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# International Journal of Applied Sciences and Biotechnology

A Rapid Publishing Journal

**ISSN 2091-2609**

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**CODEN (Chemical Abstract Services, USA): IJASKD**

Vol-3(3) September, 2015

**Available online at:**

http://www.ijasbt.org

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http://www.nepjol.info/index.php/IJASBT/index

SEM-Biotech Publishing

Impact factor*: **1.422**
Scientific Journal Impact factor#: **3.419**
Index Copernicus Value: **6.02**
IBI Factor 2015**: **4.19**

*Impact factor is issued by Universal Impact Factor. Kindly note that this is not the IF of Journal Citation Report (JCR).
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A. Shanmugavadivu C. et al. (2015) Int J Appl Sci Biotechnol, Vol 3(3): 479-482
DOI: 10.3126/ijasbt.v3i3.13141

ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

# p53 EXPRESSION IN GLIOMAS

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## Abstract

The p53 expression on Gliomas are used for subtyping of tumours. The samples were extracted from the paraffin embedded tissue blocks. The Grade IV and Grade II were tested by IHC (ImmunoHistoChemistry) staining procedures. The primary antibody used was p53. It was found that there is a high expression of p53 in Grade IV (82%) was Score 3 and low expression p53 in Grade II (18%) in which the score was 1. This is in contrast to previous studies of subtyping of Gliomas where, classical types have low expression of p53 and proneural type have high expression of p53. This subtyping was based on score 0 - 3. Score 0 has no positive nuclei, Score 1 has 10% positive nuclei, Score 2 has 10 - 30% positive nuclei while score 3 has >30% of positive nucleus.

**Keywords:** p53; Gliomas; IHC; Staining; Scores

## Introduction

Human brain is the most complex and powerful part of the body. In the brain, 90% of the cells are glial cells. Commonly tumours are caused by mutagens and carcinogens. It may also be caused due to high stress levels, increasing pollution, toxins and magnetic energy fields. All these result in mutation of p53 gene that causes brain tumour (Smith, 2007).

Brain tumours are of two types: primary tumour which arises in the brain and secondary tumour which starts elsewhere in the body and spreads to the brain. Primary tumours can be either gliomas or non- glial tumours. Nearly, half of the primary tumours are gliomas. Gliomas are divided into: astrocytomas, ependymomas, oligodendrogliomas and mixed glioma (i.e) oligoastromas.

The World Health Organization classifies tumours by a grading system. The grading is normally characteristic of tumour location. Low grade tumours (Grade I or Grade II) are less aggressive and Grade III is more aggressive than low grade tumours and that of Grade IV is high grade tumours that are malignant and life-threatening (Smith, 2007). Since recently, there has been an effort for subtyping gliomas of various grades by immunohistochemistry as classical, proneural, mesenchymal and neural. These are protein based subtypes EGFR, p53, CD44 and MERTK (Sevtllana *et al.*, 2014).

p53 was described in 1979 which is a protein mass of 53 KDa (Matlashewari *et.al.*, 1984). It is known as "the guardian of the genome" because it prevents genome mutation. p53 is located in short arm of chromosome 17 and has 11 exons. It is the most frequently altered gene in gliomas (Arnold and David, 2010). p53 is involved in both the initiation as well as the recurring of glioblastomas (Zupanska and Kaminska, 2002).

Thus p53 is vital for Gliomas. Their expressions are studied and used for the subtyping of gliomas, in which the proneural subtype (63% Grade II) has high p53 expression and classical subtype (39% Grade IV) has low p53 expression. Other types such as mesenchymal and neural have no expression of p53 (Sevtllana *et al.*, 2014).

Glioma is a type of tumour which occurs in the brain or in the spine and it arises from glial cells. The most common site of this is the brain (Manelak and Jacoby, 2007) . Overall 2% of the tumours are brain tumours out of which 80% are gliomas (Sevtllana *et al.*, 2014).

Gliomas are classified based on their historical events as astrocytic, oligodendroglial and ependymal. According to WHO classification in astrocytic glioma grade I, diffuse astrocytomas are grade II, anaplastic astrocytomas are grade III and Glioblastoma are grade IV (Branger and Bouvier, 2005).

All gliomas show the loss of p53 which is a tumour suppressor gene (Fuci *et al., 1998).* The tumour suppressor gene regulates the cell growth and proliferation and prevents the cell division after chromosomal damage by UV or ionizing radiation, loss of which causes proliferation of mutated cells, resulting in cancer (Branger and Bouvier,

2005). In humans, p53 is located on short arm of chromosome 17 (17 p 13.1) (Arnold and David, 2010). This gene encodes a protein which could bind to DNA and regulate the genes to prevent mutation of genomes (Gilbert, 10th edition). Thus, p53, will repair the DNA. If it gets damaged it can initiate apoptosis – programmed cell death. Therefore, the main role of this p53 is anti-cancer (Bell *et al.*, 2002).Human tumour is widely caused by p53 inactivation and thus its germ line mutation in the deletion of codon 236 will be seen in brain tumours (Zupanska and Kaminska, 2002).

## Materials and Methods

### *Collection of Materials*

The samples were collected from the Department of Pathology at Meenakshi Mission Hospital and Research Centre, Madurai, Tamil nadu. The samples were retrieved from archives that belong to patients diagnosed for tumours in the year 2014. The samples are taken belong to the age group of 40 to 80 years.

Tumour specimens were obtained by surgical resection (including biopsy). Formalin-fixed, paraffin-embedded specimens were subjected to histopathological examinations. Each specimen was classified according to its grade.

### *ImmunoHistoChemistry*

The poly HRP kit p53 (BioGenex, India).

In the current study, 11 samples have been processed. The process in brief includes making the tissue section at 60ºC for 1 hour. Later these paraffin wax embedded sections were dewaxed and rehydrated. It was then rinsed with de-ionized water for 2 to 5 minutes. Antigen retrieval was done by heat induced epitope retrieval method (HIER). The tissues were taken and washed with the wash buffer. Peroxidase block activity was done with 3% hydrogen peroxidase for 10 minutes at room temperature and then wash with buffer. Power Block in added for 1 hour at room temperature. Then primary antibodies were added and incubated at room temperature for 20 minutes. Secondary antibodies (poly – HRP) were added and incubated for 30 minutes. And the DAB (to be freshly prepared as 1 drop of liquid DAB (diaminobenzidine) chromogen in 1 ml stable DAB buffer) was added and incubate for 5 minutes. Counter strain has been done with hematoxylin was then mounted on the cover slip.

All the tissue were observed (400X). The staining pattern of the cell with glial fibrillary acidic protein (GFAP) was done to confirm the phenotype of tumour cells and then nuclear p53 staining pattern was analysed. p53 nuclear staining was scored from 0 to 3 where score 0 represents the absence of strong nuclear staining, score 1 represents up to 10% cells showing strong nuclear positivity, score 2 represents 10-30% cells showing strong nuclear positivity. Score 3 representing >30% cells showing strong nuclear positivity were considered to indicate high expression of protein.

## Results and Discussion

The samples were collected from hospital archives (2014). There were 11 cases of brain tumour that were tested with p53 to analyse the expression of p53. Out of 11 cases, 10 cases were Grade IV and 1 case with Grade II. The age of patients ranged from 40 to 80 years. Out of 11, there were 10 cases that are high grade (IV) glioma (98%) and one was of Grade II tumour (2%). The maximum score obtained is 3 for 9 cases. The remaining 2 cases had a score of about 1 that include a gliosarcoma and oliogoastroacytoma (Grade II).

In 2006, it was reported that malignant glial tumours such as GBM (Glioblastomamultiforme) should be subtyped on the basis of their protein constituents. Thereafter, in 2010, Glioblastomamultiforme (GBM) was subtype into four defined molecular subgroups such as classical, mesenchymal, proneural and neural. The classical subtype has low p53 expression in GBM (Glioblastomamultiforme) (39%) while the proneural type has high p53 extension (29%) (Sevtllana *et. al.*, 2014).

In the current study, by applying the IHC technique, the protein expression was analysed in the tumour that is important in the subtyping of GBMs. The p53 were analysed based on sub divisions of tumour cells. It was carried out by using the paraffin-embedded tumour tissues for the assessment of protein expression with IHC.

The paraffin – embedded tumour tissues were obtained by using various surgical techniques of diagnostic biopsy. The treatment comprised of addition of radiotherapy or chemotherapy. The collection of samples from the patients includes all clinical and medical details such as hospital number, biopsy number, age and sex, diagnosis, grade, GFAP, Vimentin, Ki67. Where GFAP, Vimentin, Ki67 are cell proliferation makers, used in order to evaluate the effect of the tumour in pathology laboratory (Table 1).

The results were confirmed by the positivity in the nucleus of tumour cells in paraffin embedded tissues only. The presence of strong brown colour of nucleus indicates the expression of p53 in the tumour the blue colour of nucleus indicates the non – expressed p53 in the tumour cells (Fig. 1).

Among the 11 cases, 8 cases showed the maximum score of 3 which indicates there is high expression of p53 among grade IV gliomas. Only 2 cases showed score1 which shows low expression of p53. According to the study, the proneural type which show high p53 expression was more among grade IV GBM. This is in contrast to the literature of 2014 which reported that classical type with loss of p53 is more among GBM Grade IV. However, number of samples are low so they may not be statistically significant. Further this study, more number of samples could have been studied to draw more meaningful conclusions.

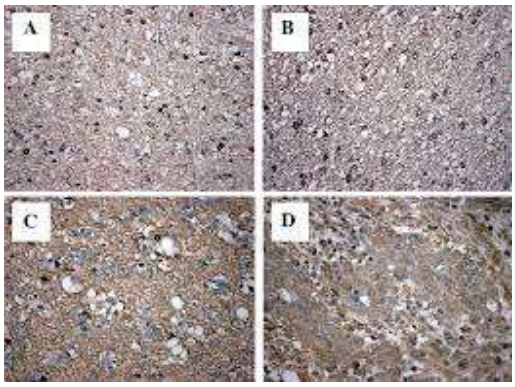


**Fig 1:** A shows score 0, B shows score 1, C shows score 2, D shows score 3 of Gliomas

**Table 1**: Details of samples collected from the patients.

| S.N. | HOSPITAL NUMBER | BIOPSY NO. | AGE & SEX | DIAGNOSIS | GRADE | p53 | GFAP (Glial fibrillary acidic protein) | VIMENTIN | KI67 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 680419 | S2995 | 56/M | OLIGOASTOCYTOMA | II | 1 | -ve | | -ve |
| 2 | 687884 | S3052 | 55/M | GLIOBLASTOMA MULTIFORMAS | IV | 3 | | | |
| 3 | 687883 | S3097 | 54/M | GLIOBLASTOMA MULTIFORMAS | IV | 3 | | | |
| 4 | 693137 | S3407 | 66/M | GLIOBLASTOMA MULTIFORMAS | IV | 3 | | | |
| 5 | 657835 | S3471 | 57/M | GLIOBLASTOMA MULTIFORMAS | IV | 1 | +ve | +ve | |
| 6 | 410684 | S368 | 46/M | GLIOBLASTOMA MULTIFORMAS | IV | 3 | | | |
| 7 | 352530 | S383 | 52/F | GLIOSARCOMA | IV | 3 | Strong +ve | +ve | 30% less +ve |
| 8 | 661019 | S903 | 60/M | GLIOBLASTOMA MULTIFORMAS | IV | 3 | | | |
| 9 | 621865 | S3366 | 44/M | RECURRENT HIGH GLIOMA | IV | 3 | +ve | | |
| 10 | 659331 | S760 | 52/M | GLIOBLASTOMA MULTIFORMAS | IV | 3 | | | |
| 11 | 677279 | S2180 | 76/M | GLIOBLASTOMA MULTIFORMAS | IV | 3 | Strong +ve | | |

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