



HIF 1 α – a promising target for the treatment of meningiomas

Shristi Butta¹, Manoj Kumar Gupta²

1) Department of Pathology, NRS Medical College, Kolkata, West Bengal, India

2) Department of Tropical Medicine, Medical College, Kolkata, West Bengal, India

Abstract

Background. Meningiomas are the most frequent tumors of the brain and spinal cord with a potency to recur in around one third of the cases and invade surrounding tissue. Hypoxia driven factors like HIFs (Hypoxia inducible factors) are implicated in tumor cell growth and proliferation.

Aim. This study aims at determining the association of HIF 1 α with different histopathological grades and types of meningiomas.

Methods. This prospective study was conducted on 35 patients. The patients presented with headache (65.71%), seizures (22.86%) and neurological deficits (11.43%). They underwent surgical excision and surgical tissue samples of these patients were histopathologically processed and microscopically graded and typed. Immunohistochemistry was performed using anti-HIF 1 α monoclonal antibody. The nuclear expression of HIF 1 α was graded as <10%: negative, 11-50%: mild to moderate positive, >50%: strong positive.

Results. Of the 35 cases so examined 20% were recurrent; 74.29% were WHO grade I with meningothelial type (22.86%), being the commonest; 57.14 % revealed mild to moderate positivity for HIF 1 α , while strong positivity was noted in 28.57%. Significant association was found between WHO grade and HIF 1 α ($p=0.0015$) and between histopathological types and HIF 1 α ($p=0.0433$). Furthermore, HIF 1 α was also significantly associated with the recurrent cases ($p=0.0172$).

Conclusion. HIF 1 α appears to be a marker and a promising target for effective therapeutics in meningiomas.

Keywords: HIF 1 α , meningioma, promising, target

Introduction

Hypoxia is frequently associated with cancer where neoplastic tumor cells proliferate beyond their vascular supply. These tumor cells adapt to the hypoxic environment by producing hypoxia inducible factors like HIF 1 α [1]. The hypoxia-inducible factors (HIFs) subsequently stimulate VEGF (Vascular Endothelial Growth Factor) that in turn allows for the proliferation of new blood vessels. Neovascularization is thus an extreme necessity for the growth and proliferation of any tumor. New blood vessels supply oxygen to the tumor cells, in the absence of which tumor cells become necrotic and

undergo apoptosis [2].

HIF was discovered in 1995 by Semenza and Wang [3]. It is considered as a “master regulator of oxygen homeostasis” [4-7]. The Nobel Prize in Physiology/Medicine in 2019 was awarded to the trio William Kaelin Jr., Peter J. Ratcliffe and Gregg L. Semenza for their work on HIF.

HIF is the prime factor implicated in controlling the hypoxia driven pathways and is primarily involved in tumor progression and therapeutic resistance. HIF can thus become a promising target for novel cancer therapeutics [8].

Meningiomas are the most common brain tumors. These tumors may either be Grade I (benign), grade II,

DOI: 10.15386/mpr-2059

Manuscript received: 01.02.2021

Received in revised form: 03.05.2022

Accepted: 29.12.2022

Address for correspondence:

Manoj Kumar Gupta
mkgupta.pintu@gmail.com

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License <https://creativecommons.org/licenses/by-nc-nd/4.0/>

or grade III (benign), with several subtypes. Some of these entities possess a potential to recur and/or metastasize. The association of VEGF with growth and proliferation of meningioma is well known. However, the role of HIF in meningioma has been studied less frequently.

In this magic we aim to ascertain the association of HIF with the WHO (World Health Organization) histopathological grades and types of recurrent and non-recurrent meningiomas.

Methods

This prospective study was conducted in the department of Pathology of a tertiary care hospital in West Bengal, India, over a period of 12 months. The study included 35 patients who presented with headache, seizures or neurological deficit. CT scans (computerized tomography) revealed an intracranial space occupying lesion. Surgical excision was carried out at the department of neurosurgery. The surgical biopsies were sent to our department for histopathological diagnosis. FFPE (Formalin fixed paraffin embedded) tissue samples were histopathologically processed and microscopically graded and typed according to the WHO classification. Immunohistochemistry was performed using Anti-HIF 1 alpha monoclonal antibody. Kidney tissue was used as the positive control. Nuclear expression of HIF 1 alpha was considered as a positive expression for hypoxia.

Sections were deparaffined using xylene and rehydrated in graded alcohol. Heat induced epitope retrieval (HIER) was carried out in a pressure cooker using Tris EDTA buffer (pH: 9.0). To avoid non-specific background staining, endogenous peroxidase activity was blocked by subjecting the sections to 3% hydrogen peroxide in methanol for 20 minutes. The sections were then washed with 0.1M phosphate buffered saline (PBS) to reduce the surface tension and also to prevent non-specific binding. Further, the sections were incubated overnight at 4 degree centigrade with the primary antibody (mouse monoclonal Anti -HIF 1 a antibody) to allow for optimal binding of the antibody to the antigenic epitopes. The sections were further rinsed in PBS to allow removal of excess primary antibody. Biotinylated secondary antibody diluted with antibody diluent was further added to the sections and incubated at 37° C for 30 minutes. This was followed by washing in PBS. Subsequently DAB chromogen was added and incubated for 10 minutes in a dark room. Sections were then counterstained with haematoxylin. The HIF 1 a nuclear expression was graded according to Reszec et al. [13] ≤10% nuclear positivity was considered negative (-), 11%–50% as mild to moderate positivity (+) and ≥51% as strong positivity (++)

Statistical analysis

Statistical analysis was performed using Epi info TM version 7.2.4.0. Epi info is the software of CDC (Centers for disease control and prevention). Probability

value or the p value <0.05 was considered as statistically significant.

Results

Among the 35 cases examined, 20 % of the cases were recurrent, while 80% of the cases were non-recurrent. The most common age group was 40-60 years with 42.86% of the cases belonging to this age group. Females (74.29%) had a higher predilection compared to males (25.71%). Cerebral convexity and parasagittal regions were the most common sites (31.43% each). Headache was the most common symptom accounting for 65.71% followed by seizures (22.86%) and neurological deficit (11.43%). However, no significant association was found between WHO grade and symptom (p=0.45). 74.29% of the cases belonged to WHO grade I, while 11.43% belonged to WHO grade II and 14.29% belonged to WHO grade III. Meningothelial meningiomas (22.86%) were the most common histological type, followed by transitional meningioma (20%); other histological types included fibroblastic, microcystic, angiomatous, psammomatous, clear cell, atypical, papillary and anaplastic (Figure 1). On immunohistochemistry with HIF 1a, 57.14% of the cases showed positivity in 11-50% of the cells, 28.57% showed positivity in >50% of the cells and 14.29% were negative for HIF 1a (Figure 2, Table I). Significant statistical correlation was found between WHO grade and HIF 1a immunoexpression (p = 0.0015) (Table II). All cases of WHO grade III meningiomas showed strong positivity (++) in >50% of the cells, while 50% of grade II meningiomas and 11.54% of grade I meningiomas showed strong positivity. 69.23% of grade I meningiomas and 50% of grade II meningiomas showed mild to moderate positivity (+) in 11-50% of the cells. Statistically significant correlation was found between histopathological types of meningioma and HIF 1a immunoexpression (p=0.0433). 10 cases showed positivity in >50% of the cells. These included 3 cases of anaplastic, 2 atypical, 3 angiomatous and 2 cases of papillary meningiomas. Significant association was found between HIF 1a immunoexpression and recurrent cases (p=0.0172) (Table III). Out of the 7 recurrent cases, 5 cases (71.43%) showed HIF 1 alpha immune-positivity in >50% of the cells. Significant association was found between recurrent cases and histopathological types of meningiomas (p=0.0394); however, no statistically significant association was found between WHO grade and recurrence (p=0.43). Of these 7 cases, 3 were angiomatous, 1 clear cell, 2 papillary and 1 psammomatous meningioma.

Table I. Frequency of HIF 1 a immunoexpression.

HIF 1	Frequency	Percentage
<10% cells (negative)	5	14.29%
11-50% cells (positive +)	20	57.14%
>50% cells (positive ++)	10	28.57%
Total	35	100.00%

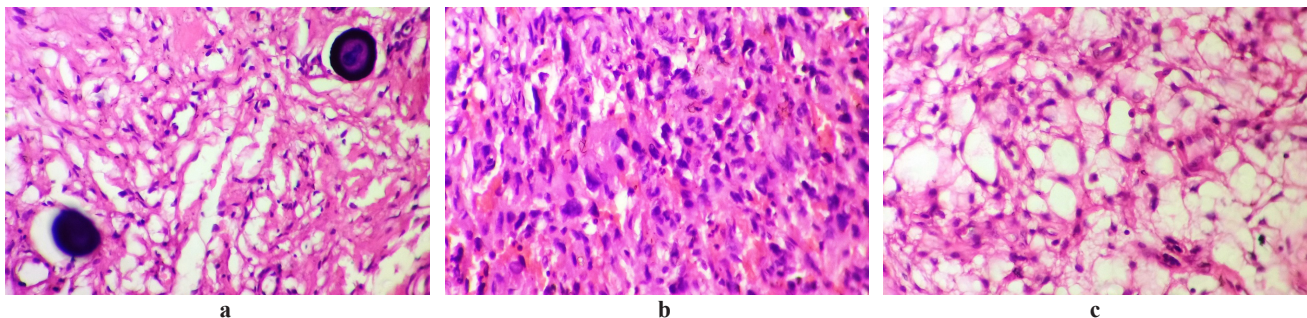


Figure 1. a: Fibroblastic meningioma (H&E, 400X); **b:** Atypical meningioma (H&E, 400X); **c:** Microcystic meningioma (H&E, 400X).

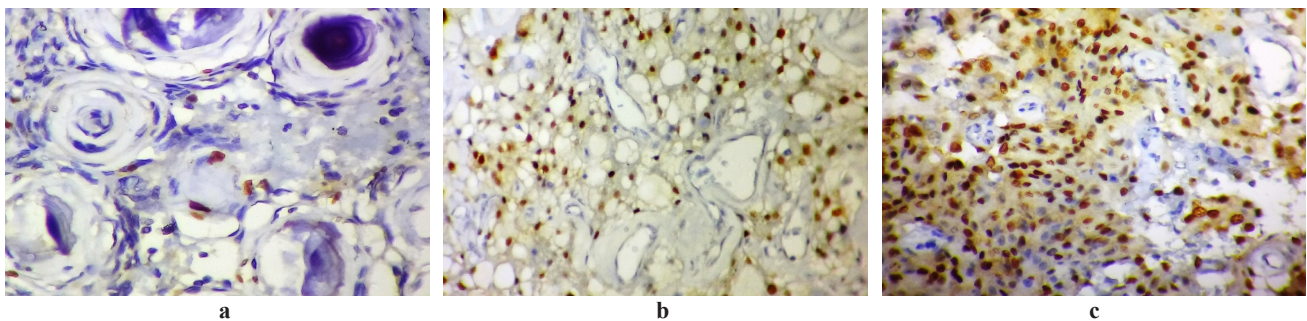


Figure 2. a: HIF 1 alpha negative with <10% cells showing nuclear positivity (HIF 1a, 400X); **b:** HIF 1 alpha mild to moderate positive with 11-50% cells showing nuclear positivity (HIF 1a, 400X); **c:** IF 1 alpha strongly positive with >50% cells showing nuclear positivity (HIF 1a, 400X).

Table II. Association between WHO grade of meningiomas and HIF 1 a immunoeexpression.

WHO grade	HIF 1			Total
	<10% cells (negative)	11-50% cells (positive +)	>50% cells (positive ++)	
I	5	18	3	26
Row%	19.23%	69.23%	11.54%	100.00%
Col%	100.00%	90.00%	30.00%	74.29%
II	0	2	2	4
Row%	0.00%	50.00%	50.00%	100.00%
Col%	0.00%	10.00%	20.00%	11.43%
III	0	0	5	5
Row%	0.00%	0.00%	100.00%	100.00%
Col%	0.00%	0.00%	50.00%	14.29%
TOTAL	5	20	10	35
Row%	14.29%	57.14%	28.57%	100.00%
Col%	100.00%	100.00%	100.00%	100.00%

Probability value= 0.0015

Table III. Association between Recurrent cases and HIF 1 a immunoeexpression.

Recurrence	HIF 1			Total
	<10% cells (negative)	11-50% cells (positive +)	>50% cells (positive ++)	
No	5	18	5	28
Row%	17.86%	64.29%	17.86%	100.00%
Col%	100.00%	90.00%	50.00%	80.00%
Yes	0	2	5	7
Row%	0.00%	28.57%	71.43%	100.00%
Col%	0.00%	10.00%	50.00%	20.00%
TOTAL	5	20	10	35
Row%	14.29%	57.14%	28.57%	100.00%
Col%	100.00%	100.00%	100.00%	100.00%

Probability value= 0.0172

Discussion

The HIF 1 has 2 subunits - the alpha and the beta. The beta subunit is integrally expressed while the alpha subunit shows oxygen dependency. Under conditions of normoxia the HIF 1 alpha is rapidly degraded by ubiquitylation and proteosomal degradation. This process is under the control of the enzyme prolyl hydroxylase which hydroxylates HIF 1 α that binds to VHL (Von Hippel Lindau) suppressor protein and undergoes subsequent degradation [9,10].

A number of studies in the past have tried to explore the role of hypoxia in cancer progression and metastasis. Here in this study we have tried to ascertain the role of hypoxia driven factor HIF 1 alpha in the various grades and histological types of meningiomas to establish whether this novel marker can be a promising target for cancer therapeutics in meningiomas.

Meningiomas are one of the very common tumors of the CNS. These are graded according to the WHO classification as grade I, II and III, respectively. While grade I tumors are considered benign with comparatively good prognosis, grade II tumors are considered atypical and grade III as malignant. Grading based on histopathology has shown limitations in determining the disease prognosis as not only grade II and III meningiomas but a group of grade I meningiomas have also shown recurrence following surgery and subsequent resistance to radiotherapy. This has therefore demanded for the need of immunohistochemical markers which can ascertain tumor prognosis [11,12].

Cancerous growth of tumor cells results in hypoxia. This hypoxia driven factor HIF 1 α subjects the tumor cells to elaborate factors responsible for neoangiogenesis, invasion and metastasis [13]. Neoangiogenesis allows for increased tumor cell survival and proliferation. This may thus be an important mechanism responsible for tumor resistance and subsequent recurrence.

HIF 1 α is translocated to the nucleus and shows nuclear expression under conditions of hypoxia while in conditions of normoxia it is degraded and shows some cytoplasmic positivity [14].

There are several target genes of HIF, like VEGF (Vascular endothelial growth factor), IGF 2 (Insulin like growth factor 2), TGF- α , PI3K, GLUT 1, GLUT 3, MMP 2, Cathepsin D, Urokinase plasminogen activator receptor, Fibronectin, vimentin etc [15]. VEGF is one of the most important targets of HIF as it is implicated in angiogenesis and subsequent cell survival and proliferation in meningiomas [16]. Further, IGF 2 and TGF- α are growth factors implicated in cell proliferation via HIF 1 α induction [17,18]. Furthermore, mutations in PTEN and stimulation of PI3K have also been found to be associated with increased tumor cell growth via increase in HIF 1 α . It has also been shown by Peter et al. [19] that higher grade meningiomas showed PTEN mutations while lower grade meningiomas did not. PTEN mutations have

been associated with increased HIF 1 α immunoreactivity.

We found that strong HIF 1 α immunoreexpression was associated with grade III and II meningiomas. However, a group of grade I meningiomas also revealed strong positivity. This probably represents the aggressive group with higher potency to recur and undergo therapeutic resistance. Further, we also found that most of the recurrent cases of meningiomas had a strong HIF 1 α immunoreexpression. Mei et al. [20] also found a similar observation while studying 160 patients of meningiomas with HIF 1 α positivity being 62.5% and 42.9% in high and low grade meningiomas, respectively. HIF 1 α immunoreexpression is associated with hypoxia and tumor resistance. Hypoxic environment leads to necrosis and stimulates HIF that activates angiogenesis and cell proliferation [21]. We found that besides high grade meningiomas (grade II and III), increased HIF 1 α positivity was found in a subset of angiomatous meningioma (WHO grade I). This observation suggested that VEGF, an angiogenic marker was involved in tumor recurrence and proliferation. Hence, it is clear that not only high grade meningiomas, but also tumors showing increased angiogenicity had a higher potency to recur. Preusser et al. [22] also had a similar observation whereby he illustrated that enhanced immunoreexpression of VEGF, or in other words HIF 1 α , was associated with poor prognosis. Further, Jensen et al. [23] also emphasized that meningiomas with enhanced immunoreexpression for HIF 1 α had a poor overall survival and an increased potency to convert from low grade to high grade.

As discussed previously, that HIF driven factors, such as matrix metalloproteinases and vimentin, are associated with tumor invasion and metastasis, it is clear that hypoxia plays a preliminary role in tumor resistance and disease recurrence [24]. Hence, blocking the activity of HIF 1 α might help in controlling tumor cell proliferation, tumor progression, tumor resistance and recurrence at its incipient stage. Sun et al. [25] demonstrated that intratumoral injection of antisense HIF 1 α plasmid led to decreased VEGF activity and reduced the microvascular density. The efficacy of antisense therapy was enhanced by addition of immunotherapy that resulted in NK and T cell mediated cytotoxicity.

Conclusions

HIF 1 α is an extremely potent transcription factor involved in tumor cell growth and proliferation. The role of HIF 1 α in meningiomas seems to be crucial as it is shown that its expression is linked to a higher chance of recurrence. This property is not only associated with grade II and III meningiomas, but a small group of grade I meningiomas also share the same attribute. HIF 1 α appears to be a good and promising marker and a valuable target for effective therapeutic strategy in meningiomas.

References

1. Hong SS, Lee H, Kim KW. HIF-1 α : a valid therapeutic target for tumor therapy. *Cancer Res Treat.* 2004;36:343-353.
2. Nishida N, Yano H, Nishida T, Kamura T, Kojiro M. Angiogenesis in cancer. *Vasc Health Risk Manag.* 2006;2:213-219.
3. Wang GL, Jiang BH, Rue EA, Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O₂ tension. *Proc Natl Acad Sci U S A.* 1995;92:5510-5514.
4. Semenza GL. Hypoxia-inducible factors in physiology and medicine. *Cell.* 2012;148:399-408.
5. Semenza GL. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer.* 2003;3:721-732.
6. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumor suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature.* 1999;399:271-275.
7. Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, et al. HIF1 α targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. *Science.* 2001;292:464-468.
8. Yu T, Tang B, Sun X. Development of Inhibitors Targeting Hypoxia-Inducible Factor 1 and 2 for Cancer Therapy. *Yonsei Med J.* 2017;58:489-496.
9. Koyasu S, Kobayashi M, Goto Y, Hiraoka M, Harada H. Regulatory mechanisms of hypoxia-inducible factor 1 activity: Two decades of knowledge. *Cancer Sci.* 2018;109:560-571.
10. Sakamoto M, Hirohashi S, Shimosato Y. Early stages of multistep hepatocarcinogenesis: adenomatous hyperplasia and early hepatocellular carcinoma. *Hum Pathol.* 1991;22:172-178.
11. Butta S, Gupta MK. Study of epidemiological aspects and hormone receptor status of meningiomas. *International Journal of Research in Medical Sciences.* 2020; 8:2482-2486.
12. Butta S, Gupta MK, Sandeep BV, Pal M, Saha SK. The role of peritumoral brain edema in ascertaining the high risk meningiomas. *International Journal of Research in Medical Sciences.* 2020;8:3938-3943.
13. Reszec J, Rutkowski R, Chyczewski L. The expression of hypoxia-inducible factor-1 in primary brain tumors. *Int J Neurosci.* 2013;123:657-662.
14. Kallio PJ, Okamoto K, O'Brien S, Carrero P, Makino Y, Tanaka H, et al. Signal transduction in hypoxic cells: inducible nuclear translocation and recruitment of the CBP/p300 coactivator by the hypoxia-inducible factor-1 α . *EMBO J.* 1998;17:6573-6586.
15. Brat DJ, Kaur B, Van Meir EG. Genetic modulation of hypoxia induced gene expression and angiogenesis: relevance to brain tumors. *Front Biosci.* 2003;8:d100-d116.
16. Reszec J, Hermanowicz A, Rutkowski R, Turek G, Mariak Z, Chyczewski L. Expression of MMP-9 and VEGF in meningiomas and their correlation with peritumoral brain edema. *Biomed Res Int.* 2015;2015:646853.
17. Krishnamachary B, Berg-Dixon S, Kelly B, Agani F, Feldser D, Ferreira G, et al. Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. *Cancer Res.* 2003;63:1138-1143.
18. Feldser D, Agani F, Iyer NV, Pak B, Ferreira G, Semenza GL. Reciprocal positive regulation of hypoxia-inducible factor 1 α and insulin-like growth factor 2. *Cancer Res.* 1999;59:3915-3918.
19. Peters N, Wellenreuther R, Rollbrocker B, Hayashi Y, Meyer-Puttitz B, Duerr EM, et al. Analysis of the PTEN gene in human meningiomas. *Neuropathol Appl Neurobiol.* 1998;24:3-8.
20. Mei T, Wang Z, Wu J, Liu X, Tao W, Wang S, et al. Expression of GLUT3 and HIF-1 α in Meningiomas of Various Grades Correlated with Peritumoral Brain Edema. *Biomed Res Int.* 2020;2020:1682352.
21. Kaynar MY, Sanus GZ, Hnimoglu H, Kacira T, Kemerdere R, Atukeren P, et al. Expression of hypoxia inducible factor-1 α in tumors of patients with glioblastoma multiforme and transitional meningioma. *J Clin Neurosci* 2008;15:1036-1042.
22. Preusser M, Hassler M, Birner P, Rudas M, Acker T, Plate KH, et al. Microvascularization and expression of VEGF and its receptors in recurring meningiomas: pathobiological data in favor of anti-angiogenic therapy approaches. *Clin Neuropathol* 2012;31:352-360.
23. Jensen R, Lee J. Predicting outcomes of patients with intracranial meningiomas using molecular markers of hypoxia, vascularity, and proliferation. *Neurosurgery.* 2012;71:146-156.
24. Unruh A, Ressel A, Mohamed HG, Johnson RS, Nadrowitz R, Richter E, et al. The hypoxia-inducible factor-1 α is a negative factor for tumor therapy. *Oncogene.* 2003;22:3213-3220.
25. Sun X, Kanwar JR, Leung E, Lehnert K, Wang D, Krissansen GW. Gene transfer of antisense hypoxia inducible factor-1 α enhances the therapeutic efficacy of cancer immunotherapy. *Gene Ther.* 2001;8:638-645.