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SHORT COMMUNICATION

# Biallelic *TP53* gain of function mutations in rapidly progressing solid tumors

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**Abstract**

Recent studies are discovering *TP53* mutations with gain of function (GOF) properties that promote tumorigenesis via a variety of mechanisms. To our knowledge, all reported compound mutations are allelic. We identified two patients with biallelic GOF *TP53* mutations in their tumors and a third with allelic compound variants. The correlation with p53 expression was also examined. Genomic DNA was extracted from formalin-fixed, paraffin-embedded tissue and mutational analysis was performed using Ion AmpliSeq™Cancer HotSpot Panel V2. Biallelic GOF mutations (p.R273H and p.R273C) were identified in a 19-year-old male with glioblastoma (allele frequencies 94% and 48%) and a 54-year-old with pT3 penile squamous cell carcinoma (allele frequencies 19% and 27%). Immunohistochemistry showed nuclear accumulation of p53. The third patient, a 62-year-old female with metastatic lung adenocarcinoma, had allelic p.P278S (GOF) and p.R283L (non-GOF) variants at frequencies of 61% but with null staining for p53. Germline testing for Patient 1 confirmed wildtype *TP53.* No other variants were discovered among the genes tested in these cases. All patients succumbed within two years of diagnosis despite aggressive treatment. In conclusion, implementation of *TP53* mutation analysis in clinical practice may predict patient outcome, and inhibition of GOF p53 could represent an attractive target for therapy.

**Keywords** *TP53*, Gain of function mutation, Next generation sequencing.



## Introduction

*TP53* is a well-known tumor suppressor and the most frequently mutated gene in human cancer [1]. Unlike other tumor suppressors, which are typically inactivated by biallelic deletions or truncations, the loss of tumor suppressor activity of the p53 protein is mainly due to missense mutations in exons 4–9 of the DNA binding domain (DBD) in concert with either a loss of heterozygosity or a dominant-negative (DN) effect of a mutant allele over the remaining wild-type allele [2–5]. About one third of these missense mutations occur in the six hotspots codons within the DBD (R175, G245, R248, R249, R273, and R282) that abrogate normal p53 tumor suppressor activity in favor of an oncogenic profile promoting tumorigenesis and disease progression [6,7]. Evidence of such gain of function (GOF) mutations have been obtained predominantly from cell cultures and animal studies, and little is known about their clinical relevance and behavior in human cancer [8,9]. Oncogenic mutations usually present as a single variant. Primary and secondary double mutations in oncogenes have been documented but are generally less frequent and less well-studied. In colorectal cancer, double codon *KRAS* mutations accounted for just over 1% of *KRAS*-mutated cases [10]. The presence of a *RET* double mutation in multiple endocrine neoplasia type 2 is thought to, but not yet proven to, confer additive oncogenic properties [11]. *KIT* double mutations are usually therapy-related [12,13], and all characterized somatic double mutations in *EGFR* are reported to be allelic [14].

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We herein report two cases with biallelic GOF *TP53* mutations, including one patient with glioblastoma (GBM) presenting at an unusually young age and another patient with a penile squamous cell carcinoma (SqCC), a tumor that accounts for less than 1% of cancers in men in the United States [15]. We also describe a third patient found to have allelic *TP53* compound mutations.

## Materials and methods

### Case report

#### Case 1

Patient 1 was a 19-year-old male with new-onset generalized tonic-clonic seizures. Brain magnetic resonance imaging (MRI) showed a non-enhancing left temporoparietal lesion with associated edema, which was deemed inoperable due to the location. Biopsy results showed anaplastic astrocytoma, IDH1-wildtype by immunohistochemistry (IHC), WHO grade III. He was treated with radiation therapy. A second biopsy 20 months later showed GBM, IDH1-wildtype by IHC, WHO grade IV and molecular studies were performed. The patient died 6 months later.

#### Case 2

Patient 2 was a 54-year-old male who presented with dysuria, severe penile pain, and red lesions on the glans. A biopsy of the glans showed invasive SqCC, and subsequent urethrectomy and penectomy revealed a pT3 poorly differentiated SqCC. He was treated with chemoradiation but developed bilateral lung metastases and died 20 months after initial diagnosis.

#### Case 3

Patient 3 was a 63-year-old female with persistent right upper quadrant pain. She was a former smoker (2 pack/day, quit for 8 years) and received a prophylactic proctocolectomy for a family history of familial adenomatous polyposis. Abdominal imaging revealed liver masses and biopsy confirmed metastatic lung adenocarcinoma. Prior molecular testing performed at an outside laboratory did not find *EGFR* mutations or *ALK* or *ROS1* fusions. No information on the mutational status of the *APC* gene was available. Subsequent imaging showed a lung mass with mediastinal lymphadenopathy. She received chemotherapy and died 10.5 months after initial diagnosis.

### Nucleic acid isolation

One H&E stained and 10 unstained sections (6 µm) were cut from a chosen optimal formalin-fixed, paraffin-embedded tissue block with areas of interest then manually scraped using a razor blade. Genomic DNA (gDNA) was extracted using the RNeasy FFPE mini kit (Qiagen, Valencia, CA) according to manufacturer's instructions. QIAamp DNA blood kit (Qiagen) was used for extraction of DNA from peripheral blood. The concentration of nuclei acids was determined using a Qubit 2.0 fluorimeter (Life Technologies, Carlsbad, CA).

### Next generation sequencing (NGS)

Mutational analysis was performed using the Ion Torrent AmpliSeq™ Cancer Hotspot Panel v2 (Thermo Fisher Scientific, Waltham, MA) according to manufacturer's instructions. Briefly, 10 ng of gDNA was used for library preparation. The libraries were bar-coded, clonally amplified, and sequenced on an Ion S5XL (Thermo Fisher). The data were analyzed using the Torrent Suite Software followed by a laboratory-developed pipeline. The assay has an analytic sensitivity of 5% for single nucleotide variants (SNV).

### Immunohistochemistry studies

IHC for p53 was performed by our immunopathology laboratory using a monoclonal antibody at 1:100 dilution (Dako M7001, Agilent, Santa Clara, CA) on FFPE tissue sections cut at 3 µm.

## Results and discussion

All *TP53* mutations were SNVs and located in exon 8 at codons R273, R278, and R283, which encode part of the p53 DBD (Table 1). *TP53* mutations were the only variants detected by the 50-gene targeted NGS panel. Germline testing on Patient 1's peripheral blood confirmed wildtype *TP53* but was unavailable in the others. IHC for p53 showed strong and diffuse nuclear staining in the tumors from patients 1 to 2. Despite having a limited number of neoplastic cells in Patient 3's tumor section, a null staining pattern for p53 was nonetheless observed (Table 1 and Fig. 1).

The tetrameric p53 protein is primarily known for its role in detecting DNA damage and mediating downstream pathways that determine whether the cell undergoes successful

**Table 1** Compound *TP53* mutations in three patients.

| Case | Age | Gender | Diagnosis | Variants; exon (EX); frequency | Effect | Relationship | IHC |
|---|---|---|---|---|---|---|---|
| 1 | 18 | Male | GBM | *c.817C > T*, p.R273C; EX8; 48% | GOF | Trans | Strong, diffuse |
| | | | | *c.818G > A*, p.R273H; Ex8; 94% | GOF | | Nuclear |
| 2 | 54 | Male | SqCC of penis | *c.817C > T*, p.R273C; EX8; 27% | GOF | Trans | Strong, diffuse |
| | | | | *c.818G > A*, p.R273H; EX8; 19% | GOF | | Nuclear |
| 3 | 63 | Female | AdenoCa of lung | *c.832C > T*, p.P278S; EX8; 61% | GOF | Cis | Negative/ |
| | | | | *c.848G > T*, p.R283L; EX8, 61% | Non-GOF | | Insufficient |

GBM, glioblastoma multiforme; adenoCa, adenocarcinoma; GOF, gain of function.