizumab was identified in multiple phase III trials (28).

Tumor specific expression of *CD105* within vascular capillary beds was utilized in some therapeutic strategies (20, 29-33).

CD105 specific monoclonal antibodies can be fused to radioactive isotope or a toxic compound, which target tumor vasculature and inhibit tumor growth in pre-clinical mouse models of breast cancers (20, 29, 31, 33).

Zhong Zheng Jian et al., (5) demonstrated that expression of *CD105* in peritumoral area was also of prognostic value in addition to those of the neoplastic tissue. Therefore, new therapeutic strategies are directed against tumor mass and can also target invasive cells in peritumoral normal appearing brain (6), which are probably beneficial to prevent tumor recurrence.

Finally, as most of other studies the current study also concluded that MVD quantified by *CD105* had positive correlation with tumor grade. Also, regarding the potential utility of anti *CD105* antibody for target therapy, the current study supposed that expression of *CD105*, especially in low-grade glioma, can serve as a basis for selective treatment option in combination with the current standard care in glioma. This approach can potentially improve treatment efficacy.

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Conflict of interests

The authors declared no conflict of interest.

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