ONCOLOGY

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

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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 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 1α 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 1α, while strong positivity was noted in 28.57%. Significant association was found between WHO grade and HIF 1α (p=0.0015) and between histopathological types and HIF 1α (p=0.0433). Furthermore, HIF 1α was also significantly associated with the recurrent cases (p=0.0172).

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

Keywords: HIF 1α, 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 alpha [1]. The hypoxia-inducible factors (HIFs) subsequently stimulate VEGF (Vascular Endothelial Growth Factor) that in turn allows for the proliferation of new blood vessels. Neoangiogenesis 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,

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or grade III (malignant), 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 study 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, endogeneous 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 α 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 37^0 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 conterstained with haematoxylin. The HIF 1 α 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

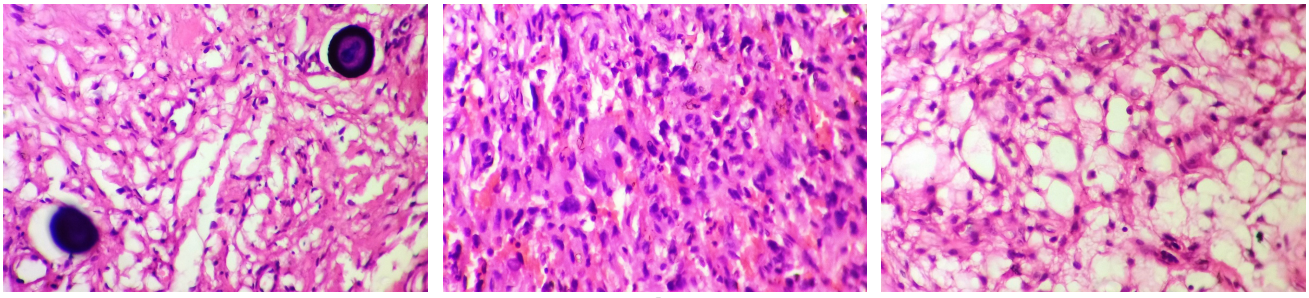
Statistical analysis was performed using Epi info TM version 7.2.4.0. Epi info is the software of CDC (Centers for disease control and prevention). Probability value or the p value <0.05 was considered as statistically significant.

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 1α, 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 1α (Figure 2, Table I). Significant statistical correlation was found between WHO grade and HIF 1α 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 1α 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 1α 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 α 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%



a b c

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

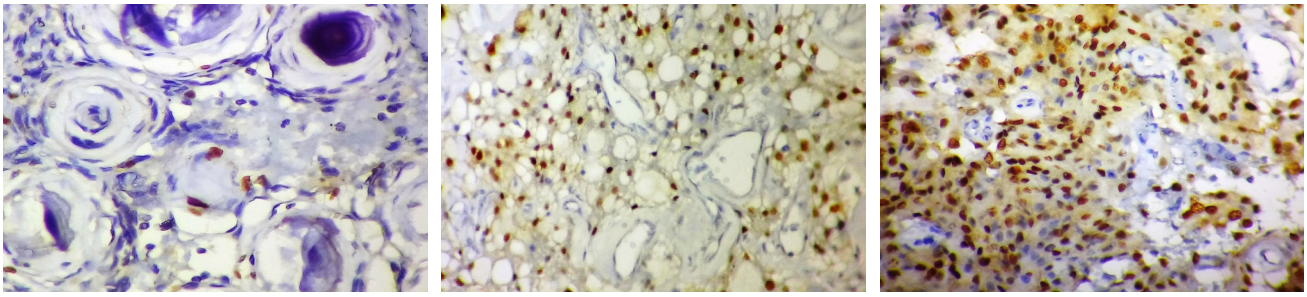


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Figure 2. a: HIF 1 alpha negative with <10% cells showing nuclear positivity (HIF 1α, 400X); **b:** HIF 1 alpha mild to moderate positive with 11-50% cells showing nuclear positivity (HIF 1α, 400X); **c:** IF 1 alpha strongly positive with >50% cells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

Table II. Association between WHO grade of meningiomas and HIF 1 α immunoexpression.

	HIF 1			
WHO grade	**<10% cells (negative)**	**11-50% cells (positive +)**	**>50% cells (positive ++)**	**Total**
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 α immunoexpression.

	HIF 1			
Recurrence	**<10% cells (negative)**	**11-50% cells (positive +)**	**>50% cells (positive ++)**	**Total**
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 Hipple 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 1α 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 1α. 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 1α immunoreactivity.

We found that strong HIF 1α immunoexpression 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 1α immunoexpression. Mei et al. [20] also found a similar observation while studying 160 patients of meningiomas with HIF 1α positivity being 62.5% and 42.9% in high and low grade meningiomas, respectively. HIF 1α immunoexpression 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 1α 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 immunoexpression of VEGF, or in other words HIF 1α, was associated with poor prognosis. Further, Jensen et al. [23] also emphasized that meningiomas with enhanced immunoexpression for HIF 1α 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 HIF1α 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 1α 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 1α is an extremely potent transcription factor involved in tumor cell growth and proliferation. The role of HIF 1α 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 1α appears to be a good and promising marker and a valuable target for effective therapeutic strategy in meningiomas.

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