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Expression of E-cadherin gene (CDH1) in Glioblastoma Multiforme

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Abstract

Astrocytoma is one of the most common forms of primary brain tumor and glioblastoma is mostly occurred astrocytoma. Due to their invasiveness in the brain parenchyma, they are less amenable to surgical removal and current chemotherapy regimens, with a high mortality rate. Cell adhesion molecule (CAM) E-cadherin (CDH1) downregulation has been implicated in many tumors of higher grade in different systems and organs featuring invasion and metastasis. Cadherins are calcium-binding proteins that promote cell adhesion and tissue homeostasis. The cadherin superfamily includes classical cadherin, protocadherins, desmoglein, desmocollins and others. Classical cadherins include Epithelial (E-cadherin), Neuronal (N-cadherin), placental (P-cadherin) and retinal (R-cadherin), which are the first members of the superfamily to be identified (CDH1, CDH2, CDH3, and CDH4). This study aimed to investigate the level of E-cadherin gene expression in high-grade astrocytoma, that is, glioblastoma multiforme. Therefore, the aim of the study was to find out the level of E-cadherin gene expression in glioblastoma multiforme. In this cross-sectional study, 12 (Twelve) formalin-fixed paraffin-embedded tissues were taken as cases. 3 (Three) non tumorous brain tissue and 1(one) fresh brain tissue were taken as control. Histological features were studied under a light microscope with H& E stain. Expression of the CDH1 gene was analyzed by real-time PCR using the comparative Ct method. The change in E-cadherin expression was measured by fold change in comparison with the control brain tissue. The data was tabulated and a statistical analysis was performed. All tumors showed downregulation of the CDH1 gene in comparison with non-tumorous control tissue. It could be concluded that marked downregulation of the E-cadherin gene occurs with tumors of higher grade due to hypermethylation of the gene promoter.

Keywords: E-cadherin gene, CDH1, Glioblastoma.

Introduction

The literature on human cancer cells and their altered behavior in tumor cells is an important step towards understanding the astrocytic brain tumor. Astrocytic brain tumors are the most common primary Central Nervous System (CNS) neoplasms and are classified according to their lineage of origin and behavior into four World Health Organization (WHO) grades.[1]

A common feature of all malignant tumors is the ability of cancer cells to invade neighboring and distant sites, leading to the formation of metastases and progression of tumor malignancy. This capacity arises as cells lose the ability to be adherent and gain an increased potential to invade, a process highly associated with the loss of expression of E-cadherin. Past studies have provided evidence that E-cadherin is a broad-acting tumor suppressor and is regarded as a major determinant of tumor progression and invasion in epithelial cancers.[2]

The control of cellular adhesion and motility is a crucial mechanism responsible for tumor initiation and progression. E-cadherin is considered one of the important molecules in cell-cell adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in regions of cell-cell contact known as adherens junctions. The human epithelial (E)-cadherin gene CDH1 maps to chromosome 16q22.1. Besides its role in normal cells, this highly conserved gene contributes to malignant cell transformation, especially in tumor development and progression. The suppression of

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E-cadherin expression is regarded as one of the main molecular events responsible for dysfunction in cell-cell adhesion.[3] Analysis of a variety of epithelial carcinoma cell lines suggested that E-cadherin expression could be correlated inversely with invasive potential.[4]

Gliomas are therapeutically challenging because their infiltrative (diffuse) growth pattern essentially prevents surgical cure, and the majority of these gliomas are resistant to standard chemotherapeutic and radiotherapeutic approaches. Nonetheless, some tumors are therapeutically sensitive and rare cures are affected. Paradoxically, these rare successes draw attention to the essential limitation of current glioma classification schemes. Responding tumors may be histologically indistinguishable from non-responding ones[5], as well as the degree of invasiveness of astrocytoma does not necessarily correlate with the grade of malignancy.[6]

Several important genetic alterations in gliomas have been known for some time; new technologies have allowed much deeper genetic and epigenetic analyses of larger numbers of glioma samples, leading to many novel discoveries in the past few years.[7] The acquisition of the invasive phenotype of gliomas probably involves alteration in cell-to-cell adhesion, an increase in cell motility, and an increase in secretion of proteolytic enzymes. A loss of E-cadherin correlates with an enhanced invasiveness of epithelial cells; conversely, transfection of epithelial cells or fibroblasts with E-cadherin suppresses their invasion in vitro.[8] Glioblastoma multiforme (GBM) is one of the most frequently diagnosed primary brain cancers in adults, where E-cadherin is found to be downregulated.[9] Primitive neuroectodermal tumors, i.e., medulloblastoma, further reveal the hypermethylation and the downregulation of the CDH1 gene.[10]

Tumors from different systems of the human body have been reported to show downregulation of E-cadherin. This includes gastric carcinoma[2], colorectal adenoma and carcinoma[11], pancreatic carcinoma[12], prostatic carcinoma and urinary bladder carcinoma[13-15], breast carcinoma[16], and non-small cell carcinoma of the lung.[17,18] The knowledge of molecular abnormalities in gastric adenocarcinoma gives an insight into the evolution of the tumor.[19]

However, there are several reports that show no clear downregulation of E-cadherin expression. This discrepancy might relate to the fact that E-cadherin could be rendered nonfunctional by various mutations in either the cadherin or the catenins[4], which implies that immunohistochemistry might under-estimate the extent of cadherin impairment in malignant carcinomas.

Considering the abovementioned facts, this study was intended to find out the extent of expression of the E-cadherin molecule at the protein level in high-grade astrocytoma (glioblastoma multiforme).

Materials and methods

This was a cross-sectional observational study carried out at the Department of Pathology, Mymensingh Medical College (MMC), Mymensingh, and Real Time PCR experiment was carried out in the Department of Microbiology and Immunology Laboratory, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. The sample size was 12. The studied materials were both paraffin-fixed brain tissue and archival paraffin blocks. Five tissues were taken after receiving informed written consent from the patients/ patient's attendant, who underwent craniotomy with total or partial resection of tumor operation in the Department of Neurosurgery, MMCH, during March 2017 to December 2018, and diagnosed as glioblastoma multiforme in the Department of Pathology, MMC. Seven paraffin blocks of glioblastoma multiforme were collected from the Department of Pathology, National Institute of Neurosciences (NINS), with proper clearance. For reference value and as an external calibrator, 3 non-tumorous brain tissue samples were also obtained from the Department of Neurosurgery, MMCH, by taking informed written consent, who underwent surgery for vascular/traumatic cause, and 1 fresh brain tissue sample was collected from an unidentified postmortem case with permission of the Department of Forensic Medicine, MMC.

Ethical Clearance

Ethical clearance for all the collected samples was taken from the Institutional Review Board of Mymensingh Medical College (MMC) and the Ethical Review Board of National Institute of Neurosciences (NINS)

The five resected specimens were received in 10% formalin and kept for overnight fixation. The next day, the specimens were examined during grossing and tissues were embedded accordingly. Tissue processing was performed manually following standard protocol for paraffin embedding. For microscopic examination, sections were stained with the hematoxylin and eosin (H/E) staining method by manual staining following standard protocol. The collected seven paraffin blocks were sectioned, stained and studied in the department of Pathology at MMC. Slides of all cases were

examined, classified and graded according to the WHO 2016 CNS tumor classification.[20]

Microscopic evaluation:

We aimed to investigate the histologic parameters of prognostic value: nuclear atypia, mitotic activity, microvascular proliferation (MVP), necrosis and cellularity. Among these criteria, mitotic activity and necrosis were defined by their presence or absence.[21]

0 = Absent
1 = Present

Nuclear atypia, Microvascular proliferation (MVP) and cellularity were defined according to their degree of expression.[21]

Nuclear atypia (Hyperchromasia and/or obvious variation in size and shape).[22]

1 = Mild
2 = Moderate
3 = Marked

Microvascular proliferation (Vascular lamina surrounded by haphazardly arranged or piled up endothelial cells often showing atypia).[23]

1 = Present only very focally or in a few low-power fields
2 = Present in many low-power fields
3 = Present in most low-power fields

Cellularity (Mean cell count from three high-power fields).[24]

1 = < 200 cell/ HPF
2 = 200-400 cell/ HPF
3 => 400 cell/ HPF

Procedure of Real Time polymerase chain reaction (RT-PCR) study:

E-cadherin expression was examined by the following basic steps:

1. Extraction of mRNA from formalin-fixed brain tumor and control tissue.
2. Construction of cDNA from the extracted mRNA
3. The constructed cDNA is amplified by real-time PCR
4. Relative expression levels of target sequences were determined by the comparative Ct method.[25]

Results

We included 12 high-grade astrocytomas in our study. Among them, 9 (75%) were male patients and 3 (25%) were female patients (Figure 01). Mean age was 53.42 with an age range of 34-70 years. The Real Time PCR technique was used to assess the gene expression level using 4 non-tumorous brain tissues as reference. We found that 100% of our sample expressed the CDH1 gene and in all of the cases, their expression was reduced than the reference (Figure 02). This downregulation was expressed as the number of times downregulation than control and was divided into three categories, i.e., 0-20 times, 21-100 times, and 101+ times downregulation. In case of prognostically important histologic factors, vascular proliferation, mitotic activity, necrosis and cellularity were assessed. But no histologic factor was found to be significant with our E-cadherin down-expression category (Tables 01, 02, 03, 04).

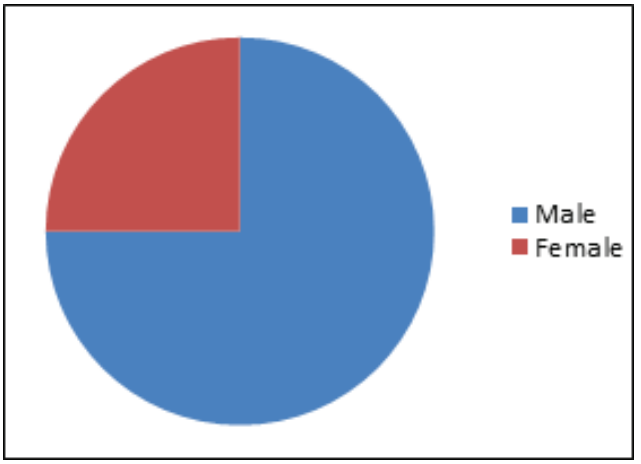

Figure 01: Distribution of patients according to sex

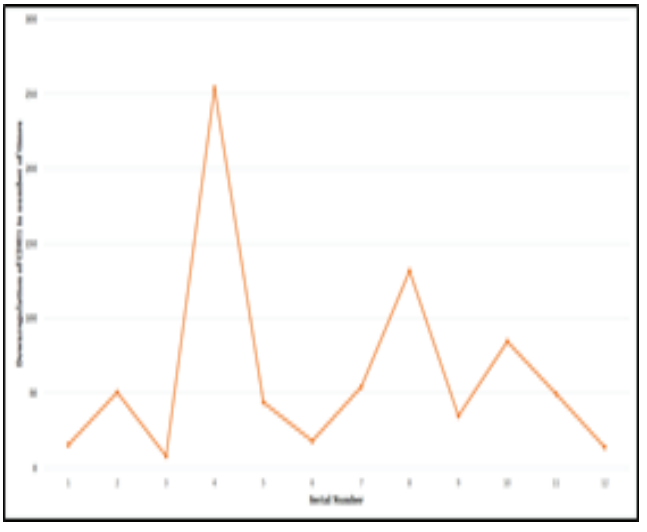

Figure 02: Expression status of E-cadherin in GBM case

Table 01: Distribution between vascular proliferation and downregulation of E-cadherin

Vascular proliferation	Downregulation of E-cadherin			Total	P-value
	0-20 times	21-100 times	101+ times		
Mild	0 (0%)	1 (100%)	0 (0.0%)	1 (100.0%)	0.503
Moderate	2 (33.3%)	2 (33.3%)	2 (33.3%)	6 (100.0%)	
Marked	2 (40%)	3 (60%)	0 (0.0%)	5 (100.0%)	
Total	4 (33.3%)	6 (50%)	2 (16.7%)	12 (100.0%)	

Table 02: Distribution between Mitotic activity and downregulation of E-cadherin

Mitotic activity	Downregulation of E-cadherin			Total	P-value
	0-20 times	21-100 times	101+ times		
Present	4 (33.3%)	6 (50%)	2 (16.7%)	12 (100%)	NA
Absent	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Total	4 (33.3%)	6 (50%)	2 (16.7%)	12 (100%)	

Table 03: Distribution between Necrosis and downregulation of E-cadherin

Necrosis	Downregulation of E-cadherin			Total	P-value
	0-20 times	21-100 times	101+ times		
Present	4 (33.3%)	6 (50%)	2 (16.7%)	12 (100%)	NA
Absent	0 (0%)	0 (0%)	0 (0%)	0 (100%)	
Total	4 (33.3%)	6 (50%)	2 (16.7%)	12 (100%)	

Table 04: Distribution between Cellularity and downregulation of E-cadherin

Cellularity	Downregulation of E-cadherin			Total	P-value
	0-20 times	21-100 times	101+ times		
Mild	0 (0%)	0 (0%)	0 (0%)	0 (100%)	0.83
Moderate	1 (25%)	2 (50%)	1 (25%)	4 (100.0%)	
Marked	3 (37.5%)	4 (50%)	1 (12.5%)	8 (100.0%)	
Total	4 (33.3%)	6 (50%)	2 (16.7%)	12 (100.0%)	

Discussion

E-Cadherin is one of the most important and most investigated cell adhesion molecules (CAMs). CAMs are under the scrutiny of researchers because it is one major suppressor for the malignant cell to become locally invasive and metastasize to distant sites. Malignancies that metastasize to distant sites or diffusely invade the surrounding parenchyma are the main cause of mortality. So, the search for CAMs statement in various human malignancies is ongoing with the hope that some means would be discovered to sustain these molecules intact so that the cancer cells remain localized.

In the present study, the samples range from 34-70 years of age group and the mean age was 53.4. A group of authors found in their study that the mean age was 56 years.[26] Another researcher found an age range from 13-77 years.[27] All these studies show little variation from the present study may be due to different sample sizes, variation in tumor type and different regions.

In the current study, among the 12 cases majority, 9 (75%), were male patients and 3 (25%) were female patients. Another study shows that among 92 cases, 53

(57.61%) were male patients and 39 (42.39%) were female patients.[28]

The current study aimed to observe the E-cadherin gene expression status in high-grade astrocytoma (GBM) to find any relation between the WHO-prescribed histologic parameters of prognostic importance with this gene expression. It was observed that the expression of the E-cadherin gene in all the 12 cases is always downregulated than the non-tumorous control tissue used as reference.

To understand the regulation of E-cadherin expression, the main histologic features of prognostic importance for astrocytoma diagnosis, e.g., cellularity, atypia, microvascular proliferation and mitosis, were analyzed and categorized. Interobserver variability and lack of reproducibility due to the subjective histologic criteria often create a dilemma/confusion.[29,30] But no histologic feature is found to be significantly associated with downregulation of E-cadherin.

A number of studies claimed that there was no relation between E-cadherin expression and astrocytoma of various grades, or that E-cadherin is not at all associated with human brain tissue.[31-33] The contrast between the current study findings and the above-mentioned studies may be due to the method of E-cadherin analysis. Most of their works were based on immunohistochemistry, whereas the current study used RT-PCR. Immunohistochemistry has its own limitations in interpreting positive staining by a semi-quantitative method, which is also subjective and tissue processing, as well as staining quality, could influence the result.[34,35] A genetically mutated nonfunctional but antigenically active molecule may be falsely positive; on the contrary, low-level expression might be missed by immunohistochemistry.[36] Absence of positive immunostaining in the abovementioned studies may be due to these reasons, whereas RT-PCR is highly sensitive and specific in detecting very low levels of gene expression.[37,38]

Limitations of the study

The study had a few limitations. First, the sample size was small, which could have resulted in inflated effect sizes and confidence intervals. It is to be noted that the collection of the samples took over 1 year and the limitation of logistics contributed to it. Second, as the study used cross-sectional data, it restricts the results from inferring causality. Third, the molecular testing was performed in a diagnostic PCR lab. Thus, there could be a possible unwanted lack of sophistication and optimization of environmental support. Due to a lack of infrastructural setup and financial constrain, a few steps in the laboratory had to be carried out manually, such as manual routine tissue processing and deparaffinization for mRNA extraction. Finally, E-cadherin expression is low, heterogeneous, and biologically insignificant in GBM.

Conclusion

It is found in this study that E-cadherin gene expression is down-regulated markedly in high-grade astrocytoma. It can be inferred that the lack of E-cadherin gene expression is the hallmark of the malignant potential of astrocytomas and may correlate with progression and dissemination in the brain parenchyma. E-cadherin is linked with several major signaling pathways, including Wnt/β-catenin, NF-κB, receptor tyrosine kinase (RTK) and GTPase signaling pathways. E-cadherin can regulate the cellular response generated by external signals that the cell receives. Thus, it can regulate migration, proliferation, apoptosis and cell differentiation. These warrant a further probing into the E-cadherin gene and its mode of action. Further investigation should be conducted to identify the method of blocking E-cadherin downregulation in tumors, which might be one of the important future approaches in gene therapy. Thus, targeting this molecule is found to be a logical path to prevent the metastasizing potential of almost any epithelial tumor. Furthermore, advancements in genomic technologies, including next-generation sequencing, hold promise for uncovering novel therapeutic targets. By integrating these strategies, we may be able to develop more effective treatment, ultimately improving the survival rate and quality of life for GBM patients.

Conflict of interest

There was no conflict of interest among the authors. All authors read the final manuscript and approved it.

References

1. Pećina-Šlaus N, Kafka A. Wnt signaling and astrocytic brain tumors. 2015.
2. Carneiro P, Fernandes MS, Figueiredo J, Caldeira J, Carvalho J, Pinheiro H, Leite M, Melo S, Oliveira P, Simões-Correia J, Oliveira MJ. E-cadherin dysfunction in gastric cancer-Cellular consequences, clinical applications and open questions. FEBS Letters. 2012 Aug 31;586(18):2981-9.
3. Pećina-Šlaus N. Tumor suppressor gene E-cadherin and its role in normal and malignant cells. Cancer Cell International. 2003 Jan 1;23(3):1-7.

4. Ahmed et all 1997 Ahmad A, Hart IR. Mechanisms of metastasis. Critical reviews in oncology/hematology. 1997 Dec 31;26(3):163-73.
5. Louis DN, Holland EC, Cairncross JG. Glioma classification: a molecular reappraisal. The American journal of pathology. 2001 Sep;159(3):779.
6. Giese A, Bjerkvig R, Berens ME, Westphal M. Cost of migration: invasion of malignant gliomas and implications for treatment. Journal of Clinical Oncology. 2003 Apr 15;21(8):1624-36.
7. Cohen AL, Holmen SL, Colman H. IDH1 and IDH2 mutations in gliomas. Current neurology and neuroscience reports. 2013 May 1;13(5):345.
8. Barami K, Lewis-Tuffin L, Anastasiadis PZ. The role of cadherins and catenins in glioma genesis. Neurosurgical focus. 2006 Oct 1;21(4):1-4.
9. Schiffer, D., Mellai, M., Annovazzi, L., Caldera, V., Piazzi, A., Denysenko, T. and Melcarne, A., 2014. Stem cell niches in glioblastoma: a neuropathological view. BioMed Research International, 2014.
10. Frühwald MC, O'Dorisio MS, Dai Z, Tanner SM, Balster DA, Gao X, Wright FA, Plass C. Aberrant promoter methylation of previously unidentified target genes is a common abnormality in medulloblastomas–implications for tumor biology and potential clinical utility. Oncogene. 2001 Aug;20(36):5033-42.
11. Kroepil F, Fluegen G, Totikov Z, Baldus SE, Vay C, Schauer M, Topp SA, am Esch JS, Knoefel WT, Stoecklein NH. Down-regulation of CDH1 is associated with expression of SNAI1 in colorectal adenomas. PLOS One. 2012 Sep 28;7(9): e46665.
12. Zhao L, Wang YX, Xi M, Liu SL, Zhang P, Luo LL, Liu MZ. Association between E-cadherin (CDH1) polymorphisms and pancreatic cancer risk in the Han Chinese population. International journal of clinical and experimental pathology. 2015;8(5):5753.
13. Umbas R, Schalken JA, Aalders TW, Carter BS, Karthaus HF, Schaafsma HK, Debruyne FM, Isaacs WB. Expression of the cellular adhesion molecule E-cadherin is reduced or absent in high-grade prostate cancer. Cancer research. 1992 Sep 15;52(18):5104-9.
14. Bringuier PP, Umbas R, Schaafsma HE, Karthaus HF, Debruyne FM, Schalken JA. Decreased E-cadherin immunoreactivity correlates with poor survival in patients with bladder tumors. Cancer research. 1993 Jul 15;53(14):3241-5.
15. Gravdal K, Halvorsen OJ, Haukaas SA, Akslen LA. A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progression of prostate cancer. Clinical cancer research. 2007 Dec 1;13(23):7003-11.
16. Brzozowska A, Sodolski T, Duma D, Mazurkiewicz T, Mazurkiewicz M. Evaluation of prognostic parameters of E-cadherin status in breast cancer treatment. Annals of Agricultural and Environmental Medicine. 2012;19(3).
17. Zheng SY, Hou JY, Zhao J, Jiang D, Ge JF, Chen S. Clinical outcomes of downregulation of E-cadherin gene expression in non-small cell lung cancer. Asian Pac J Cancer Prev. 2012 Jan 1;13(4):1557-61.
18. Yang YL, Chen MW, Xian L. Prognostic and clinicopathological significance of downregulated E-cadherin expression in patients with non-small cell lung cancer (NSCLC): a meta-analysis. PLOS One. 2014 Jun 30;9(6): e99763.
19. Dewan K, Madan R, Sengupta P. Correlation of Lauren's histological type and expression of E-cadherin and HER-2/neu in gastric adenocarcinoma. Internet Journal of Pathology and Laboratory Medicine. 2016 Dec 21;2(1).
20. Louis DN, Perry A, Reifenberger G, Von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW. The 2016 World Health Organization classification of tumors of the central nervous system: a summary. Acta neuropathologica. 2016 Jun 1;131(6):803-20.
21. Coons SW, Johnson PC, Scheithauer BW, Yates AJ, Pearl DK. Improving diagnostic accuracy and interobserver concordance in the classification and grading of primary gliomas. Cancer: Interdisciplinary International Journal of the American Cancer Society. 1997 Apr 1;79(7):1381-93.
22. Daumas-Duport C, Scheithauer B, O'Fallon J, Kelly P. Grading of astrocytomas: a simple and reproducible method. Cancer. 1988 Nov 15;62(10):2152-65.
23. Burger PC, Green SB. Patient age, histologic features, and length of survival in patients with glioblastoma multiforme. Cancer. 1987 May 1;59(9):1617-25.
24. Tove LL, Hanssøn HA, Stein S, Sverre H. Prognostic value of histological features in diffuse astrocytomas WHO grade II. International journal of clinical and experimental pathology. 2012;5(2):152.

25. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 2001 Dec 1;25(4):402-8.
26. Burger PC, Vogel FS, Green SB, Strike TA. Glioblastoma multiforme and anaplastic astrocytoma pathologic criteria and prognostic implications. Cancer. 1985 Sep 1;56(5):1106-11.
27. Nikuševa-Martić T, Beroš V, Pećina-Šlaus N, Pećina HI, Bulić-Jakuš F. Genetic changes of CDH1, APC, and CTNNB1 found in human brain tumors. Pathology-Research and Practice. 2007 Nov 12;203(11):779-87.
28. Bar JK, Zub L, Lis-Nawara A, Noga L, Jelen M, Paradowski B. Expression and interactions between cell adhesion molecules CD44v6 and E-cadherin in human gliomas. Adv Clin Exp Med. 2014 Sep 1;23(5):827-34.
29. Kros JM. Grading of gliomas: the road from eminence to evidence. Journal of Neuropathology & Experimental Neurology. 2011 Feb 1;70(2): 101-9.
30. Prayson RA, Agamanolis DP, Cohen ML, Estes ML, Kleinschmidt-DeMasters BK, Abdul-Karim F, McClure SP, Sebek BA, Vinay R. Interobserver reproducibility among neuropathologists and surgical pathologists in fibrillary astrocytoma grading. Journal of the neurological sciences. 2000 Apr 1;175(1):33-9.
31. Asano K, Kubo O, Tajika Y, Huang MC, Takakura K, Ebina K, Suzuki S. Expression and role of cadherins in astrocytic tumors. Brain tumor pathology. 1997 Mar 1;14(1):27-33.
32. Asano K, Kubo O, Tajika Y, Takakura K, Suzuki S. Expression of cadherin and CSF dissemination in malignant astrocytic tumors. Neurosurgical review. 2000 Apr 1;23(1):39-44.
33. Utsuki S, Sato Y, Oka H, Tsuchiya B, Suzuki S, Fujii K. Relationship between the expression of E-, N-cadherins and beta-catenin and tumor grade in astrocytomas. Journal of Neuro-Oncology. 2002 May 1;57(3):187-92.
34. Gown AM. Diagnostic immunohistochemistry: what can go wrong and how to prevent it. Archives of pathology & laboratory medicine. 2016 Sep;140(9):893-8.
35. Kim SW, Roh J, Park CS. Immunohistochemistry for pathologists: protocols, pitfalls, and tips. Journal of pathology and translational medicine. 2016 Nov;50(6):411.
36. Ha NH, Faraji F, Hunter KW. Mechanisms of metastasis. In Cancer Targeted Drug Delivery 2013 (pp. 435-458). Springer, New York, NY.
37. Sinn HP, Schneeweiss A, Keller M, Schlombs K, Laible M, Seitz J, Lakis S, Veltrup E, Altevogt P, Eidt S, Wirtz RM. Comparison of immunohistochemistry with PCR for assessment of ER, PR, and Ki-67 and prediction of pathological complete response in breast cancer. BMC Cancer. 2017 Dec;17(1):1-0.
38. Wallander, M.L., Geiersbach, K.B., Tripp, S.R. and Layfield, L.J., 2012. Comparison of Reverse Transcription-Polymerase Chain Reaction, Immunohistochemistry, and Fluorescence In Situ Hybridization Methodologies for Detection of Echinoderm Microtubule-Associated Protein like 4–Anaplastic Lymphoma Kinase Fusion–Positive Non–Small Cell Lung Carcinoma: Implications for Optimal Clinical Testing. Archives of pathology & laboratory medicine, 136(7), pp.796-803.