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Frequency of Somatic TP53 Mutations in Combination with Known Pathogenic Mutations in Colon Adenocarcinoma, Non–Small Cell Lung Carcinoma, and Gliomas as Identified by Next-Generation Sequencing

Zahra Shajani-Yi, Francine B. de Abreu, Jason D. Peterson and Gregory J. Tsongalis

Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center and Norris Cotton Cancer Center, Lebanon, NH; The Theodore and Audrey Geisel School of Medicine at Dartmouth, Hanover, NH

Abstract

The tumor suppressor gene *TP53* is the most frequently mutated gene in human cancer. It encodes p53, a DNA-binding transcription factor that regulates multiple genes involved in DNA repair, metabolism, cell cycle arrest, apoptosis, and senescence. *TP53* is associated with human cancer by mutations that lead to a loss of wild-type p53 function as well as mutations that confer alternate oncogenic functions that enable them to promote invasion, metastasis, proliferation, and cell survival. Identifying the discrete *TP53* mutations in tumor cells may help direct therapies that are more effective. In this study, we identified the frequency of individual *TP53* mutations in patients with colon adenocarcinoma (48%), non–small cell lung carcinoma (NSCLC) (36%), and glioma/glioblastoma (28%) at our institution using next-generation sequencing. We also identified the occurrence of somatic mutations in numerous actionable genes including *BRAF*, *EGFR*, *KRAS*, *IDH1*, and *PIK3CA* that occurred concurrently with these *TP53* mutations. Of the 480 tumors examined that contained one or more mutations in the *TP53* gene, 219 were colon adenocarcinomas, 215 were NSCLCs, and 46 were gliomas/glioblastomas. Among the patients positive for *TP53* mutations diagnosed with colon adenocarcinoma, 50% also showed at least one mutation in pathogenic genes of which 14% were *BRAF*, 33% were *KRAS*, and 3% were *NRAS*. Forty-seven percent of NSCLC patients harboring *TP53* mutations also had a mutation in at least one actionable pathogenic variant with the following frequencies: *BRAF:* 4%, *EGFR*: 10%, *KRAS:* 28%, and *PIK3CA:* 4%. Fifty-two percent of patients diagnosed with glioma/glioblastoma with a positive *TP53* mutation had at least one concurrent mutation in a known pathogenic gene of which 9% were *CDKN2A,* 41% were *IDH1*, and 11% were *PIK3CA.*

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Introduction

Sequencing of the cancer genome has shown that the tumor suppressor gene *TP53* is the most frequently mutated gene in human cancer [1–3]. *TP53* is located on chromosome 17p13 and composed of 19,149 base pairs and 11 exons (NM_000546.5). It encodes p53, a DNA-binding transcription factor that regulates multiple genes involved in DNA repair, metabolism, cell cycle arrest, apoptosis, and senescence by controlling their transcription rate. *TP53* is well known as a tumor suppressor gene, associated with human cancer by mutations that lead to a loss of wild-type p53

Abbreviations: NSCLC, Non–small cell lung carcinoma; NGS, Next Generation Sequencing; LOF, Loss of Function; IARC, International Agency for Research of Cancer; FFPE, Formalin-Fixed, Paraffin-Embedded; H&E, Hematoxylin and Eosin.

Address all correspondence to: Gregory J. Tsongalis, PhD, Department of Pathology and Laboratory Medicine, Dartmouth-Hitchcock Medical Center, One Medical Center Drive, Lebanon, NH 03756. Received 28 July 2017; Revised 14 December 2017; Accepted 18 December 2017

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function (loss of function, LOF) [4]. In healthy cells, p53 is present at low concentrations; however, when cells are subjected to hypoxic stress or DNA damage, p53 inhibits its own degradation, allowing for a rapid concentration increase. p53 can then bind to promoter DNA, stimulating the transcription of several genes that repair damaged cells. If the damage is significant and cannot be repaired, p53 then induces apoptosis, preventing tumor growth. Cells with mutated p53 protein cannot bind to the DNA promoters, and subsequently, mutant/damaged cells are not repaired or destroyed. These cells lose their tumor suppressor activity, allowing for tumor proliferation [4–6].

Mutations in *TP53* have been found across the entire coding region, and tumor suppressor activity is lost by different mechanisms depending on the type of mutation [7]. According to the International Agency for Research of Cancer, there are currently greater than 7000 unique coding mutations within the *TP53* gene with >70% being missense mutations. The vast majority (90%) of all mutations are reported to be in the DNA binding region (base pairs: 102-292), with six point mutations (R175, G245, R248, R249, R273, R282) accounting for nearly 30% of all mutations [8]. These six mutations are referred to as "hotspot mutations" that disrupt tumor suppressor activity by 1) conformational point mutations that have a global effect on the tertiary protein fold (R175, G245, R249, R282) or 2) contact mutations that have a localized effect that disrupt binding to DNA (R248, R273) [3,7,9,10]. In addition to these tumor suppressor properties, many *TP53* mutations are now known to have oncogenic properties that enable them to promote invasion, metastasis, proliferation, and cell survival [11,12]. They do so by interacting with different DNA promoters than wild-type p53, subsequently upregulating different sets of genes. Cell-line studies have demonstrated that the hotspot mutations all exhibit some form of oncogenic activity [12]. Oncogenes are seen as more promising targets for treatment than tumor suppressor genes; the latter requires restoring normal function to the mutant gene, which has proved to be increasingly difficult. Clinical trials using gene replacement therapy have, to date, reported limited efficacy [13].

In this study, we identified the frequency of individual *TP53* mutations in patients with colon adenocarcinoma, non–small cell lung carcinoma (NSCLC), and glioma/glioblastoma at our institution using next-generation sequencing. We also identified the occurrence of somatic mutations in numerous actionable genes including *BRAF*, *CDKN2A, EGFR*, *IDH1, KRAS*, *NRAS,* and *PIK3CA* that occurred concurrently with these *TP53* mutations.

Materials and Methods

A total of 1211 formalin-fixed, paraffin-embedded (FFPE) surgical and cytology tumor samples with adenocarcinoma or poorly differentiated carcinoma histology were received for somatic mutation screening at the Dartmouth-Hitchcock Medical Center from 2013 to 2016. Our cohort consisted of 456 cases of colon adenocarcinoma, 592 cases of NSCLC, and 163 cases of glioma/glioblastoma. Hematoxylin and eosin slides were reviewed by an attending pathologist for tumor content and percent cellularity. Samples with tumor cellularity below 10% were not submitted for testing.

DNA was extracted using the Gentra Pure Gene Kit (Qiagen) (2013-August 2015) and QIAmp DNA FFPE Kit (Qiagen) (August 2015-June 2016). DNA quantification and DNA quality were performed using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen) and KAPA hgDNA Quantification and QC Kit (KAPA Biosystems), respectively. Validation between the two extraction methods yielded 100% concordance (data not shown). Samples with DNA concentrations below 1.7 ng/μl and quality ratio (Q129/Q41) below 0.4 were not screened. Quality ratio (Q129/Q41) was determined by dividing the quantification of 129-bp amplicons by the quantification of 41-bp amplicons.

Library preparation was performed using the Ion AmpliSeq Cancer Hotspot Panel v2 (CHPv2) (Life Technology) as described in previous studies [14,15]. Libraries were quantified, normalized, pooled, and sequenced using the Ion 318 Chip v2 or Ion 316 Chip v2 on the PGM Sequencing Platform. Variants were identified using the Variant Caller Plugin (v4.0.2) available in the Torrent Suite, and Golden Helix SVS (v7.7.8) was used to assess quality and functional predictions.

For this study, deidentified patients positive for *TP53* mutations and diagnosed with colon adenocarcinoma, NSCLC, or glioma/glioblastoma were selected. The *TP53* gene encodes the tumor suppressor protein p53 with three domains: a transactivation domain (exons 2-3), a DNA binding domain (exons 4-8), and an oligomerization domain (exons 9-10) [8]. The CHPv2 covers all 3 domains and 7 of the 11 exons: 2 (transactivation domain), 4-8 (DNA binding domain), and 10 (oligomerization domain).

Results

TP53 mutations were identified in 480 of 1211 patient samples (40%) with greater than 300 independent mutations. A total of 522 *TP53* mutations were detected in 480 tumors. Of these, 455 were single nucleotide variants (SNVs) and 67 were insertions or deletions (INDELs). Of the 480 tumors examined that contained one or more mutations of the *TP53* gene, 219 were colon adenocarcinomas (SNVs: 200, INDELs: 34), 215 were NSCLCs (SNVs: 205, INDELs: 28), and 46 were gliomas (SNVs: 50, INDELs: 5). Although SNVs and INDELs were observed in this cohort of patients, this study focuses only on a subset of SNVs.

Colon Adenocarcinoma

Forty-eight percent (219/456) (48%) of colon adenocarcinoma cases were found to have one or more mutations in *TP53*. Two hundred and thirty-four *TP53* mutations were identified in 219 tumors with 79% (185/234) of mutations found in the DNA binding region. Twenty-three percent (53/234) of all mutations were hotspot mutations. Over 130 discrete *TP53* mutations were identified. The most common mutations in our patient population were hotspot residues R248 (19/234, 8%) and R273 (13/234, 6%) (Table 1). Variants in clinically actionable genes were observed in 50% of tumors (109/219) harboring one or more *TP53* mutations, including variants in the following pathological genes at the following

Table 1. Summary of *TP53* Mutations Identified in Each Tumor Type and Prevalence of Mutations in Clinically Actionable Genes

	TP53 Mutation	*TP53* Hotspot Residues	Most Common *TP53* Mutation	Prevalence of Actionable Gene Mutations	Actionable Gene Mutations (>5%)
Colon	219/456 (48%)	54/234 (23%)	R248 (8%) R273 (6%)	109/219 (50%)	*KRAS* (33%) *BRAF* (14%)
NSCLC	215/592 (36%)	26/233 (11%)	V157 (3%) R158 (3%)	101/215 (47%)	*KRAS* (28%) *EGFR* (10%)
Glioma	46/163 (28%)	13/55 (24%)	R273 (13%)	24/46 (52%)	*IDH1* (41%) *PIK3CA* (11%) *CDKN2A* (9%)

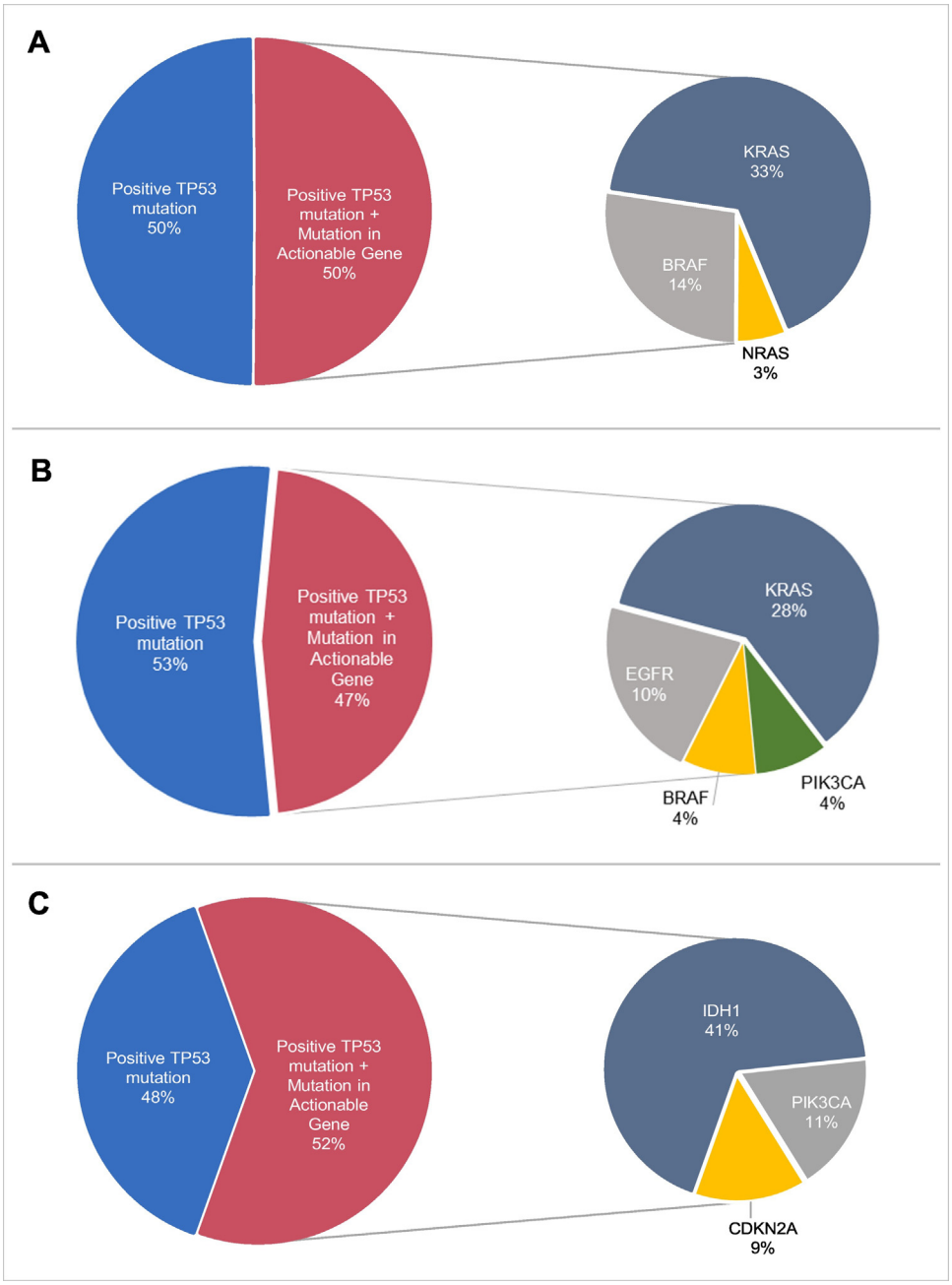

Figure 1. Frequency of *TP53* mutations identified in each tumor type as well as frequency of concurrent *TP53* mutations with mutations in clinically actionable genes. (A) Colon adenocarcinoma. (B) NSCLC. (C) Glioma/glioblastoma. Some tumors exhibited mutations in more than one clinically actionable gene.

frequencies: *BRAF* (30/219, 14%), *KRAS* (73/219, 33%), and *NRAS* (7/219, 3%). Thirty-three percent (36/109) of tumors exhibited only one pathogenic mutation in addition to a *TP53* mutation, of which 28% (10/36) were *BRAF*, 69% (25/36) were *KRAS*, and 3% (1/36) were *NRAS*. Sixty-seven percent (73/109) of tumors with pathogenic variants had more than one mutation in addition to *TP53*. Figure 1*A* shows a summary of *TP53* mutations identified in patients diagnosed with colon adenocarcinoma, and Figure 2*A* shows the frequency of *TP53* mutations detected concurrently with mutations in clinically actionable genes (*BRAF*, *KRAS*, and *NRAS*), respectively.

NSCLC

Thirty-six percent (215/592) of NSCLC cases were found to have one or more mutations in *TP53*. Two hundred and thirty-three *TP53* mutations were found in 215 tumors, with 82% (191/233) of these mutations found in the DNA binding region. Eleven percent (26/233) of all mutations were found in hotspot residues. Over 160 discrete *TP53* mutations were identified. Interestingly, in our patient population, the two most common mutations were V157 (7/233, 3%) and R158 (8/233, 3%), which are not considered hotspot residues (Table 1). In addition to *TP53* mutations, concurrent

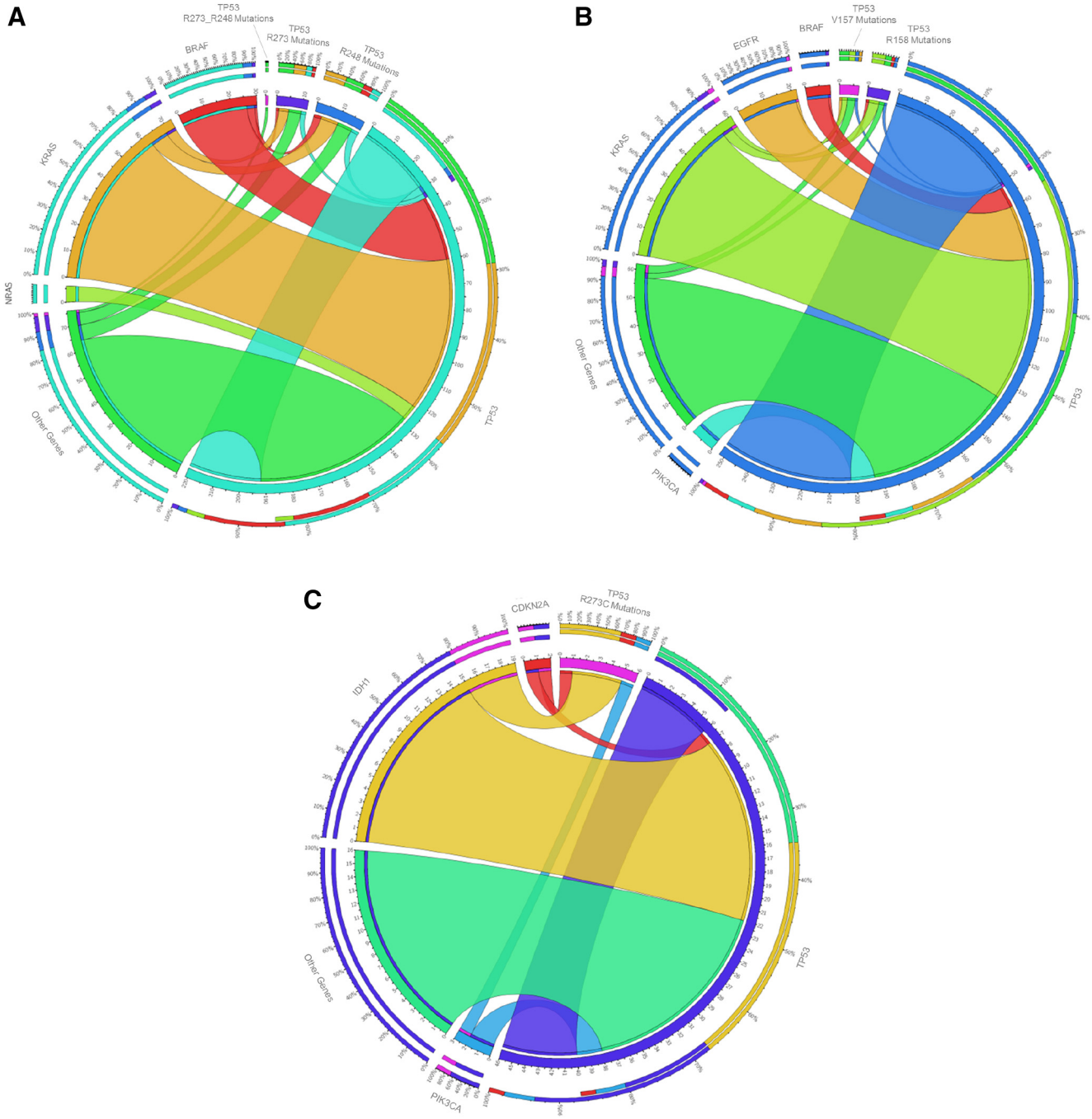

Figure 2. Circos plots for each tumor type showing the frequency of *TP53* mutations that occurred concurrently with clinically actionable genes. (A) Colon adenocarcinoma samples. (B) NSCLC. (C) Glioma/glioblastoma. The graphs are divided into specific *TP53* mutations (colon: R273, R248, and R273_R248; NSCLC: V157 and R158; glioma: R273C), other *TP53* mutations (other than the ones described above), actionable genes, and nonactionable genes (or "other genes"). Each category is represented by a unique color within the inner circle. For example: in graph A, the actionable genes are *KRAS*, *BRAF*, and *NRAS*. *KRAS* is represented in orange. Approximately 5% of the *KRAS* mutations were identified concurrently with *TP53* R273 mutations (purple), 10% with *TP53* R248 mutations (blue), and 85% with other *TP53* mutations (turquoise). *BRAF* is represented in red. Approximately 3% of the *BRAF* mutations were identified concurrently with *TP53* R273 mutations (purple), 6% with *TP53* R248 mutations (blue), and 91% with other *TP53* mutations (turquoise). *NRAS* is represented in light green. Approximately 6% of the *NRAS* mutations were identified concurrently with other *TP53* mutations (turquoise). The other gene category is represented in dark green. Approximately 2% of mutations detected in nonactionable genes were identified concurrently with *TP53* R273_R248 mutation (burgundy), 8% with *TP53* R273 mutations (purple), 10% with *TP53* R248 mutations (blue), and 84% with other *TP53* mutations (turquoise).

variants in clinically actionable genes were observed in 47% of tumors (101/215): *KRAS* (61/215, 28%), *EGFR* (22/215, 10%), *PIK3CA* (9/215, 4%), and *BRAF* (9/215, 4%). Sixty-five percent (66/101) of tumors exhibited only one pathogenic mutation, of which 62% (41/66) were in *KRAS*, 21% (14/66) were in *EGFR*, 9% (6/66) were in *BRAF*, and 6% (4/66) were in *PIK3CA*. Thirty-five percent (35/101) of tumors

with pathogenic variants had more than one mutation in addition to *TP53*. Figure 1*B* shows a summary of *TP53* mutations identified in patients diagnosed with NSCLC, and Figure 2*B* shows the frequency of *TP53* mutations detected concurrently with mutations in clinically actionable genes (*KRAS*, *EGFR*, *PIK3CA*, and *BRAF*), respectively.

Glioma/Glioblastoma

Twenty-eight percent (46/163) of glioma/glioblastoma cases were found to have one or more mutations in *TP53*. Fifty-five *TP53* mutations were identified in 46 tumors, with 82% (45/55) mutations found in the DNA binding region. Twenty-four percent (13/55) of all mutations were hotspot mutations, and 48 discrete *TP53* mutations were found. The most common mutation in our patient population was in a hotspot residue, R273C, which was present in 13% (6/46) of patients (Table 1). In addition to *TP53* mutations, concurrent variants in clinically actionable genes were observed in 52% (24/46) of tumors, including variants in the following clinically actionable genes: *IDH1* (19/46, 41%), *CDKN2A* (4/46, 9%), and *PIK3CA* (5/46, 11%). Sixty-seven percent (16/24) of tumors with pathogenic variants exhibited only one pathogenic mutation, of which 94% (15/16) were in *IDH1* and 6% (1/16) were in *PIK3CA*. Thirty-three percent (8/24) of tumors with pathogenic variants had more than one mutation in addition to *TP53*. The most common combination of multiple gene mutations was found in *IDH2* and *PIK3CA* in two patients (25%, 2/8). Figure 1*C* shows a summary of *TP53* mutations identified in patients diagnosed with glioma/glioblastoma, and Figure 2*C* shows the frequency of *TP53* mutations detected concurrently with mutations in clinically actionable genes (*IDH1, IDH2, CDKN2A,* and *PIK3CA*).

Discussion

Here we report one of the largest studies looking at individual *TP53* mutations in 456 cases of colon adenocarcinoma, 592 cases of NSCLC, and 163 cases of glioma/glioblastoma. We determined that the frequency of *TP53* mutations in our patients varied with cancer type, with an identifiable *TP53* mutation found in 48% of colon adenocarcinomas, 36% of NSCLCs, and 28% of gliomas. *TP53* mutational frequencies have previously been reported to range from 33% to 52% (colorectal adenocarcinomas) [16–19], 22% to 60% (NSCLC) [19–23], and 25% to 28% (gliomas) [1,24]; however, variances in the cohort size, the number of exons sequenced, and cancer stage included in these studies render comparisons to our study impossible. Variances in cancer subtype grouping also complicate any attempts at a direct comparison. For example, in our study, we classified all NSCLC patients as adenocarcinoma (ADC) or squamous cell carcinoma (SCC). Several studies have reported that *TP53* mutations are found less frequently in ADC than SCC [1,23,25,26].

We also determined that mutations in the hotspot residues were prevalent in 24% patients with glioma/glioblastoma and in 23% of patients of colon adenocarcinoma but only 11% of patients with NSCLC. The most frequent *TP53* mutations for both colon adenocarcinoma and glioma/glioblastomas were "hotspot" residues; however, interestingly, this was not the case for NSCLC. Across all tumor types, numerous discrete mutations spanned all three domains, with ~80% of all mutations occurring in the DNA binding domain. The most prevalent *TP53* mutation was found in glioma patients, with 13% having an identifiable R273C mutation. The most common *TP53* mutations in patients with colon adenocarcinoma and NSCLC were found at lower frequencies of 8% and 3%, respectively.

The role of *TP53* mutational status as a predictive marker for treatment response and prognostic marker for survival has been mixed, and the conclusions have varied depending on study size, cancer stage, the method used to assess mutational status, and the type of tumor studied [20,27,28]. The majority of recent studies have shown that *TP53* mutations are associated with poorer overall survival in NSCLC patients [20,21,29,30]. In particular, stage I NSCLC patients with mutant *TP53* have a statistically significant worse overall survival than patients with wild-type *TP53* [21]. Patients with *TP53* mutations also have a poorer overall response to therapy with increased resistance to radiation and to adjuvant cisplatin therapy. Treatment with cisplatin is used after surgery and chemoradiotherapy in stage I to stage III and is a common first-line therapy in stage IV NSCLC [20,27,30]. Co-clinical trials in engineered mouse models have demonstrated that the loss of *TP53* or *STK11* impaired the response of *KRAS*-mutant cancers to docetaxel monotherapy and that the addition of selumetinib provided substantial benefit for mice with lung cancers caused by *KRAS* and *KRAS/TP53* mutations [31]. *TP53* mutational status has also been reported to have prognostic and predictive value in breast carcinoma, advanced sarcomas, and ovarian cancers [32–34]. *TP53* has also been reported as a strong marker for predicting the effect of adjuvant 5-fluorouracil in stage III colon cancer [35]. Mutational status information has the potential to be immensely valuable for developing personalized therapies.

In colon tumors, the two most frequent mutations in our cohort were contact mutations: point mutations of arginine 248 to glutamine (R248Q) and arginine 273 to cysteine (R273C). R273 mutations have been reported to show increased resistance to paclitaxel, cisplatin, and doxorubicin [36–38]. Patients with metastatic sarcoma or metastatic colorectal cancer who were positive for *TP53* mutations in exons 5-8 (A159fs, R213*, R175H, H179R, H193R, V216M, G245S, and R273C) had better clinical outcomes (longer median progression-free disease and median overall survival) in response to treatment with pazopanib and vorinostat [39]. Knowledge of discrete mutational status has the potential to allow clinicians to direct treatment and use alternate first-line therapies when indicated.

We also identified somatic mutations in numerous actionable genes that occurred concurrently with these *TP53* mutations. Knowledge of both mutational status and concurrent actionable gene mutational status has the potential to allow clinicians to direct treatment and use alternate first-line therapies as indicated. Differences in drug sensitivities in response to concurrent mutations are already under way in KRAS mutant lung adenocarcinomas [40].

P53 is an attractive target for cancer therapeutics. It has a high mutational frequency (~40%-50%) across all different cancer types; however, the mutational frequency can vary significantly, ranging from >95% in ovarian serous carcinoma to <10% in hematopoietic malignancies [1,2,41,42]. Different discrete p53 mutations alter p53 function by various mechanisms and exhibit varying oncogenic activity [42]. Multiple studies have suggested that these mutations are not biologically equivalent and that some mutations lead to phenotypes that are more aggressive or to tumors that are more resistant to therapy [41]. To date, despite the uncovering of numerous discrete mutations in tumors, only a handful of mutations have been studied in depth. Very few studies have looked at the

frequency of individual *TP53* mutations in various tumor types along with the prognostic and predictive value of individual mutations [32,35]. NSCLC patients with mutations that were predicted to disrupt the structure of p53 and who were treated with chemotherapy had decreased overall survival rates compared to untreated patients with the same type of mutations. This phenomenon was not seen in patients with wild-type p53 [28]. Identification of both discrete *TP53* mutation and concurrent known actionable genes present in the tumor may assist clinicians in directing therapy and aid in predicting chemotherapy/radiotherapy resistance and response.

It has been suggested that the binary model representation of *TP53* mutations should be replaced with a functional classification based on the activity of the mutation [3]. To this end, our study provides details on the frequency of individual mutations in a large cohort of patients with colon adenocarcinomas, NSCLCs, and gliomas.

The CHPv2 panel used in this study is designed to assess hotspot regions within 50 genes that are frequently mutated in human cancers; subsequently, one of the limitations of this study is that the CHPv2 panel does not sequence the entire coding region of the *TP53* gene. The panel covers 7 of the 11 exons of the *TP53* gene, including the 4 exons that make up the DNA-binding region. To date, most reports have focused on detecting mutations/deletions/insertions within the DNA-binding region, and 90% of mutations have been reported to be located within the DNA-binding region; however, this could be due to the scarcity of studies that look at the whole gene. As more studies look at an increased number of exons, the mutational frequency of the binding region could decline. In this study, the mutational frequency within the DNA binding region was 79%, 82%, and 82% for colon adenocarcinoma, NSCLC, and glioma/glioblastoma tumors, respectively.

Another limitation of this study is that all patient data were deidentified and clinical data were not available; hence, no conclusions on the phenotypic profile of the discrete mutations can be made. Future studies correlating the molecular profile of the tumors with the clinical presentation, treatment, and progression-free survival would be of extraordinary value. Correlating outcomes with discrete *TP53* mutations is complicated by the vast number of discrete mutations found in colon adenocarcinoma, NSCLC, and gliomas and will require extensive computational effort; however, this also increases the potential to select targeted therapies in patients with a particular molecular profile.

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