Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 32, issue 1, 2022, pp. 5 - 9

IMMUNOEXPRESSION OF P53, IDH1 AND ATRX IN GLIOMAS

Lydia KHIREDDINE [1*], Amina BOUCHEMAL[1], Abderrezak GHIDOUCHE [2], Souhil TLIBA [3,4], Fatiha BEDJOU [1]

[1]Université de Bejaia, Faculté des Sciences de la Nature et de la Vie, Laboratoire de Biotechnologies Végétales et Ethnobotanique, Bejaia, Algérie
[2] Université de Bejaia, Laboratoire de Geni-biologique des cancers, Bejaia, Algérie
[3]Service de Neurochirurgie, Centre hospitalo-universitaire, Bejaia, Algérie
[4] Université de Bejaia, Faculté de Medecine, Bejaia, Algérie

Abstract: Gliomas account for 45% of all intracranial tumors. Technologies, allowed better molecular analysis leading to the discovery of IDH1 (Isocitrate dehydrogenase) mutations. IDH1 mutations are often associated with ATRX (alphathalassemia/mental retardation syndrome Xlinked) and P53. In this study, we analyzed their expression and correlation with grade. 28 samples were included in the study. Immunohistochemistry for IDH1, ATRX, and P53 was done and reported. Statistical analysis was done in order to compare their immunoexpression in different grades of gliomas. A total of 28 gliomas were included, excluding pilocytic astrocytoma. IDH1, ATRX, and P53 positivity was seen in both Diffuse glioma and Glioblastoma, at almost the same frequencies. A combined analysis of expression of IDH1 and ATRX, and P53 with WHO grade showed statistically no significant association. Neither IDH1, ATRX, and P53 immunoexpression nor their combination at two and three variants showed significant differences according to the grade in this study.

Keywords: glioblastoma, diffuse gliomas, immunohistochemistry, grade, profile.

INTRODUCTION

Glioma is the most common type of tumor originating in the brain. It represents about 45 percent of all brain tumors. Gliomas originate from the glial cells that surround and support neurons, it also infiltrates the adjacent tissues. The most aggressive form is Glioblastoma (GBM). Despite therapeutic advances, patient survival remains unfavorable with a 5-year survival of 12 to 14 months. The World Health Organization (WHO) (2007), according to histological parameters defines gliomas as grade I, grade II, and grade III astrocytic tumors, grade II and III oligodendrogliomas, and grade IV Glioblastomas. Molecular biology studies have provided new insights into the oncogenesis and progression of gliomas. Hence, in 2016, the WHO updated the classification of gliomas, integrating new molecular features, thus splitting gliomas into two main groups, based on positive expression of wild-type isocitrate dehydrogenase (IDH1+) and negative or mutated expression of IDH1 protein (IDH1-). IDH1 mutation occurs in the early stages of glioma formation and may affect DNA demethylation and lead to tumorigenesis, the exact mechanism remains unclear. There are many different biomarkers that are often associated with IDH1 such as P53 mutation, 1p/19q codeletion specific to oligodendroglioma, or alpha-thalassemia/mental retardation syndrome X-linked (ATRX) mutation; it commonly occurs in low-grade astrocytomas and secondary Glioblastomas (GBMs), but are rare in primary GBM. Glial tumors are now classified into three basic categories, pilocytic astrocytoma (WHO grade I), Glioblastoma (WHO grade IV), and Diffuse gliomas, which include astrocytomas and oligodendrogliomas (WHO grade II and III), it was adopted in the 2016 update of WHO classification of Central nervous System tumors. Biomarkers are biological characteristics that can be measured and assessed, to indicate normal and pathological processes. Many Technologies are used to detect biomarkers, they can be genomic or proteomic. Immunohistochemistry (IHC) is one of the most used techniques, because of its availability, but also reliability and precision.

The primary objective of this investigation is to delineate the protein expression patterns of P53, IDH1, and ATRX in gliomas comprehensively. Furthermore, the study aims to discern whether alterations in the protein expression of any of these specific markers or their collective variants exhibit associations with the histological grade of gliomas.

MATERIAL AND METHODS

Tumor Specimens

Tumor specimens were aseptically obtained intraoperatively from the Neurosurgery Department. Ethical clearance for the utilization of human samples in all experiments was secured from the institutional ethical review board. Subsequent to surgical resection, segments of the excised tumors were allocated for storage, while the remaining tissue underwent formalin fixation and paraffin embedding for conventional histopathological examination and immunohistochemical analyses.

Immunohistochemistry

A cohort comprising 28 glioma biopsies, comprising 18 cases of glioblastoma multiforme (GBM) and 10 cases of diffuse glioma (DG), was employed for the study. Immunohistochemical analyses for IDH1, P53, and ATRX were executed on formalin-fixed paraffin-embedded tumor specimens, followed by sectioning into 5-micron slices using a microtome. The application of specific antibodies adhered to the

*Correspondance: Khireddine Lydia, Université de Bejaia, Faculté des Sciences de la Nature et de la Vie, Laboratoire de Biotechnologies Végétales et Ethnobotanique, 06000 Bejaia Algérie. Email: khireddinelydia@gmail.com.

supplier's recommended protocols. IDH1 R132H (Dianova, dilution 1:40), ATRX (Sigma, dilution 1:300), P53 (Dako, dilution 1:50). Labeled streptavidin biotin kit (Universal) was used as a detection system (Dako, Denmark).

Statistical analysis

Statistical assessment of the variance in P53, IDH1, and ATRX immunoexpression between Glioblastoma and Diffuse gliomas was conducted utilizing the Chi-square test and Fisher's exact test. A significance threshold of $P < 0.05$ was employed to denote statistical significance in the observed differences.

RESULTS

In the present study, a total of 28 gliomas biopsies were studied. The gliomas were diagnosed histopathologically, followed by IHC on the following markers: P53, ATRX, and IDH1. Of the 28 gliomas samples, 75% were P53+, 50% were IDH1-, and only 32.14% of our cohort presented a loss of ATRX (Tab I).

The results show P53 positivity at 77.77 % and 70 % in GBM and DG respectively. the variance in P53 expression in Glioblastoma and Diffuse gliomas remains negligible; P-value: 0.674. This result suggests that P53 overexpression is grade-independent in this study (Tab I).

the percentage of samples, expressing the mutated form of IDH1 in Glioblastoma and Diffuse glioma, is slightly different 44.44% and 60% respectively, moreover, this difference is statistically not significant. The expression of IDH1 mutation in IHC does not seem to be correlated to the grade (Tab I). In our cohort, only 32.14% of gliomas show a loss of ATRX as mentioned above, indeed, only 22.22% of Glioblastomas tested show a loss of ATRX, vs 50% in Diffuse gliomas. The statistical analysis reveals no significant difference concerning the expression of loss of ATRX according to the grade.

Table 1.

The results of IDH1, P53, and ATRX immunoexpression in gliomas

Features	Glioma N (%)	Diffuse glioma N (%)	Glioblastoma N (%)	*P value* DG vs GBM
Number of biopsies	28	10	18	
IDH1 Mutation	14 (50)	6 (60)	8 (44.44)	0.694
P53 Overexpression	21 (75)	7 (70)	14 (77.77)	0.674
Loss of ATRX	9 (32.14)	5 (50)	4 (22.22)	0.209

Table II presents immunohistochemical results of two protein pairs, ATRX/IDH1, ATRX/P53, and IDH1/P53, in both Glioblastomas and Diffuse gliomas. In GBM samples, the three largest combinations were IDH1+/ATRX+ (55.55%), IDH1+/P53+ (50%), and ATRX+/P53+ (61.11%). The smallest subgroups were IDH1-/ATRX- (22.22%), IDH1-/ATRX+ (22.22%), ATRX-/P53- (5.55%), and IDH1+/P53- (5.55%). IDH1-/ATRX- (40%), IDH1+/ATRX+ (40%), IDH1-/P53+ (40%), and ATRX+/P53+ (40%) were found to be the largest subgroup in Diffuse gliomas, however, ATRX-/P53+ and IDH1+/P53+ were also similarly high at (30%). The three smallest subgroups were IDH1+/ATRX- (4.3%), ATRX-/P53+ (6.7%), and IDH1+/P53+ (4.9%).

As shown in Table II, a combined three-protein immunohistochemical analysis revealed six different molecular variants in Glioblastomas. About (50%) of the samples were consisted of wildtype protein expression of IDH1 and ATRX, and mutated expression of P53, i.e., IDH1+/P53+/ATRX+ while (5.55%) were wildtype protein expression group, i.e., IDH1+/P53-/ATRX+ which represents also the smallest subgroup. the same molecular six variant found in Glioblastoma were found in Diffuse glioma as well. In the same way as in GBM IDH1+/P53+/ATRX+ was the most important subgroups were IDH1-/P53+/ATRX- (30%) and IDH1+/P53+/ATRX+/interestingly, this subgroup was the most important in Glioblastoma samples as well (Tab II).

Table 2.

Combined analysis of two and three protein pairs in Glioblastoma and Diffuse glioma

Immunohistochemistry	Variants	Glioblastoma (%)	Diffuse glioma (%)
IDH1/P53	***IDH1-/P53+***	27.77	***40***
	IDH1-/P53-	16.66	20
	IDH1+/P53-	5.55	10
	IDH1+/P53+	***50***	30
ATRX/P53	***ATRX+/P53+***	***61.11***	***40***
	ATRX+/P53-	16.66	20
	ATRX-/P53-	5.55	10
	ATRX-/P53+	16.66	30
IDH1/ATRX	IDH1-/ATRX+	22.22	20
	IDH1-/ATRX-	22.22	***40***
	IDH1+/ATRX+	***55.55***	***40***
IDH1/P53/ATRX	IDH1-/P53+/ATRX+	11.11	10

Immunohistochemistry	Variants	Glioblastoma (%)	Diffuse glioma (%)
	IDH1-/P53+/ATRX-	16.66	***30***
	IDH1+/P53+/ATRX+	***50***	***30***
	IDH1-/P53-/ATRX-	5.55	10
	IDH1+/P53-/ATRX+	5.55	10
	IDH1-/P53-/ATRX+	11.11	10

DISCUSSIONS

In this series, an attempt was made to compare the immunoexpression of P53 IDH1 and ATRX in Glioblastoma and Diffuse gliomas individually, as well as in different combinations. Immunohistochemically, we found ATRX loss in 32.4%, P53 overexpression in 75 %, and IDH1 mutated form in 50% of our cohort. P53 is a pivotal biomarker frequently overexpressed in almost 50% of gliomas and particularly in astrocytic tumors, even if our findings are slightly higher, it still comparable. In our study aberrant expression of P53 was found highly similar in GBM and Diffuse glioma 77.77% and 70% respectively. The statistical analysis shows no significant difference. In the same way, many Immunohistochemical studies report an equivalent rate of P53 overexpression; indeed, it was detected at 35 to 60% of grade II & III glioma (DG), and 45 to 70% of Glioblastomas (Litofsky *et al*., 2008). In contrast to Jin Yueling's meta-analysis, which encompassed 1322 glioma cases REF, the incidence of P53 immuno-positivity is notably elevated in high-grade gliomas compared to lower-grade gliomas (63.8% versus 41.6%). Gliomas are recognized for their potential to undergo increasing anaplastic changes over time. Research has demonstrated that low-grade tumors exhibiting P53 overexpression have a propensity to progress to higher grades. However, it's noteworthy that not all gliomas exhibiting progression show immunoreactivity to P53.

IDH1 mutations exist in at least 70% gliomas, particularly seen in (WHO) grade II and III glioma, and secondary Glioblastomas. It is now believed that IDH1 mutation is responsible for the initiation of gliomas genesis. Similarly, in this study, 50% of gliomas biopsies were IDH1-. In our Glioblastoma cohort 44.44% were IDH1-; all of them were secondary Glioblastomas (data not shown). IDH1 mutated form was observed in 60 % of Diffuse glioma, the frequency of IDH1 mutation is variable in DG, varying from 50 to 90%, thus still concords with our results. It is reported in the literature that IDH1 mutation is rare in primary GB <10%, in our study, none of the primary Glioblastoma biopsies showed IDH1 mutation, however, there seems to be no difference according to the grade of malignancy.

ATRX mutation is characteristic of astrocytic differentiation, which can be determined by loss of nuclear ATRX expression on immunohistochemistry. It occurs more frequently in DA 60–70% and like IDH1 mutation, is very rare in primary GB 4–6%. Loss of ATRX is almost mutually exclusive with co-deletion 1P 19Q, which occur in oligodendroglioma. Our findings align with the literature cited above in fact, in our cohort 50% of grade II and III glioma were ATRX-, 100% of anaplastic astrocytoma were also ATRX-, and all oligodendrogliomas biopsies were ATRX+ (data no shown). Furthermore, we found ATRX loss in only 22.22% in GBM, our findings align with the study conducted by Ikemura and colleagues (2016) Specifically, their research reported the presence of ATRX negativity in only 12.7% of Glioblastoma cases, corroborating our observations. Conversely, Cai and colleagues (Cai *et al*., 2015) have documented a more pronounced decrease in ATRX expression, particularly in primary Glioblastoma (GBM) and anaplastic gliomas compared to grade II gliomas. They have posited this reduced ATRX expression as a potential marker indicative of malignancy. The strong association of IDH1 mutation and ATRX mutation has been effectively documented. ATRX mutations also correlate with other prominent features including P53 mutations and occur most often in astrocytic tumors. In Glioblastomas, some studies have identified a small percentage that is IDH1 wild-type and has a loss of ATRX expression.

In this study, only grade II and III gliomas (DG) and GBM were compared. In combination of two variants, the most common profiles in GBM are IDH1+/P53+, ATRX+/P53+, and IDH1+/ATRX+, however in DG; IDH1-/P53+, ATRX+/P53+, IDH1-/ATRX- and IDH1+/ATRX+ are the most widespread. In three variants combination IDH1+/P53+/ATRX+ is the most found profile in GBM but also in DG. The IDH1-/P53+/ATRX+/profile is also found in DG at an equal percentage. Interestingly, this study shows that in grade II and III gliomas and GBM in the most cases share the same immunohistochemical signatures, which suggests that the immunoexpression of IDH1, ATRX, and P53 is independent of the grade in our cohort. Otherwise, it was previously observed in various studies that some relationship exists between aberrant expression of ATRX, P53, and IDH1 proteins in gliomas, Indeed, the investigation by Sarma et al. (2021). demonstrated a noteworthy disparity in both the ATRX/IDH1 combination and the ATRX/IDH1/P53 combination with respect to glioma grade. It is crucial to acknowledge that our study, while providing valuable insights, remains in its preliminary stage and is constrained by a limited sample size. Additionally, the unavailability of pilocytic astrocytoma (grade I) represents a limitation in the comprehensiveness of our analysis.

CONCLUSION

In conclusion, the immunohistochemical signature study conducted on a cohort of 28 glioma biopsies on P53, ATRX and idh1, suggests that the identified signature appears to be independent of the tumor grade. However, caution is warranted given the relatively small sample size. While the initial findings provide intriguing insights, the potential influence of a larger cohort merits exploration to enhance the robustness and generalizability of the results.

AUTHORS CONTRIBUTIONS

Conceptualization: KL. and BA.; methodology, KL.; data collection LK and BA; data validation, TS., GA. and BF.; data processing KL.; writing—original draft preparation KL.; writing—review and editing: BF.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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