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Immunohistochemistry is Not a Sufficient Method of Determining p53 Mutation in Glioblastoma

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Introduction

Tumor protein 53 (p53) is a nuclear phosphoprotein involved in fundamental pathways of cellular growth and differentiation, including transient arrest of the cell cycle in the G1 phase. Due to its eminent role in cell cycle regulation, p53 has several mechanisms to restrict uncontrolled cell division. As such, tumor protein 53 gene mutations are of momentous significance in neurooncology as aberrant p53 expression in glioblastoma has been associated with worse patient outcomes and decreased chemosensitivity to temozolomide. Currently, p53 immunohistochemistry (IHC) is used as a detection assay for the presence of mutant *TP53* in gliomas, with many using a 10% nuclear staining threshold to imply p53 mutation positivity. Here, we reexamined the correlation between p53 immunoreactivity and the *TP53* mutation status attained by DNA Sanger sequencing to question whether IHC is sensitive and specific enough in the determination of this potentially important mutation.

Methods

Forty-one freshly resected glioblastoma tumor samples were collected between April 2016 and May 2018. Each sample underwent standard laboratory p53 IHC staining using the DO-7 antibody clone. A >10% immunoreactivity threshold was used as a surrogate for prediction of *TP53* mutation status. Sanger gene sequencing was utilized to determine the actual TP53 mutation status in these samples and compared to the results of IHC staining.

Results

Of 41 histologically confirmed glioblastoma samples, 27 (66%) were immunopositive for p53 mutation via laboratory IHC and 14 (34%) were immunonegative. Utilizing gene sequencing, we identified only nine samples (22%) with actual *TP53* mutations. The sensitivity and specificity in using >10% p53 IHC surrogacy as a predictor of TP53 mutation status were 89% and 41%, respectively. The positive-predictive and negative-predictive values were 30% and 93%, respectively. Raising the p53 IHC positivity threshold from >10% to >40% would have increased the positive predictive value from 30% to 73%, respectively. Under the same parameters, the negative predictive value would have also increased from 93% to 97%. Even with a >40% threshold for p53 IHC positivity, the sensitivity of our study would have remained unchanged at 89%: 8 of the 9 mutations demonstrated p53 staining of >40%. Moreover, the specificity of our study would have increased from 41% using the >10% p53 IHC threshold, to 91% with a >40% p53 IHC cut-off.

Subject	p53 %	Mutation	EGFR	R72P	Age	Gender	Race
1*	90	H179R	-	No	31	M	W
2	5		+	No	66	M	W
3	10		-	Yes	83	M	W
4	25		-	Yes	63	F	W
5	30		+	No	73	M	W
6	5		-	Yes	66	F	W
7	2		-	Yes	64	M	W
8	80	R273C	+	Yes	48	M	W
9	5		-	Yes	54	F	W
10	5		+	No	54	F	B
11	60		-	Yes	68	F	W
12	1		-	Yes	79	M	W
13	10		-	No	76	M	W
14	5		+	No	62	F	W
15	40		+	Yes	59	M	W
16	10		-	No	77	F	W
17	20		-	No	64	F	W
18	20		-	Yes	64	F	W
19	25		+	Yes	66	M	W
20	20		-	No	75	F	W
21	5	P36P	+	No	81	F	W
22	40	A158H	-	Yes	73	F	W
23	1		-	No	54	M	W
24	70	R273H	-	Yes	37	M	W
25	10		-	No	76	M	B
26	25		+	No	77	F	W
27	10		+	Yes	54	M	W
28	5		-	No	64	F	W
29	0		-	Yes	84	M	W
30	60		-	Yes	62	M	W
31	25		-	Yes	65	M	W
32	80	Y234D	-	Yes	80	M	W
33	5		-	Yes	85	M	W
34	5		-	No	54	F	W
35	5		-	No	71	F	B
36	90	M246T	-	Yes	31	M	H
37	10		+	Yes	58	F	W
38	20		-	Yes	40	F	W
39	30		-	Yes	74	M	W
40	75	N235D	-	Yes	63	F	W
41	90	C176Y	-	Yes	65	M	W

* Prior surgical debulking of a grade 3 anaplastic astrocytoma

Conclusions

In the current study, we found that utilizing a >10% p53 IHC threshold for predicting *TP53* mutation status in glioblastoma samples is insufficient. Implementing this threshold, we demonstrated a remarkably low positive-predictive value. Our data suggests that raising the threshold to 40% would increase both the positive and negative predictive values as well as the specificity without changing the sensitivity of the assay.

Learning Objectives

to understand that antibody staining for p53 mutation in glioblastoma may have high rates of false positivity.

to understand that gene sequencing is a preferred method for determining p53 mutational positivity.

References

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