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Markers of Molecular Pathways in Brain Tumours: Role of CD15 and P53

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Abstract
Introduction: Brain tumours remain a challenging group of diseases and understanding their underlying molecular mechanisms could identify novel therapeutic targets. Previous studies have shown that the CD15 gene, a possible oncogene controlled by the Shh-Gli1 signalling pathway, can be used to identify cancer stem cells in brain tumours. P53 is a key participant in the development and growth of brain tumours, and P53 mutations are common in these tumours.
Aim: To study the expression of CD15 and P53 in primary human brain tumour samples.
Material and methods: In the study, CD15 and P53 immunohistochemistry was used to examine brain tumour tissues taken from eight individuals.
Results: According to the findings, CD15 immunostaining was strong in two cases, weak in five cases, and absent in one case. Four cases showed strong immunostaining for P53, and nuclear atypia was observed in three cases.
Conclusion: The study findings highlight the potential role of CD15 and P53 in brain tumour development and progression, and further research could provide new insights into the underlying mechanisms of these tumours and identify novel therapeutic targets.
Keywords: Brain tumours**,** immunohistochemistry, CD15, P53, cancer stem cell

INTRODUCTION

Brain tumours are a challenging and complex group of diseases that can have devastating effects on patients and their families. Despite advancements in treatments, the prognosis for brain tumours remains poor, and there is a need for new therapies and better understanding of the underlying molecular mechanisms.

CD15 is considered one of the stem cell markers and behaves as potential oncogene, which would be further regulated by Shh-Gli1 signaling pathway. According to reports, anaplastic astrocytes and human glioblastoma multiforme (GBM) cells do not typically express the CD15 gene (1). But some research found that a specific cancer cell type expresses CD15 (2,3). In medulloblastoma cells, CD15 is now thought to be a distinguishing hallmark of cancer stem cells (CSCs) (4).

Another, critical player in the development and progression of brain tumours is the tumour suppressor protein P53. In response to DNA damage or other stress signals, P53, a key regulator of cell growth, causes cell cycle arrest, DNA repair, or apoptosis, which prevents the formation of cancer (5). The most prevalent genetic changes in gliomas are P53 mutations (6) which need Hh signaling for growth and maintenance (7). A recent work demonstrated that there is a negative feedback loop between P53 and Gli1 in the mouse. In the mouse brain and numerous cell lines, Gli1 overexpression resulted in a dramatic rise in Mdm2 expression and a reduction in P53 (8). These studies suggested that alterations can occur through mutations or deletions in the P53 gene, or through alterations in the expression or function of downstream targets of P53.

Understanding the molecular markers associated with the CD15 and P53 pathway and their role in brain tumour development and progression could provide new insights into the underlying mechanisms of these tumours and potentially identify novel therapeutic targets. In this study, we investigated the CD15 and P53 expression in primary human brain tumours.

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MATERIALS AND METHODS

The present study was conducted in Department of Physiology and Interdisciplinary Brain Research Center (IBRC) in Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh from 2015 to 2017. Design of the study was cross sectional and total eight patients were taken. They were selected as cases for further study who met the inclusion and exclusion criteria. Informed consent was obtained from each patient in written after explaining the procedure to the subject prior. A detailed medical history and physical examination were performed on each subject enrolled in the study according to a predesigned proforma. Selected cases of Primary Brain Tumour were assessed to perform expression analysis and molecular profiling. Experiments were approved by the Institutional Ethics Committee, Faculty of Medicine, AMU, Aligarh on 28.12.2015. Clinical data were obtained of all patients included in the study and were also evaluated by medical records for exclusion of Secondary Brain Tumour.

Brain Tumour samples were collected from the Department of Neurosurgery, Operation Theatre, Jawaharlal Nehru Medical College & Hospital, Aligarh Muslim University, Aligarh. After resection of brain tumour, samples were collected in tube containing 40% PFA (Paraformaldehyde) for Immuno-histochemical analysis and stored at room temperature (37˚C). Formalin-fixed paraffin-embedded tumour blocks were used for the immunohistochemical staining for CD15 and P53. Deparaffinization of 4-µm-thick sections in xylene was the first step in the procedure, which was then followed by a graded ethanol immersion for hydration. Epitope retrieval solution (0.01 M citrate buffer, pH 6.0) was used for antigen retrieval in a microwave for 20 minutes, and endogenous peroxidase was inhibited by immersing the sections in 0.3% hydrogen peroxide for 10 minutes. Subsequently, the sections were incubated with primary antibodies against CD15 and P53 for 30 minutes at room temperature. The reactions were visualized by 3,3'-diaminobenzidine (DAB), followed by haematoxylin counterstaining. A Nikon microscope with a high-power field (40X) was used for microscopic analysis. In tissue sections, mouse or rabbit antibodies attached to an antigen were detected using the Ultra vision Quanto-Detection system. Both the paranuclear membrane and cell membrane exhibited CD15 staining. P53 labelling was limited to the nuclei of tumour cells; no cytoplasmic staining was seen, and endothelial cells did not exhibit positive staining.

RESULTS

CD15 Immunostaining:

Among the 8 cases of brain tumour examined, 2 cases showed strong immunostaining for CD15. The immunopositive cells exhibited blue stained crowded nuclei. In 5 cases, weak immunostaining for CD15 was observed in the cell membrane while the nuclei were not stained. In 1 case, nuclear atypia was observed, which is characteristic of tumour cells, but no immuno-positive cells were found.

P53 Immunostaining:

Among the 8 cases examined, 4 cases showed strong immunostaining for P53. In 1 case, weak immunostaining for P53 was observed in the nuclei. In 3 cases, nuclear atypia was observed, which is characteristic of tumour cells, but total lack of immunoreactivity in the cells.

Overall, these results indicate that CD15 and P53 markers are expressed in brain tumour cells, with varying degrees of immunoreactivity. These findings suggest that further studies are needed to look into the potential clinical use of these markers in the detection and management of brain tumours.

DISCUSSION

The present study investigated the expression of CD15 and P53 in a small group of brain tumour specimens, with the aim of identifying potential markers for the diagnosis and prognosis of these tumours. Our results showed that 2 out of 8 cases exhibited strong immunostaining for CD15, indicating high expression of this marker in these tumours. In contrast, weak immunostaining was observed in 5 cases, while 1 case showed no immune-positive cells. These findings suggest that CD15 expression may be a variable and heterogeneous feature in brain tumours, and that further studies are needed to clarify its clinical significance.

Interestingly, one case showed nuclear atypia, a characteristic feature of tumour cells, but no immuno-positive cells were detected. This suggests that CD15 may not be a universal marker for brain tumours, and that other markers may need to be investigated in cases where CD15 expression is absent or low. This finding is supported by a study of Kenney-Herbert E *et al*., 2015, which found that CD15 is not a reliable cell-surface marker capable of differentiating a population that is tumorigenic or stem cells (9).

In addition to CD15, our study also investigated the expression of P53 in the brain tumour specimens. We found that 4 out of 8 cases showed strong immunostaining for P53 while in 1 case, weak immunostaining for P53 was observed. In 3 cases, characteristic of tumour cells was seen, but lack of immunoreactivity. These results suggest that there might be heterogenous group of primary brain tumours patients.

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Although the role of CD15 in the development and evolution of brain tumours is not fully understood, earlier studies have suggested that it might play a role in carcinogenesis and stem cell self-renewal. Our study shows that CD15 may have a role in brain tumours and it may be a useful diagnostic and prognostic marker in certain cases.

A well-known tumour suppressor gene P53, on the other hand, regulates the cell cycle and inhibits the growth of cancer. Nonetheless, p53 mutations occur frequently in brain tumours, as it is well documented. In addition to p53 gene loss or mutation, the stability of the p53 protein, gene expression, and transactivation potential are all disrupted in brain tumour cells. Both gain-of-function of mutant P53 and loss of function of P53 are linked to the formation of brain tumours (10).

Overall, our study provides important insights into the expression of CD15 and P53 in brain tumours and suggests that these markers may have clinical significance in the diagnosis and prognosis of these tumours. However, further studies are needed to confirm and extend these findings, and to identify other potential markers that may be useful in the management of brain tumours.

Limitations of the study

Although our study's results are promising, there are number of issues that need to be considered. First, our results might not be as generalizable to bigger populations due to the small sample size. Second, other potential markers or biological pathways that might be implicated in the development of brain tumours were not examined; the study was restricted to analysing the expression of CD15 and P53 in cases of brain tumours. Finally, the study was limited to immunohistochemistry-based analysis, which has its own limitations such as subjectivity in interpretation and potential variability in staining quality.

CONCLUSION

In conclusion, our study examined the expression of CD15 and P53 in a small group of patients with brain tumours. The results suggest that CD15 and P53 may play important roles in the molecular pathways of brain tumours. CD15 was expressed in a subset of tumour cases and was associated with strong immunostaining and the presence of blue-stained crowded nuclei. P53 staining was observed in most cases, with varying levels of intensity. According to these results, P53 and CD15 may be used as prognostic and diagnostic indicators for tumours of brain.

These results need to be confirmed by larger cohort studies. In addition, the identification of markers like CD15 and P53 may advance our knowledge of the molecular processes underlying the development of brain tumours and may pave the way for the creation of more potent targeted treatments for these lethal cancers.

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