

*Original Investigation*

# A Novel Candidate Molecule in the Pathological Grading of Gliomas: ELABELA

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## ABSTRACT

**AIM:** To investigate the possible role of ELABELA (ELA) in the histopathological grading of gliomas.

**MATERIAL and METHODS:** We retrospectively assessed pathological specimens of patients who underwent surgery for intracranial space-occupying lesions. Only primary glioma specimens were included in this study. We enrolled 11 patients histologically diagnosed with low-grade glioma and 22 patients with high-grade glioma. The ELA antibody was applied to 4–6- $\mu$ m-thick sections obtained from paraffin blocks. Histoscores were calculated using the distribution and intensity of staining immunoreactivity. An independent sample t-test was used for two-point inter-group assessments, whereas one-way analysis of variance was used for the other assessments.  $p < 0.05$  was considered statistically significant.

**RESULTS:** The histoscores of the control brain, low-grade glioma, and high-grade glioma tissues were found to be 0.08, 0.37, and 0.92, respectively. The difference in ELA immunoreactivity between the control brain tissue and glioma tissue was statistically significant ( $p < 0.05$ ). In addition, a statistically significant increase was observed in ELA immunoreactivity in high-grade glioma tissues compared with that in low-grade glioma tissues ( $p < 0.05$ ).

**CONCLUSION:** ELA has an angiogenetic role in the progression of glial tumors. ELA, which is an endogenous ligand of the apelin receptor, activates the apelinergic system and causes the progression of glial tumors. Further studies with a large number of patients are necessary to investigate the angiogenetic role of ELA in glial tumors.

**KEYWORDS:** Apelin, Apelin receptor, Astrocytoma, Elabela, Glioma

## INTRODUCTION

Gliomas are the most common primary malignant central nervous system (CNS) tumors in adults. They are either astrocytic, oligodendrocytic, or a combination of these two cell types (14). In addition, gliomas are characterized by genetic and morphological complexities and can diffusely infiltrate into the normal brain parenchyma (13,17).

ELABELA (ELA) is an endogenous peptide ligand of the apelin receptor (AR) and plays a critical role in zebrafish embryonic development (7,30). Reportedly, AR was first cloned as an orphan G-protein-coupled receptor (GPCR) and has 30% homology with angiotensin receptor-1 (29). In addition, its ligand, apelin, was isolated from bovine stomach extract in 1998 (37). The AR is a class-A GPCR that was first described by O'Dowd (29). Both apelin and its receptor are expressed



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in various eukaryotes, including humans, and some other studies have also been conducted in cells or tissue cultures of rodent or other animal models. High levels of apelin have been observed in the CNS, and both its receptor and ligand are widely present throughout the peripheral tissue (2,9,19).

Apelin regulates numerous different physiological functions such as fluid homeostasis, angiogenesis, and energy metabolism (1,22,34). Reportedly, apelin acts as a vascular chemo-attractant molecule as the vascular endothelial growth factor (VEGF) (31) and induces angiogenesis in the progression of tumors (20,21,24). The activation of VEGF signaling stimulates new vessel formation for the growing tumor, as these new vessels provide oxygen and nutrients for tumor growth (15). Thus, ELA is a hormone that stimulates angiogenesis and, at the same time, provides homeostasis of energy metabolism through the apelinergic system.

Because high-grade gliomas are still one of the most lethal forms of cancer, it is imperative to establish possible molecular mechanisms underlying their growth/progression patterns. Thus, the present study aimed to investigate the possible role of ELA in the histopathological grading of gliomas that are radiologically and pathologically diagnosed.

## ■ MATERIAL and METHODS

### Patient Selection

This study was approved by Firat University Ethical Committee (Date: 29.09.2016, Approval No: 14-07). We retrospectively assessed pathological specimens of patients who underwent surgery for intracranial space-occupying lesions between 2015 and 2017. Only primary glioma specimens were included in this study. We enrolled 11 patients histologically diagnosed with low-grade glioma (grade I, n=1; grade II, n=10) and 22 patients with high-grade glioma (grade III, n=11; grade IV, n=11) in this study. As a control group (n=10), the cerebral tissues which were obtained from the patients who underwent surgery for hematoma or tumor and the pathological reports confirmed the absence of tumor or vasculopathy, were used.

### Immunohistochemistry

An independent pathologist re-evaluated all pathological preparations. The ELA antibody (ELA primary antibody, H-007-19, 1/200; Phoenix Pharmaceuticals, Inc., CA, USA) was applied to 4–6- $\mu$ m-thick sections obtained from paraffin blocks. All preparations were evaluated and photographed using a light microscope (Leica DFC295, Germany). Histochemical scores were calculated using the distribution (0.1: <25%; 0.4: 26%–50%; 0.6: 51%–75%; 0.9: 76%–100%) and intensity (0: no staining; +0.5: very little staining; +1: little staining; +2: medium; +3: very strong) of staining immunoreactivity (histoscore=distribution $\times$ intensity).

### Statistical Analysis

The data were determined as mean and standard deviation. Statistical analysis was conducted using SPSS v.22 package program. An independent sample t-test was used for two-point inter-group assessments, whereas one-way analysis

of variance was used for the other assessments.  $p < 0.05$  was considered statistically significant.

## ■ RESULTS

The results of immunohistochemical staining for ELA immunoreactivity using light microscopy revealed ELA immunoreactivity in the neurons and glial cells of the control brain tissue (Figure 1A, B). The histoscores of the control brain tissue, low-grade glioma tissue, and high-grade glioma tissue were 0.08, 0.37, and 0.92, respectively (Table I). Compared with the control brain tissue, we observed a statistically significant difference in ELA immunoreactivity in low-grade glioma tissue (Figure 1C-E) and high-grade glioma tissue (Figure 1F-K) ( $p < 0.05$ ). In addition, a statistically significant increase was observed in ELA immunoreactivity in high-grade glioma tissue compared with that in low-grade glioma tissue (Figure 2, Table I) ( $p < 0.05$ ). However, there were no differences in ELA immunoreactivity between anaplastic astrocytomas and glioblastomas (GBMs).

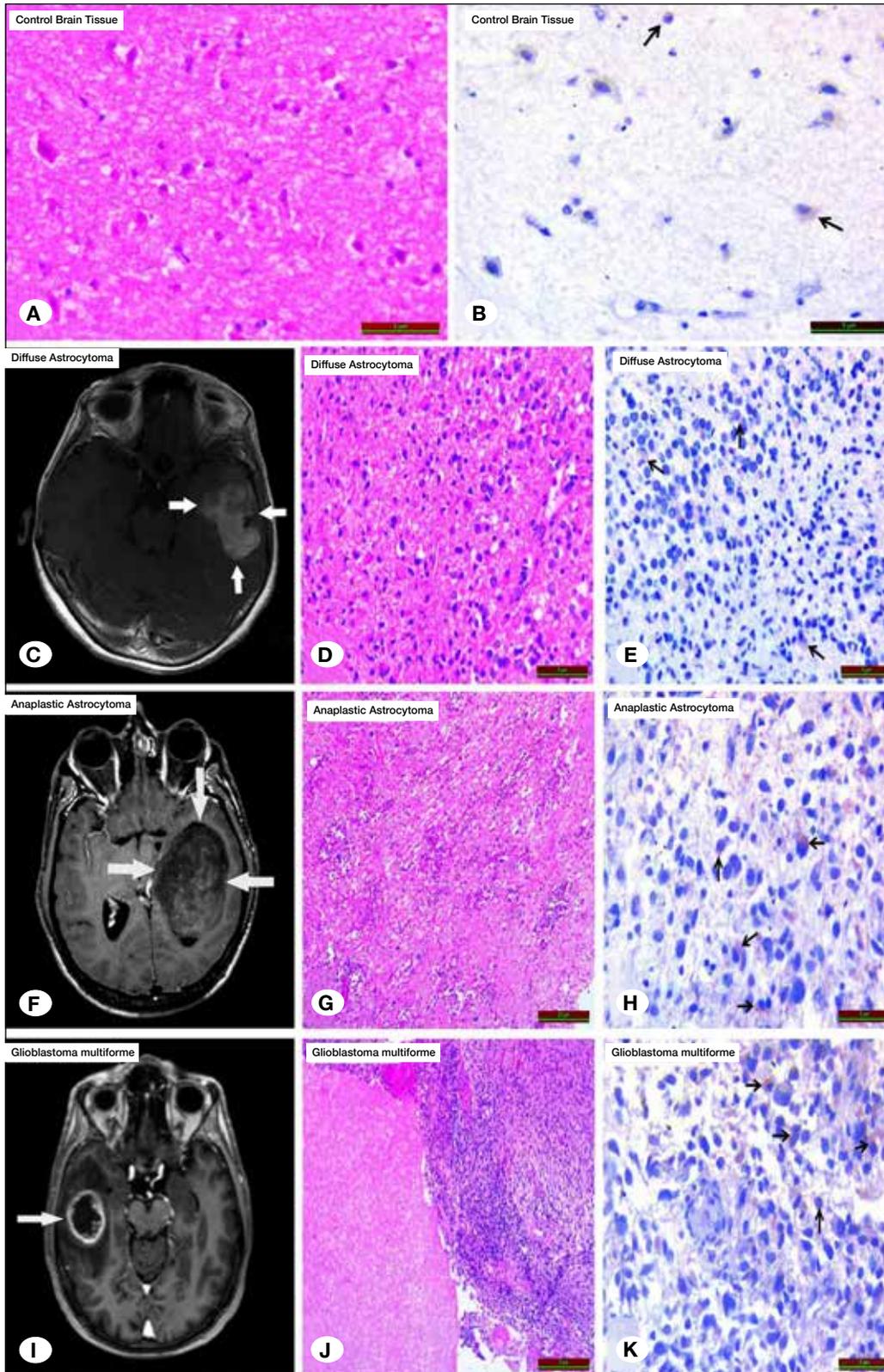
## ■ DISCUSSION

Although primary malignant CNS tumors are reported in 2% of all cancers, morbidity and mortality rates do not concur. Reportedly, malignant CNS tumors are the leading cause of mortality in children and are the third most common cause of cancer-related deaths in adolescents and adults aged 15–34 years (8). However, they are most commonly reported in patients aged above 45 years (23).

The World Health Organization (WHO) grades gliomas as I–IV based on their malignant behavior. The main histological types of gliomas in adults are astrocytomas (grades I–IV), oligodendrogliomas (grades II–III), and oligoastrocytomas (grades II–III). GBMs (grade IV) are the most common gliomas reported in adults, whereas supratentorially located low-grade gliomas are the most common in children (4). In addition, meningiomas (30%) are the most common benign tumors, and GBMs (20%) are the most frequent malignant tumors among primary CNS tumors. Reportedly, gliomas account for 40% of all CNS tumors and 78% of malignant CNS tumors (5,26).

Pilocytic astrocytomas constitute 2%–6% of CNS tumors, with the cerebellum, optic region, brain stem, and infundibulum as the residential areas. Histopathologically, the biphasic pattern of the tumor creates compact and loose areas (25) where mitosis is minimal or absent. In addition, the Ki-67 proliferation index is <1% in pilocytic astrocytomas (35). Diffuse astrocytomas account for 10% of all gliomas and are more common in males aged 30–40 years. They are often supratentorial, frontal, and temporal. Histopathological examination reveals well-differentiated neoplastic fibrils and gemistocytic astrocytes in a microcystic tumor matrix. It exhibits more cellular and nuclear atypia than the normal brain tissue. Furthermore, mitotic activity is usually absent or minimal (25,33).

Anaplastic astrocytomas are grade III tumors, as classified by the WHO, located in the cerebral hemispheres similar to diffuse astrocytomas. Histopathologically, there is diffuse increased cellularity, nuclear atypia, and marked mitotic

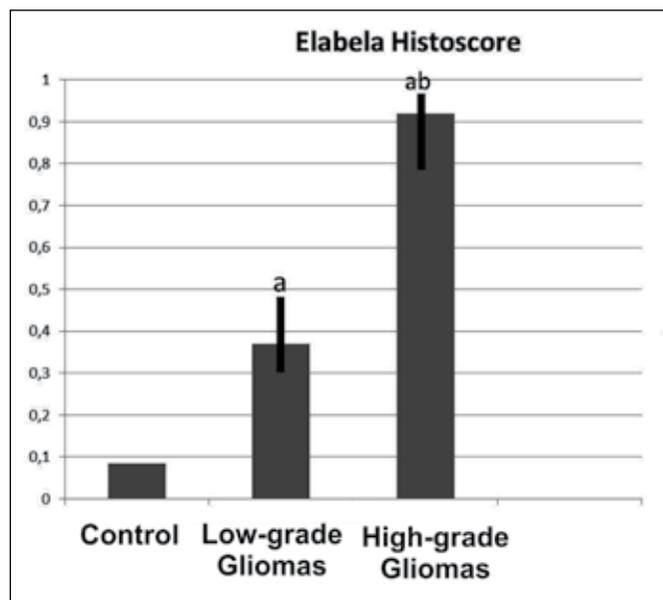


**Figure 1:** **A)** Hematoxylin-eosin (H&E X400) and **B)** immunohistochemical staining of control brain tissue (X400), **C)** post-contrast image of diffuse astrocytoma case, **D)** H&E of diffuse astrocytoma (X400), **E)** immunohistochemical staining of diffuse astrocytoma (X400), **F)** post-contrast image of anaplastic astrocytoma case, **G)** H&E of anaplastic astrocytoma (X100), **H)** immunohistochemical staining of anaplastic astrocytoma (X400), **I)** post-contrast image of glioblastoma multiforme case, **J)** H&E of glioblastoma multiforme (X100) and **K)** immunohistochemical staining of glioblastoma multiforme (X400) (black arrows: ELABELA immunoreactivity).

**Table I:** Elabela Immunoreactivity of Tissues with Statistical Analysis

Tissues	Histoscore		p
	Benign Cell		
	Neuronal cells	Glial cells	
Control	0.09±0.15	0.08±0.13	-
Low-grade gliomas	-	-	0.37±0.11 <sup>a</sup>
High- grade gliomas	-	-	0.92±0.18 <sup>ab</sup>

*a: compared with control, b: compared with low-grade, values are given as mean ± standard deviation.*



**Figure 2:** ELABELA histoscores of control brain tissue and glioma tissues.

activity. Abnormal mitotic activity is present; however, no microvascular proliferation and necrosis are reported. Anaplastic astrocytomas may progress to GBMs (25,33). GBMs are WHO grade IV tumors and the most prevalent brain tumors and malignant gliomas (5), with central necrosis in 80% of the tumor mass. GBMs are poorly differentiated anaplastic cellular gliomas that comprise pleomorphic astrocytic tumor cells with marked mitotic activity and nuclear atypia. While both microvascular proliferation and necrosis are diagnostic, tumor necrosis is an essential feature of GBMs that indicates aggressive behavior (25,33).

In this study, we assessed 11 patients with a histologically verified low-grade glioma (grade I, n=1; grade II, n=10) and 22 with a histologically verified high-grade glioma (grade III, n=11; grade IV, n=11), based on the WHO classification (26). The histoscores of the control brain tissue, low-grade glioma tissue, and high-grade glioma tissue were 0.08, 0.37, and 0.92, respectively. We observed increased immunoreactivity in low-grade glioma tissues compared with that in control brain tissues. In addition, the increase of ELA immunoreactivity in

high-grade gliomas was higher than that in low-grade gliomas, which exhibits the importance of vascular proliferation and of ELA as its activator.

ELA is a peptide containing 54 amino acids, including a secretory signal with a mature form containing 32 amino acids, and its transcripts are found in human pluripotent stem cells, kidney, and prostate (7). Wang et al. investigated the functionality of ELA and its relationship with AR signaling in the mammalian system. They demonstrated that ELA is expressed in human embryonic stem cells, induced pluripotent stem cells, and adult kidney tissue. In addition, they supported that ELA is a natural hormone in the human system that functions during and after embryonic development (38). Both AR and apelin are expressed in some tissues such as those of the heart, lungs, vascular endothelium, kidneys, and brain. Studies have demonstrated that the apelinergic system has a broad range of biological functions such as homeostasis of the cardiovascular system and fluid metabolism (6,10). Apelin is an endogenous ligand of the GPCR, with some isoforms such as apelin-36, apelin-17, and apelin-13 (27,32). Apelin and AR are widely expressed in many types of cells and tissues such as neurons, glial cells, hypothalamic tissues, colonic tissues, and skeletal muscle (28). Reportedly, apelin stimulates the proliferation and migration of retinal endothelial cells (18). A study investigating the role of apelin in diabetic retinopathy demonstrated a high vitreous concentration of apelin in the eyes of patients with proliferative diabetic retinopathy, as shown by immunofluorescence staining of apelin in the endothelial cells of the fibrovascular membranes (36).

Hao et al. demonstrated that high AR expression was significantly associated with higher rates of tumor invasion and local lymph node and distant metastasis in gastric cancer (11). Currently, clinically approved angiogenesis inhibitors primarily target the VEGF signaling pathway, thereby inhibiting tumor vascularization (3,39). The overexpression of apelin and its receptor is further detected in highly proliferating microvessels in primary GBM tissues compared with that in normal brain tissues (16). A recent and important study regarding the apelinergic system and glioma growth reported by Harford-Wright et al. concluded that apelin is a critical factor in glioma growth (12). In addition, the authors concluded that inhibition of the apelinergic system is associated with a reduction in tumor volume, vascularization, and proliferation and an increase in apoptosis. This study also supports the results and proposed

pathophysiological mechanism of our study. Although there are some studies that have reported the association between the interaction of ELA and the apelinergic system, to the best of our knowledge, our current study is the first to report the aggressivity of gliomas and the role of ELA in this behavior.

## ■ CONCLUSION

ELA has an angiogenetic role in the progression of glial tumors. ELA, which is an endogenous ligand of the AR, activates the apelinergic system and causes the progression of glial tumors. Further studies with a large number of patients are necessary to investigate the angiogenetic role of ELA and apelin in glial tumors.

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