Sniffing Out the Carcinogenic Spy: The Identification of p53 and PTEN Mutations in Glioblastoma

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Abstract

In order to have a better understanding of molecular signature in cancer for efficient cancer therapy, this study aims to identify the most frequently mutated genes in brain cancers through public database. Here, we chose glioblastoma (GBM) and glioma as our models and analyzed public data in cBioPortal database. We found that two tumor suppressor genes PTEN and p53 are the two most frequently mutated genes in GBM and glioma. And R273H and R248Q are mutation hotspots of p53; while R130 and T319 are easily mutated sited of PTEN. At the same time, we also took one GBM cell line to verify the mutation sites of p53 and PTEN. However, due to COVID-19 and time limitation, this part is not fully characterized.

Background

- Glioblastoma (GBM) is an aggressive (grave IV) malignant brain tumor in adults, causing increased intracranial pressure, resulting in headaches and focal or progressive neurologic deficits (Davis, 2016).
- It is the MOST COMMON primary malignant brain tumor with ONLY A 6.8 PERCENT FIVE-YEAR SURVIVAL RATE and an average length of survival between twelve and eighteen months (Davis, 2016; National Brain Tumor Society, 2020).
- TP53 (p53) is a well-known tumor suppressor located on chromosome 17p13.1, that has been linked with a genetic vulnerability to the development of tumors - more than 50% of human cancers have p53 mutations (American Cancer Society, 2014; Zhang et al., 2018).
- PTEN, another well-known tumor suppressor located on chromosome 10, prevents tumor growth and survival and promotes chromosomal stability and DNA repair that has been linked with a genetic vulnerability that promotes tumorigenesis and resistance to anti-cancer therapeutics (Dillon &Miller, 2014).
- I conducted this study to determine the correlation between mutations in the tumor suppressor genes, p53 and PTEN, and the emergence of glioblastoma.

Materials & Methods

cBioPortal Database used in this study:
https://www.cbioportal.org/

Primers for the tumor suppressor genes, p53 and PTEN, were designed by NCBI primer design (https://www.ncbi.nlm.nih.gov/tools/primer-blast/).
DNA was from GBM cell line. To amplify the DNA, PCR was performed, and the products were then analyzed by agarose gel electrophoresis and then submitted for Sanger sequence.

*Due to COVID-19, I only did quick DNA extract at home, and the rest of experiments were conducted by staffs at DNA learning center.

Results

	Most Prevalent	Males (1629 samples)	Females (1102 samples)	Race	TP-53 Survival (985 samples)	PTEN Survival (397 samples)	IDH1(1057 samples)
Diagnosis Age	50-55 (10.5%)	50-55 (10.8%)	50-55 (10.7%)	N/A	25-30 (11.7%)	50-55 (16.4%)	30-40 (30.3%)
Sex	Male (57.3%)	N/A	N/A	N/A	Male (58.0%)	Male (61.3%)	Male (55.2%)
# of samples with PTEN mutation	(397/2254) (17.6%)	(251/1320) (19.0%)	(144/867) (16.4%)	N/A	135/985 (13.7%) Both led to 44.5% survival w/ 55 and male (61.3%) and 50-55 (14.2%)	22/1057 (1.6%)	
# of samples with TP-53 Mutation	(985/ 2254) (43.7%)	(621/1738) (44.3%)	(364/867) (42.0%)	N/A	N/A	141/397 (35.5%)	660/1057 (62.2%)
Tumor Site	N/A (80.4%) Supratentorial, Frontal Lobe (11.5%)	N/A (81.1%) Supratentorial, Frontal Lobe (10.7%)	N/A (78.1%) Supratentorial, Frontal Lobe (13.5%)	N/A	N/A (83.7%) Supratentorial, Frontal Lobe (9.7%)	N/A (96.4%) Supratentorial, Frontal Lobe (1.9%)	N/A (77.6%) Supratentorial, Frontal Lobe (15.0%)
Survival Status	1498 (57.0%)	867 (57.6%)	631 (60.4%)	N/A	560 (63.3%)	163 (45.0%)	748 (76.2%)

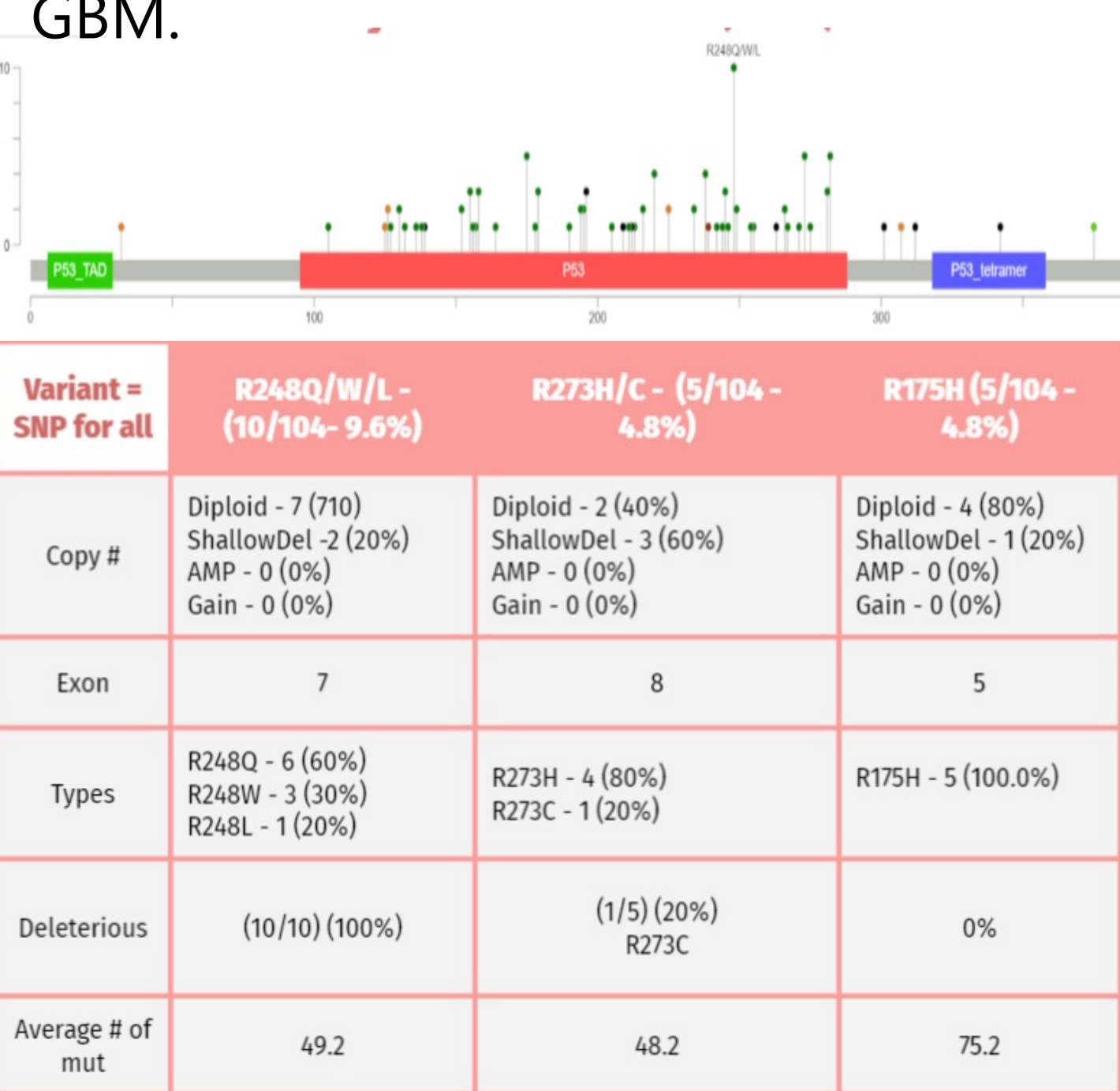**Table 1:** Shows trends in mutations for glioma.

	Most Prevalent	Males (403 samples)	Females (248 samples)	Race	Tp-53 Survival (87 samples)	PTEN Survival (96 samples)
Diagnosis Age	55-60 (13.3%)	55-60 (15.1%)	60-65 (11.3%)	N/A	70-75 (13.8%)	60-65 (17.7%)
Sex	Male (61.0%)	N/A	N/A	N/A	Male (59.8%)	Male (59.4%)
# of samples with PTEN mutation	(96/322) (29.8%)	(57/205) (27.8%)	(38/114) (33.3%)	N/A	(31/87) (35.6%) w/ male (64.5%) and 8 (25.8%) survival and (70-75 age - 19.4%)	N/A
# of samples with TP-53 Mutation	(87/322) (27.0%)	(52/205) (25.4%)	(35/114) (30.7%)	N/A	N/A	(31/96) (32.3%)
Survival Status	133 (20.5%)	78 (19.7%)	55 (22.6%)	N/A	26 (29.9%)	21 (21.9%)

Table 2: Shows trends in mutations for GBM.

Variant = SNP for all	R273C/H/L - (194/1249 w/ 26.3% duplicates)	R248 Q/W - (74/1249 w/ 25.6% Duplicates)	R175H/C (51/1249 w/ 31.3% Duplicates)
Copy #	Diploid - 164 (84.5%) ShallowDel -9 (4.6%) AMP - 3 (1.55%) Gain - 1 (0.52%)	Diploid - 64 (86.5%) ShallowDel -2 (2.7%) AMP - 0 (0%) Gain - 1 (1.35%)	Diploid - 46 (90.2%) ShallowDel -0 (0%) AMP - 0 (0%) Gain - 0 (0%)
Exon	8	7	5
Types	R273C - 143 (73.7%) R273H - 42 (21.6%) R273L - 8 (4.1%)	R248Q - 42(56.8%) R248W - 32 (43.2%)	R175H - 50(98.0%) R175G - 1 (1.96%)
Deleterious	(151/194) (77.8%)	(74/74) (100%)	(1/51) (2.0%) *the only R175G
Average # of mut	34.56	27.6	15.1

Figure 1 (top): shows all the mutations for p53 in the glioma databases. **Table 3 (bottom):** The three most common mutations for p53.

Variant = SNP for all	R248Q/W/L - (10/104- 9.6%)	R273H/C - (5/104 - 4.8%)	R175H (5/104 - 4.8%)
Copy #	Diploid - 7 (70%) ShallowDel -2 (20%) AMP - 0 (0%) Gain - 0 (0%)	Diploid - 2 (40%) ShallowDel - 3 (60%) AMP - 0 (0%) Gain - 0 (0%)	Diploid - 4 (80%) ShallowDel -1 (20%) AMP - 0 (0%) Gain - 0 (0%)
Exon	6	8	5
Types	R248Q - 6 (60%) R248W - 3 (30%) R248L - 1 (20%)	R273H - 4 (80%) R273C - 1 (20%)	R175H - 5 (100.0%)
Deleterious	10/10 (100%)	(1/5) (20%) R273C	0%
Average # of mut	49.2	48.2	75.2

Figure 2 (top): shows all the mutations for p53 in the GBM databases. **Table 4 (bottom):** The three most common mutations for p53.

- Males made up over half of all the mutation samples and had a lower survival rate compared to females. The age range 50-55 was most common. The low survival rate of PTEN of 45% in **Table 1** and 29.9% in **Table 2** led to further research into the gene.
- The mutations were concentrated in the DNA-binding domain of p53 as seen in **Figures 1-2**. **Table 4** shows that GBM had a higher average number of mutations in its genes compared to the data in **Table 3** representing lower-grade gliomas. Diploid mutations of the variant type SNP made up the bulk of the samples.
- The location of concentration of the mutations in PTEN remains unclear as seen in **Figures 3-4**. **Tables 5-6** show the same trend in the number of mutations as in p53. Diploid mutations made up the bulk of the samples. The only common mutations that were prevalent in both databases were R130*/Q/G, which was 27.7% to 28.6% deleterious.
- The data acquired from the cell line revealed mutations found in exons 5-6 of the p53 gene. Out of the 11 mutations found, there were 3 mutations that yielded no change, 2 deletion mutations, and mutations that involved a base substitution that changed the S amino acid to C or N. Regarding the PTEN mutations, mutations were found in exons 5-7. 2 of the 9 mutations discovered remain unknown. The known mutations involved different amino acids (I, K, R, F, and C) and deletion mutations involved the H amino acid.
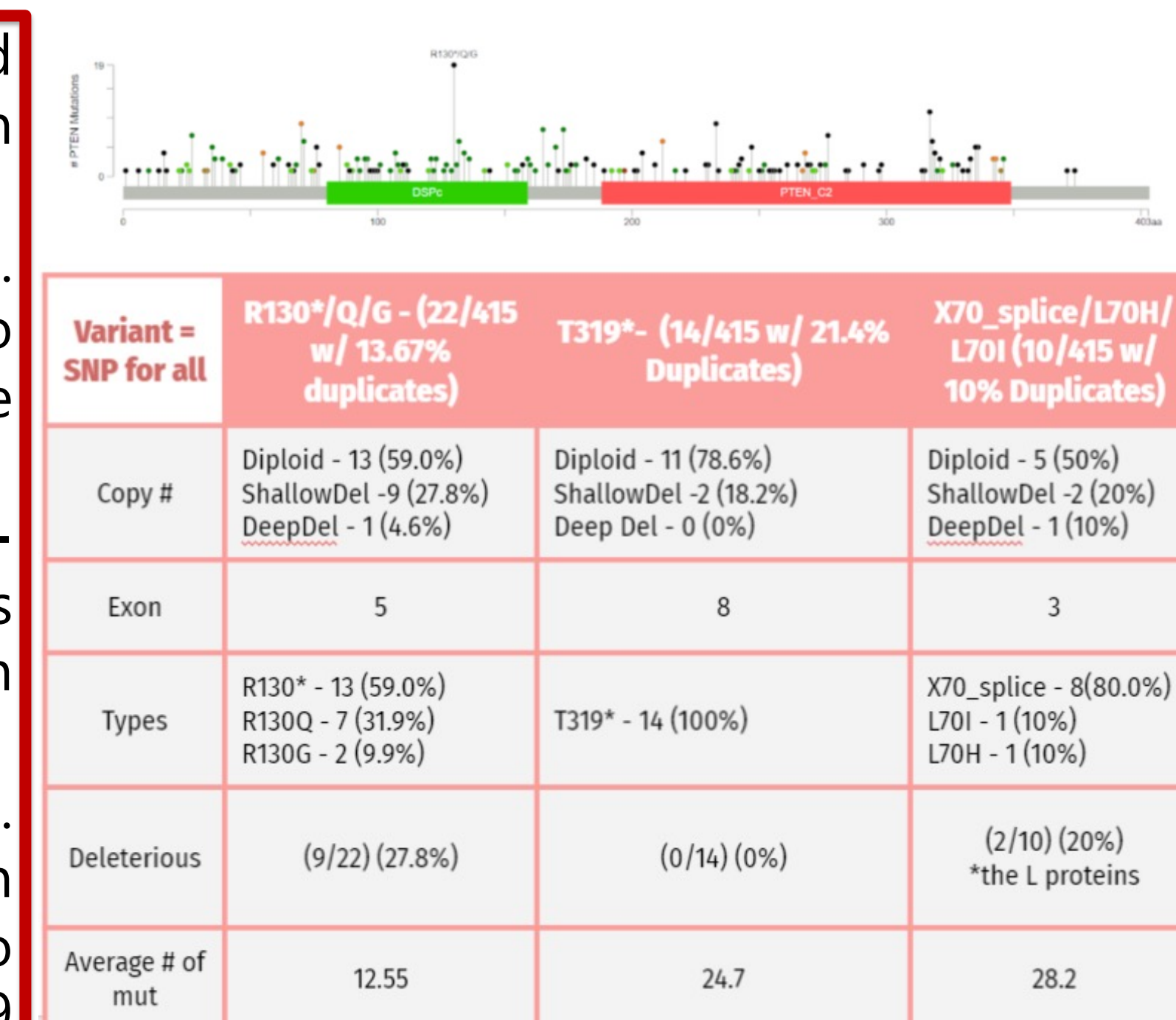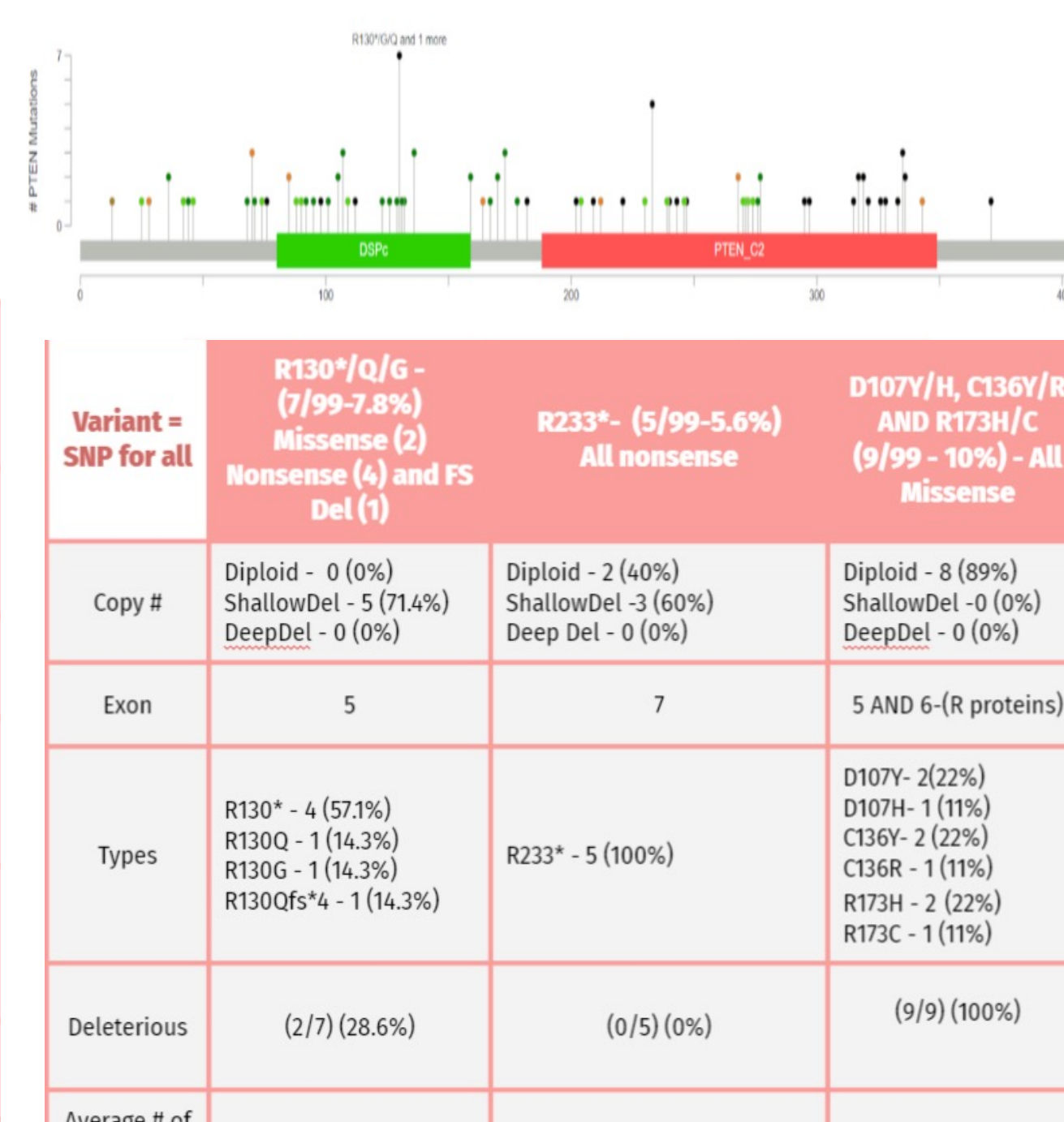

Variant = SNP for all	R130*/Q/G - (22/415 w/ 13.67% duplicates)	T319* - (14/415 w/ 21.4% Duplicates)	X70_splice/L70H/ L70I (10/415 w/ 10% Duplicates)
Copy #	Diploid - 13 (59.0%) ShallowDel - 9 (27.8%) DeepDel - 1 (4.6%)	Diploid - 11 (78.6%) ShallowDel -2 (18.2%) Deep Del - 0 (0%)	Diploid - 5 (50%) ShallowDel - 2 (20%) DeepDel - 1 (10%)
Exon	5	8	3
Types	R130* - 13 (59.0%) R130Q - 7 (31.9%) R130G - 2 (9.9%)	T319* - 14 (100%)	X70_splice - 8(80.0%) L70I - 1 (10%) L70H - 1 (10%)
Deleterious	(9/22) (27.8%)	(0/14) (0%)	(2/10) (20%) *the L proteins
Average # of mut	12.55	24.7	28.2

Figure 3 (top): shows all the mutations for PTEN in the glioma databases. **Table 5 (bottom):** The three most common mutations for PTEN.

Variant = SNP for all	R130*/Q/G - (7/99-7.8%) Nonsense (4) and FS Del (1)	R233* - (5/99-5.6%) All nonsense	D107*/H, C130*/C/R AND R130I/C (9/99 - 10%) - All Missense
Copy #	Diploid - 0 (0%) ShallowDel - 5 (71.4%) DeepDel - 0 (0%)	Diploid - 2 (40%) ShallowDel - 3 (60%) Deep Del - 0 (0%)	Diploid - 8 (89%) ShallowDel - 2 (20%) DeepDel - 0 (0%)
Exon	5	7	5 AND 6-(R proteins)
Types	R130* - 4 (57.1%) R130I - 1 (14.3%) R130S - 1 (14.3%) R130Qfs*% - 1 (14.3%)	R233* - 5 (100%)	D107*- 2(22%) R130I - 1 (11%) C130*- 2 (22%) C136R - 1 (11%) R173H - 2 (22%) R173C - 1 (11%)
Deleterious	(2/7) (28.6%)	(0/5) (0%)	(9/9) (100%)
Average # of mut	59.86	117	43.1

Figure 4 (top): shows all the mutations for PTEN in the GBM databases. **Table 6 (bottom):** The three most common mutations for PTEN.

Discussion

- The research conducted on the cBioPortal revealed that both p53 and PTEN affected people in the age groups of 50-60 the most in the glioma and glioblastoma multiforme databases. Survival rates for both dropped from the mid 50% in the glioma databases to the mid 20% in the GBM databases. On the other hand, the number of mutations increased from the mid-20s to the mid-50s from the glioma to the GBM database, possibly indicating a useful signal in identifying the emergence of GBM.
- The least fatal p53 mutation in both databases was R175H and could therefore be held less significant than other mutations for prognosis or identification of GBM. Even though R273C/H/L had deleterious effects on 77.8% of patients discovered to have that mutation in the glioma samples but only 20% for GBM, therapeutic strategies to restore p53 function by inhibiting this mutation are essential. R278Q/W/L, which had 100% deleterious in the samples in which the mutations were discovered, is an exceedingly fatal mutation that requires further research into therapeutic strategies to combat it.
- As seen in the databases, no specific mutation in PTEN prevailed as the most common or the most fatal. However, the mutations were concentrated in exons 5-7, indicating the need for further research into this region for therapeutic strategies. Further research into the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway, which represses tumor cell growth and survival, could provide more therapeutic methods in battling GBM (Dillon & Miller, 2014).
- Furthermore, the use of CRISPR gene-editing technology to target specific hotspots mentioned previously, like R248Q/W/L, could transform the battle against not only GBM but many other cancers due to its cheap cost and ability to edit "virtually any segment of DNA within the 3 billion letters of the human genome" (NCI Staff, 2020).
- The laboratory procedure involving the GBM cell line provided a few results. The mutations within PTEN involved different amino acids, affirming the trend discovered in the databases. However, either primer issues or incorrect methodology during the DNA extraction process combined with the inability to access a laboratory to perform the procedure individually may have caused the poor quality of the Sanger sequencing. Nonetheless, it was a valuable learning experience in DNA extraction, PCR, and DNA analysis.

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